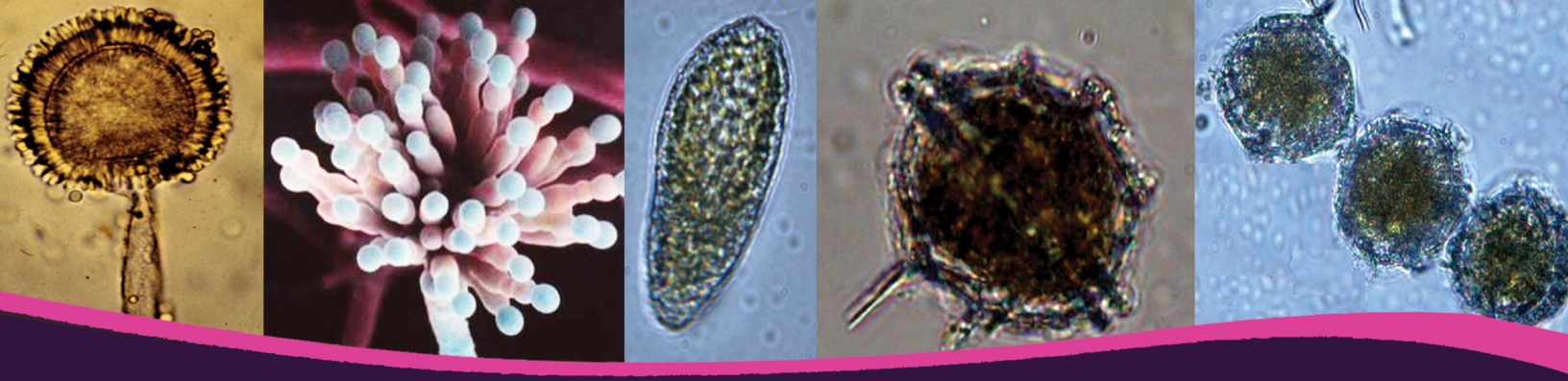

SOCIEDAD LATINOAMERICANA
DE MICOTOXICOLOGÍA
FUNDADA EN 1992

PROCEEDINGS

VI LATIN AMERICAN CONGRESS OF MYCOTOXICOLOGY
and
II INTERNATIONAL SYMPOSIUM
ON ALGAL AND FUNGAL TOXINS FOR INDUSTRY

June 27 to July 1, 2010
Hotel Fiesta Americana
Merida Yucatan

Magda Carvajal Moreno
Chairwoman

Instituto de Biología UNAM

International Agency for Research on Cancer
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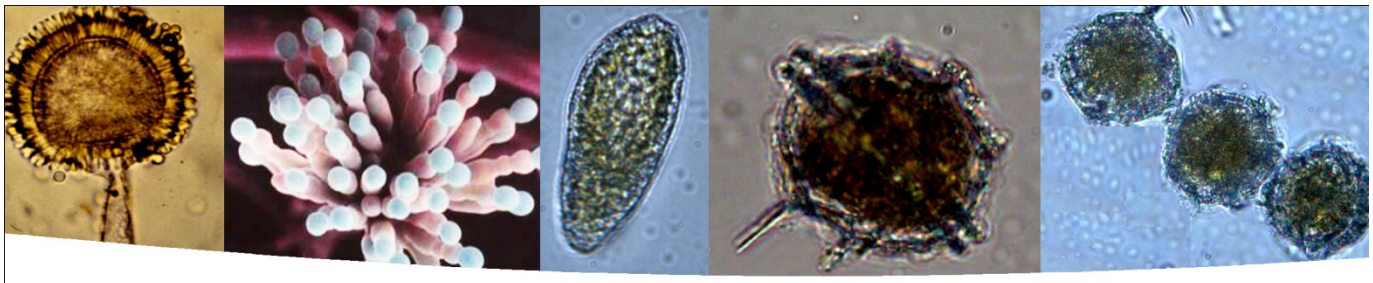

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VI LATIN AMERICAN CONGRESS OF MYCOTOXICOLOGY
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UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO
POR MI RAZA HABLARA EL ESPIRITU

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	Genoveva García	(Mexico)
	René Márquez	(Mexico)
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	Peter Scott	(Canada)
	Wayne Carmichael	(United States of America)
	Ronald Riley	(United States of America)

Scientific Commitee

Christopher P. Wild	(United Kingdom) (IARC France)
Gerald Wogan	(United States of America)
John Groopman	(United States of America)
Gordon S. Shephard	(Republic of South Africa)
Michael Quilliam	(Canada)
Vildes Scussel	(Brazil)
Gary Payne	(United States of America)
Marina Miraglia	(Italy)
Dilek Heperkan	(Turkey)
Sofia Chulze	(Argentina)
Peter Mantle	(United Kingdom)
Maya Piñeiro	(Uruguay) (FAO, Chile)

Sociedad Latinoamericana de Micotoxicología (SLAM Society)

Amaury Martínez	(República Bolivariana de Venezuela). President of the Latin American Society of Mycotoxicology.
Myrna Sabino	(Brazil). Vice-president of SLAM.
Claudio Mazzani	(República Bolivariana de Venezuela). Treasurer SLAM.
Ana Dalcero	(Argentina). Vocal of SLAM. Head of Cyted Project.

Instituto de Biología
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Tila María Pérez Ortiz. Director.
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Julio César Montero. Web Master and Graphic Designer.
Alfredo Wong. Web Server Administrator, Institute of Biology, UNAM.
Joel Villavicencio. Registration and Computing, Institute of Biology, UNAM.

Keynote Conferences

Myrna Sabino	(Brazil)	**Ronald Riley**	(United States of America)
Sofia Chulze	(Argentina)	**John Groopman**	(United States of America)
Vildes Scussel	(Brazil)	**Gordon S. Shephard**	(Republic of South Africa)
Peter Scott	(Canada)	**Michael Quilliam**	(Canada)
Timothy D. Phillips	(United States of America)	**Gary Payne**	(United States of America)
Wayne Carmichael	(United States of America)	**Marina Miraglia**	(Italy)
Christopher P. Wild	(UK (IARC France)	**Dilek Heperkan**	(Turkey)
Gerald Wogan	(United States of America)	**Peter Mantle**	(United Kingdom)
Maya Piñeiro	(Uruguay) (FAO, Chile)		

Ackowledgments

Julio César Montero, Web Master and Graphic Designer, Institute of Biology, UNAM.
Claudia Canela, Head of Admnistration Secretary. Financial Administration .Institute of Biology, UNAM.
Ramon Santiago, Administration. Institute of Biology, UNAM.
Alfredo Wong, Web Server Administrator, Institute of Biology, UNAM.
Rosalba Alonso (UNAM, Mexico) and **René Márquez** (Mexico). Organization.
Peter Scott (Canada) and **Timothy D. Phillips** (United States of America). Organization.
John Groopman (United States of America) and **Myrna Sabino** (Brazil). Honor Ceremony.
Wayne Carmichael (United States of America). Pre-congress Phycotoxin Workshop.
Anthony Lupo. (United States of America). Neogen. Pre-congress Phycotoxin Workshop.
Maya Piñeiro (FAO, United Nations). Organization of FAO Forum.
Genoveva García and **Rebeca Martínez,** Institute of Biology, UNAM. Organization.
Mariana Díaz-Zaragoza, Pável Castillo-Urueta, Institute of Biology, UNAM.
Joel Villavicencio. Registration and Computing**,** Institute of Biology, UNAM.
Universidad Autónoma de Yucatán, for the Ballet, Musical Groups, Videos.
Government of Yucatan. Secretary of Turism for Welcome Ceremony.
Sharon Valle, Turistrans Merida Travel Agency. Stands and Poster Session.
Aide-de-camp of Tourism School and **Secretary of Tourism of the Yucatan Government.**
Maritza Paredes and **Maricruz Montiel.** Fiesta Americana Hotel.Merida, Yucatán

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Ana Dalcero (Argentina). Vocal of SLAM. Head of Cyted Group.

Ackowledgments

Welcome Ceremony

Lic. Ivonne Aracelly Ortega Pacheco. Constitutional Governor of the State of Yucatán.
Dr. Carlos Arámburo de la Hoz, Scientific Research Coordinator representing **Dr. José Narro Robles.** Rector of the Universidad Nacional Autónoma de México (UNAM).
Dr. Alfredo Dajer Abimerhi. Rector of the Universidad Autónoma de Yucatán.
Dr. Christopher P. Wild. Director de IARC (Agencia Internacional de Investigación en Cáncer). Lyon Francia.
Dr. Tila María Pérez Ortiz. Director of the Institute of Biology, UNAM.
Dr. Amanda Gálvez Mariscal. Coordinator of the University Food Program (PUAL), UNAM.

Moderators

Amaury Martínez	(República Bolivariana de Venezuela)	**Myrna Sabino**	(Brasil)
Carlos Alberto Da Rocha Rosa	(Brazil)	**Guilherme Prado**	(Brazil)
Gordon S. Shephard	(Republic of South Africa)	**Mario Vega**	(Chile)
Genoveva García Aguirre	(Mexico)	**Adriana Torres**	(Argentina)
Maya Piñeiro	(FAO) (Chile)	**Valentín Rojas**	(Panama)
Lorena Delgado	(Chile)	**Miriam Etcheverry**	(Argentina).
Doralinda Guzmán de Peña	(Mexico)	**Clara Eder López**	(Argentina)
Virginia Robinson-Fuentes	(Mexico)	**Roberto Saelzer**	(Chile)
Ana María Dalcero	(Argentina)	**Andrea Patriarca**	(Argentina)
René Márquez	(México)	**Liliana Pascual**	(Argentina)
Marta Jaramillo	(República Bolivariana de Venezuela)	**Juan Carlos Medina**	(Mexico)
Claudio Mazzani	(República Bolivariana de Venezuela)	**César Mateo Flores Ortiz**	(Mexico)
Silvia Peña Betancourt	(Mexico)	**Rosalba Alonso**	(Mexico)
John Berry	(United States of America)	**Cruz Lozano Ramírez**	(Mexico)
Ivonne S. Santiago Morales	(Mexico)	**Ana Cabado**	(Spain)
Jeffrey M. van de Riet	(Canada)	**Víctor Fajer**	(Cuba)
Ruth Noemí Aguila-Ramírez	(Mexico)		

Date	Time	Auditories						
		Mycotoxins Section					Phycotoxins Section	Sa
		Yucatan 1	Yucatan 2	Yucatan III	Yucatan IV	Celestun	Valladolid	Lu
June 28	8:30-10:00	Technological Exhibition and coffee	Welcome Ceremony					
	10:00-10:30		Recess				Recess	
	10:30-12:40		Session 1:Honor Ceremony for Dr. Homero Fonseca (Brazil) by Myrna Sabino and Prof. Gerald Wogan (USA) by John Groopman.				Session 17: Cyanobacterial & Phytoplankton Toxins **Wayne W. Carmichael (USA). Keynote Conference**	Co C
	12:40-13:30		**Dr Christopher P. Wild** (IARC, France) **Keynote Conference**				Session 18: Cyanobacterial & Phytoplankton Toxins	Regi
	13:30-15:00	Recess	Recess	Recess		Recess	Recess	Re
	15:00-17:00	Poster Session I,II,III					Poster Session X: Microalgae	
	16:30-18:00	Technological Exhibition	Session 2: Aflatoxins in human health. **Keynote Conferences (USA): John Groopman, Timothy D. Phillips**	**CYTED Meeting (Project Group)**		Round Table: Definition of the Mycotoxin Problems in the Pecuary Industr Moderator:René Márquez	Session 19: Dinoflagellate and harmful microalgae	Co Ce Regi
	18:00-19:30							
June 29	08:30-10:30		Session 3: Human mycotoxins diseases: DON and Aflatoxins	Session 8: Mycotoxins in food and commodities	Session 11: Methodology **Vildes Scussel (Brazil) Keynote Conference**	Session 13: Mycotoxins in swine and livestock	Session 20: Shellfish Toxins and Diarrhetic Toxins (DSP).	Co Ce Regi
	10:30-11:00	Recess	Recess	Recess	Recess	Recess	Recess	Re
	11:00-13:30	Technological Exhibition		**Dilek Heperkan (Turkey) Keynote Conference**	Session 11: Methodology	Session 13: Mycotoxins in swine and livestock	**Mychael Quilliam (Canada) Keynote Conference**	
	13:30-15:00	Recess	Recess	Recess	Recess	Recess	Recess	Re
	15:00-17:00	Poster Session IV-VII					Poster Session XI	
	16:30-19:00	Technological Exhibition	Session 4: Advances in Fumonisins & biomarkers **Keynote conferences: Peter Scott (Canada), Jia-Sheng Wang (USA), Ronald Riley (USA)**			Session 14: Mycotoxins in poultry	Session 21: Round Table: Red Tide	Co C Regi
	19:00 21:30				**SLAM Session**			
June 30	08:30-13:30		Session 5: Ochratoxin A in food, human health and its control.	Session 9: Physiology, antibiosis, toxicicity and storage problems.	Session 12: Mycotoxin Control	Session 15: Mycotoxins in feed and its control	Visit to SISAL	Co C Regi
	10:30-11:00	Recess	Recess	Recess	Recess	Recess		
	11:00-11:40		Session 5.: OTA in food & human health and its control	**Sofia Chulze (Argentina) Keynote Conference**	Session 12: Mycotoxin Control			
	12:30-13:30		**Peter Mantle (UK) Keynote conference**			Session 15: Mycotoxins in feed and its control		
	13:30-15:00	Recess	Recess	Recess	Recess	Recess		Re
	15:00-17:00	Poster Session VIII,IX						
	16:30-19:30	Technological Exhibition	Session 6:Genomic analysis of mycotoxins **Gary Payne (USA) Keynote conference**	Session 10: Mycotoxins in Latin America		Session 16: Mycotoxins in dairy cattle and milk		
	20:00-	Transportation to Banquet: Hacienda Ochil						
July 1st	8:30-12:00		Session 7: FAO Forum "Food safety risk assessment of chemical contaminants/ mycotoxins" **Maya Piñeiro (FAO); Gordon Shephard (South Africa); Myrna Sabino (Brazil); Marina Miraglia (Italy) Keynote Conferences.**					Co C
	11:00-11:30							Regi
	12:00-12:30		Recess					
	12:30-13:00		Closing Ceremony. SLAM, UNAM.					
	13:00-19:00	Visit to Chichen Itza (Optional)						

MODERATORS

Yucatan II

Monday June 28

10:30-13:30 **Session 1**: **Honor Ceremony for Homero Fonseca & Dr. Gerald Wogan**
Moderators: **Amaury Martínez** (República Bolivariana de Venezuela)
Magda Carvajal (Mexico)

16:30-20:00 **Session 2: Aflatoxins in human health.**
Moderators: **Magda Carvajal** (Mexico)
Myrna Sabino (Brasil)

Tuesday June 29

08:30-13:30 **Session 3: Human mycotoxin diseases: DON and Aflatoxins.**
Moderators: **Carlos Alberto Da Rocha Rosa** (Brazil)
Guilherme Prado (Brazil)

16:00-19:30 **Session 4: Advances in Fumonisins and biomarkers.**
Moderators: **Gordon S. Shephard** (South Africa)
Amaury Martínez (República Bolivariana de Venezuela)

Wednesday June 30

08:30-13:30 **Session 5: Ochratoxin A in food, human health and its control.**
Moderators: **Mario Vega** (Chile)
Genoveva García Aguirre (Mexico)

16:30-19:00 **Session 6: Genomic analysis of Mycotoxins.**
Moderator: **Adriana Torres** (Argentina)

Thursday July 1

08:30–12:00 **Session 7: FAO Forum/SLAM**
Food safety risk assessment of chemical contaminants.
Moderator: **Maya Piñeiro (FAO)** (Chile)

Yucatán III

Monday 28

17:00-20:00 **CYTED Meeting (Project Group).**

Tuesday 29

8:30- 13:30 **Session 8: Mycotoxins in food and commodities.**
Moderators: **Valentín Rojas** (Panama)
Lorena Delgado (Chile)

Wednesday June 30

08:30-13:30 **Session 9: Physiology, antibiosis, toxicity and storage problems**
Moderators: **Miriam Etcheverry** (Argentina).
Doralinda Guzmán de Peña (Mexico)

17:00-19:00 **Session 10: Mycotoxins in Latin America**
Moderators: **Clara Eder López** (Argentina)
Virginia Robinson-Fuentes (Mexico).

Yucatan IV

Tuesday June 29

08:30-13:30 **Session 11: Methodology**
Moderators: **Roberto Saelzer** (Chile)
Amaury Martinez (República Bolivariana de Venezuela)

19:00-21:30 **SLAM Session**

Wednesday June 30

08:30-13:30 **Session 12: Mycotoxin Control.**
Moderators: **Ana María Dalcero** (Argentina)
Andrea Patriarca (Argentina)

Celestún

Monday June 28

17:00-20:00 **Round Table: Definition of the Mycotoxin Problems in the Pecuary Industry.**
Moderator: **René Márquez**

Tuesday June 29

08:30-13:30 **Session 13: Mycotoxins in swine and livestock.**
Moderators: **René Márquez** (México)
Liliana Pascual (Argentina)

17:00-19:00 **Session 14: Mycotoxins in poultry.**
Moderators: **Juan Carlos Medina** (Mexico)
Marta Jaramillo (República Bolivariana de Venezuela)

Wednesday June 30

08:30-13:30 **Session 15: Mycotoxin in feed and its control.**
Moderators: **Claudio Mazzani** (República Bolivariana de Venezuela)
César Mateo Flores Ortiz (Mexico)

17:00-19:00 **Session 16: Mycotoxins in dairy cattle and milk.**
Moderators: **René Márquez** (Mexico)
Silvia Peña Betancourt (Mexico)

PHYCOTOXIN SECTION
Valladolid

Monday June 28

10:30-13:30 **Phycotoxin Session 17: Cyanobacterial and Phytoplankton Toxins**
Moderators: **Rosalba Alonso** (Mexico)
John Berry (United States of America)

16:00-18:00 **Oral Phycotoxin Session 18: Dinoflagellate and harmful microalgae.**
Moderators: **Cruz Lozano Ramírez** (Mexico)
Ivonne S. Santiago Morales (Mexico)

Tuesday June 29

08:30-10:30 **Oral Phycotoxin Session 19: Shellfish Toxins and Diarrhetic Toxins (DSP).**
Moderators: **Ana Cabado** (Spain)
Jeffrey M. van de Riet (United States of America)

10:30-13:30 **Oral Phycotoxin Session 20: Methods, ecology, exposure and new toxins.**
Moderator: **Víctor Fajer** (Cuba)
Ruth Noemí Aguila-Ramírez (Mexico)

16:00-18:00 **Session 21: Round Table: Red Tide.**
Moderators: **Rosalba Alonso** (Mexico)
Jeffrey M. van de Riet (Canada)

Wednesday June 30

09:00- **Visit to Sisal.**

CONGRESS PROGRAM

June 26 and 27, 09:00-18:00: **Pre-congress Workshop on Phycotoxins. Valladolid.**

June 27, 09:00-18:00: **Pre-congress Workshop on Mycotoxins. Celestún.**

June 27, 18:00-23:00: **Welcome Cocktail Terraza, Fiesta Americana Hotel.**

Welcome Opening Ceremony.

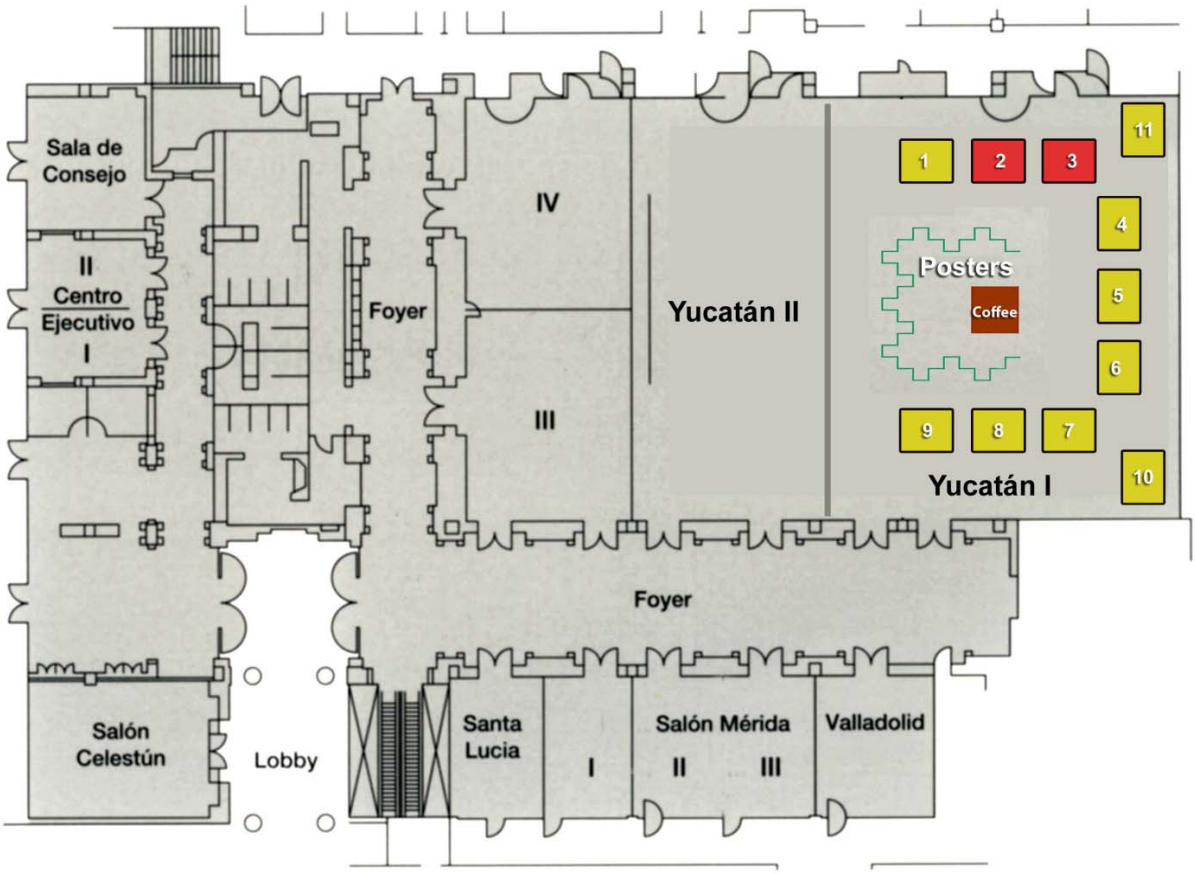
YUCATAN II

08:30-10:00

1. **Lic. Ivonne Aracelly Ortega Pacheco.** Constitutional Governor of the State of Yucatán.
2. **Dr. Carlos Arámburo de la Hoz,** Scientific Research Coordinator representing **Dr. José Narro Robles.** Rector of the Universidad Nacional Autónoma de México (UNAM).
3. **Dr. Alfredo Dajer Abimerhi.** Rector of the Universidad Autónoma de Yucatán.
4. **Dr. Christopher P. Wild.** Director de IARC (Agencia Internacional de Investigación en Cáncer). Lyon Francia.
5. **Dr. Maya Piñeiro.** Coordinator Food Safety and Agricultural Health. Senior Officer Food Safety and Quality.FAO, United Nations.
6. **Dr. Amaury Martínez**. President of the Latin American Society of Mycotoxicology.
7. **Dr. Tila María Pérez Ortiz.** Director of the Institute of Biology, Universidad Nacional Autónoma de México.
8. **Dr. Amanda Gálvez Mariscal.** Coordinator of the University Food Program (PUAL), Universidad Nacional Autónoma de México.
9. **Dr. Peter Scott.** Bureau of Chemical Safety, Health Canada. Organizing Committee.
10. **Dr. Timothy D. Phillips.** Texas A&M University, USA. Organizing Committee.
11. **Dr. Magda Carvajal Moreno**. Chairwoman. Universidad Nacional Autónoma de México.

		Participation
08:30-08:35	Welcome	**Dr. Magda Carvajal Moreno.** Chairwoman
08:35-08:40	Welcome	**Dr. Amaury Martínez** President of the Latin American Society of Mycotoxicology (SLAM).
08:40-08:45	PUAL	**Dr. Amanda Gálvez Mariscal** Food Program of the Universidad Nacional Autónoma de México.
08:45-08:50	FAO	**Dr. Maya Piñeiro** Coordinator Food Safety and Agricultural Health. Senior Officer Food Safety and Quality. United Nations.
08:50-09:00	IARC	**Dr. Christopher P. Wild,** **I**nternational Agency for Research on Cancer Director (IARC). Lyon, France.
09:00-09:10	UADY	**Dr. Alfredo Dajer Abimerhi.** Rector of the Universidad Autónoma of Yucatán.
09:10-09:20	UNAM	**Dr. Carlos Arámburo de la Hoz** **r**epresentant of Dr. José Narro Robles. Rector of the Universidad Nacional Autónoma de México.
09:20-09:30		**Lic. Ivonne Aracelly Ortega Pacheco.** Constitutional Governor of Yucatan

Map of the distribution of Auditories.



YUCATAN I MYCOTOXIN SECTION Poster Presentations (10 minutes). 15:00-17:00.				
Poster Section	**Subject**	**Groups**	**Poster numbers**	**Date**
I	**Mycotoxins in field crops.**	1	P- 1 to P-6	**Monday June 28**
		2	P-7 to P-12	
		3	P-13 to P-17	
II	**Mycoflora and Mycotoxin production.**	4	P-18 to P-23	
		5	P-24 to P-29	
III	**Mycotoxins in Commodities.**	6	P-30 to P-35	
		7	P-36 to P-40	
		8	P-41 to P-45	
IV	**Mycotoxins in poultry.**	9	P-46 to P-50	**Tuesday June 29**
		10	P-51 to P-56	
V	**Mycotoxins in swine**	11	P-57 to P-62	
VI	**Mycotoxins in bovines, rats and goats.**	12	P-63 to P-66	
VII	**Mycotoxins in Pets and their feed**.	13	P-67 to P-71	
		14	P-72 to P-75	
VIII	**Mycotoxin Methodology.**	15	P-76 to P-81	**Wednesday June 30**
		16	P-82 to P-86	
IX	**Fungal and Mycotoxin Control.**	17	P-87 to P-92	
		18	P-93 to P-97	
		19	P-98 to P-102	
		20	P-103 to P-107	
PHYCOTOXIN SECTION Poster Presentations (20 minutes).15:00-17:00				
X	**Cyanobacterial and Phytoplankton Toxins.**	21	P-108 to P-110	**Monday June 28**
XI	**Phycotoxins in the Atlantic area.**	22	P-111 to P-112	**Tuesday June 29**

Posters will be hanged on **Sunday June 27 afternoon (15:00-18:00) and will remain on Exhibition in the YUCATAN I Auditory the whole three days June 28-30 of the congress.**

Presenters should be explaining their poster in the fixed times for each Poster Section, scheduled in the Poster Program, depending on the theme.

Posters should be taken away on Wednesday 18:00-19:30 hours, and can be stored in Valladolid Auditory on Wednesday afternoon, they can be recovered on Thursday July 1, 2010 morning in the same place.

All Auditories should be emptied on Thursday July 1st, 2010, just after the Closing Ceremony.

MYCOTOXIN SECTION

Yucatan II

Monday June 28, 2010

Session 1: Honor Ceremony for Dr. Homero Fonseca (Brazil) and Prof. Gerald Wogan (USA)

08:30 -09:30 Welcome Ceremony
09:30–10:00 Video
10:00-10:30 ***Recess***

10:30-13:30 Session 1: **Honor Ceremony for Homero Fonseca & Dr. Gerald Wogan**

Moderators: Magda Carvajal (Mexico)
Amaury Martinez (R.B. Venezuela)

10:30-10:50 Homero Fonseca: biographical and scientific contributions.
Myrna Sabino (Brazil)

10:50-11:30 Peanut: Fungi, mycotoxins and phytoalexins in peanut varieties, during plant growth in the field, in Brazil.

Homero Fonseca (Brazil)
with Patricia Zorzete, Tatiana A. Reis, Benedito Corrêa

11:30-11:50 Dr. Gerald Wogan: biographical and importance of scientific contributions.

John Groopman (United States of America)

11:50-12:40 Aflatoxin and Liver Cancer: The Role of Molecular Biomarkers in Etiology and Prevention.

Dr. Gerald Wogan (United States of America)

12:40-13:30 The global health burden of Aflatoxins

Christopher P. Wild (IARC, France) **Keynote Conference.**

13:30-15:00 ***Recess***

15:00-17:00 Poster Session I

10:50-11:30 FUNGI, MYCOTOXINS AND PHYTOALEXINS IN PEANUT VARIETIES, DURING PLANT GROWTH IN THE FIELD, IN BRAZIL

Patrícia Zorzete[1], Tatiana A. Reis[1], **Homero Fonseca[2],** Benedito Corrêa[1]*

[1] Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, SP, Brazil
[2] ESALQ, University of São Paulo, Piracicaba, SP, Brazil
* Tel: +5511 30917295 correabe@usp.br

Background: Soil is a primary reservoir of many microorganisms such as fungi, including *Aspergillus*. *Aspergillus flavus* and *Aspergillus parasiticus* are the main producers of aflatoxins B_1, B_2, G_1 and G_2, biologically active secondary metabolites (Smith and Ross, 1991). Along with aflatoxins, peanuts can also be contaminated with cyclopiazonic acid (CPA), mycotoxin also produced by *A. flavus* (Lansden and Davidson, 1983). In addition, to the care of the agricultural practices, is of great importance to choose the most appropriate variety, more resistant to invasion by pests. Phytoalexins are anti-microbial agents, produced by the plant to defend itself from microorganisms at the site where the infection occurs. The plant's capacity to produce such substances can be an ally in the choice of resistant cultivars (Fritzemeier and Kindl, 1981).

Aim: The present work aimed to analyze the mycoflora, the occurrence of mycotoxins (aflatoxins and cyclopiazonic acid) and the production of phytoalexins (*trans*-resveratrol) in two varieties of peanuts (Runner IAC 886 and Caiapo), considered the most used in Brazil, during plant growth in the field.

Materials and Methods: The technique of fungi isolation utilized was of direct seeding (Berjak, 1984). From each sample of peanuts 30 g were taken for disinfection in a solution of sodium hypochlorite 0.4%, for 3 minutes, followed by washing with sterile distilled water three times for removal of external contaminants. After disinfection, were separated from the husks of grain and some peanut seeds were randomly selected and seeded directly into the Petri dishes containing medium *Aspergillus flavus* and *parasiticus* agar (AFPA). For each sample, 3 plates containing 11 kernels each, totaling 33 kernels per sample were used. For the peanut shells were used 4 boards, where they were placed 4 pairs of shells on each plate, a total of 16 shells per sample. All plates were incubated at 25 ^{o}C for 5 days and the results expressed as a percentage of total kernels and shells infected by fungi. Then, colonies of different morphological types were isolated on Sabouraud dextrose agar and subjected to identification utilizing the technique of microcultured on potato dextrose agar. The determination of aflatoxins (Truckess et al., 1991; Tarin et al., 2004), cyclopiazonic acid (CPA) (Urano et al., 1992) and *trans*-resveratrol (Felicio et al., 2001, Sanders et al., 2000) by HPLC

Results and Discussion: Our results showed in the kernels and shells, the predominance of *Fusarium* spp., followed by *Penicillium* spp. and *A. flavus*. Analyses of samples for aflatoxins showed 20% and 10% positivity for the varieties IAC 886 and Caiapó, respectively. Samples of the varieties IAC 886 and Caiapó, showed that 65% and 25%, respectively, were contaminated with CPA. The analyses of *trans*-resveratrol in peanuts variety IAC 886 and Caiapó, 6.7% and 20%, respectively, were positive.

However, *trans*-resveratrol was detected in 73.3% of the leaves of the samples in both varieties.

Conclusions: Monitoring of the variables in the field, during planting peanuts, reveals the importance of good agricultural practices during cultivation. The presence of toxigenic strains signalizes the risk of mycotoxin production and commitment of grain produced. Despite the detection of aflatoxins and CPA at very low levels, noted the importance of studies on the co-occurrence of these toxins in samples of peanuts. The search for *trans*-resveratrol need more studies in the field, looking for the benefits in the use of varieties with greater synthesis capacity of this compound.

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12:40-13:30 THE GLOBAL HEALTH BURDEN OF AFLATOXINS

Keynote Conference

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The majority of the world population is exposed to mycotoxins through consumption of contaminated food. Mycotoxins of major concern to human health include aflatoxins, fumonisins, tricothecenes (deoxynivalenol), ochratoxin A and zearalenone. In the economically wealthier parts of the world exposure is controlled or minimised by regulation and surveillance. However, in many geographic regions, including Africa, such protection is lacking. Furthermore a balance often has to be found between food security and food sufficiency. Accurate assessment of human exposure to mycotoxins is a key to assessing the associated human health consequences. Biomarkers of human exposure to aflatoxins, fumonisins and deoxynivalenol have been developed and are in various stages of validation and application to population studies. In West Africa there is a high prevalence and level of dietary exposure to aflatoxins beginning early in life and exposure has been associated with increased liver cancer risk in interaction with chronic hepatitis B virus (HBV) infection. In addition, aflatoxins cause aflatoxicosis and may be a cause of growth faltering and immune suppression in young children (Gong et al., Env. Health Per. 112:1334-1338, 2004; Turner et al. Env. Health Per. 111:217-220, 2003). Growth and immune impairment could be critical in pre-disposing children to the infections, including HBV, that result in the high morbidity and mortality in these populations. Intervention strategies to reduce exposure have been explored. In a simple post-harvest intervention in Guinea, aflatoxin contamination of the groundnut crop was reduced, resulting in a >50% reduction in exposure (Turner et al., Lancet 365: 1950-1956, 2005). Current research involves application of urinary biomarkers to study exposure and intervention strategies against fumonisin (Gong et al., Cancer Epi. Bio. Prev. 17: 688-694, 2008) and deoxynivalenol (Turner et al., Env. Health Per. 116: 21-25, 2008) in different populations. Supported by the NIEHS, USA: Grant.

Session 2: Aflatoxins in human health.

Keynote Conferences (USA): John Groopman and Timothy D. Phillips

16:30-17:20 ROLE OF AFLATOXIN AND THE HEPATITIS B VIRUS IN HUMAN HEPATOCELLULAR CARCINOMA

Keynote Conference

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Biomarker investigations have been extensively applied in the study of hepatocellular carcinoma (HCC), a major cause of cancer morbidity and mortality in many parts of the world, including Asia and sub-Saharan Africa, where there are upwards of 600,000 new cases each year. Much (~ 80%) of the burden of HCC is manifest in the developing world. Further, the median age of diagnosis and death from HCC is between 45-55 years of age in these regions. Since the occurrence of HCC is coincident with regions where aflatoxin exposure is high, efforts started in the 1960s to investigate this possible association.

Aflatoxin biomarkers of internal and biologically effective dose have been integral to establishing the etiologic role of this toxin in human HCC. In a cohort study of over 18,000 people in Shanghai, a highly significant increase in the relative risk (RR=3.4) existed for those liver cancer cases where urinary aflatoxin biomarkers were detected. The relative risk for people who tested positive for HBV was 7.3, but individuals with both urinary aflatoxins and positive HBsAg status had a relative risk for developing HCC of about 59. These results strongly supported a causal, multiplicative relationship between two major HCC risk factors, HBV and AFB_1 exposure(1, 2).

In recent years research has been facilitated by the demonstration that DNA isolated from plasma of cancer patients contains the same genetic aberrations as DNA isolated from an individual's tumor. In many cases of HCC in China and Africa a double mutation in the HBV genome, an adenine to thymine transversion at nucleotide 1762 and a guanine to adenine transition at nucleotide 1764 ($1762^{T}/1764^{A}$), has been found in tumors. The onset of these mutations has been also associated with the increasing severity of the HBV infection and cirrhosis(3).

We have followed up this initial investigation by taking advantage of a male cohort of 5581 HBsAg carriers. Six hundred sixty-seven HCC cases emerged over the course of 10-15 years in this cohort. Mutations in HBV were determined and 79.2% of these samples contained the $1762^{T}/1764^{A}$ mutation. Since the progression to HCC occurred at a relatively constant rate, we explored the relationship of HBV mutations at baseline to both time to death from HCC and age at death. Analysis showed that

samples from individuals succumbed at longer intervals after plasma collection had a higher probability of containing one of the HBV double mutations. Overall, there was an inverse relation between the $1762^{T}/1764^{A}$ mutations and age at death revealing that cases under the age of 45 were significantly more likely to have the $1762^{T}/1764^{A}$ mutations than in men who died after age 55. These findings continue to confirm the predisposing nature of mutations of HBV detected in plasma samples up to 15 years prior to death(4).

Thus, over the past thirty years, the development and application of molecular biomarkers reflecting events from exposure to manifestation of clinical disease has rapidly expanded our knowledge of the mechanisms of disease pathogenesis. These biomarkers will have increasing potential for early detection, treatment, interventions and prevention. This work was supported in part by grants P01 ES006052 and P30 ES003819 from the USPHS.

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17:20-18:10 ENTEROSORBENT THERAPY FOR POPULATIONS AT RISK FOR AFLATOXICOSIS

Keynote Conference

Timothy D Phillips [a]

with A. Robinson [a], NM Johnson [a], A Strey [b] JF Taylor [a], A Marroquin-Cardona[a], E.Afriyie-Gyawu [c], N-A Ankrah [d], JH Williams [e], PE Jolly [f], R Nachman [b], J-S Wang [g]

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A practical and effective approach to the aflatoxin problem in animals has been the inclusion of montmorillonite clays such as NovaSil (NS) in the diet. NovaSil acts as a high affinity enterosorbent that tightly sequesters aflatoxins in the gastrointestinal tract resulting in decreased aflatoxin uptake and bioavailability. In mechanistic studies, the intact dicarbonyl system in aflatoxin was shown to be essential for optimal sorption by NS.

Evidence also suggests that aflatoxins react at multiple sites on NS surfaces (especially those within the interlayer region). In a clinical trial in Ghana, we have confirmed the safety and efficacy of NS during a 3-month intervention, where clay was administered by capsules before each meal. Our results from this study also indicated that: 1) NS inclusion in the diet did not produce adverse health effects, 2) NS did not interfere with the levels of vitamins A and E, or iron and zinc and other minerals in serum, and 3) NS significantly decreased AF-albumin adducts in serum and AFM1 in urine after 3 months of intervention. In further work, urine samples from 118 study participants that were collected after 10 weeks of treatment with NS and placebo (microcrystalline cellulose) were analyzed for the presence of fumonisin B1 (FB1) using immunoaffinity column clean-up, derivatization with naphthalene-2,3-dicarboxaldehye (NDA) and HPLC analysis. NDA-FB1 derivatives from urine were confirmed using MALDI-TOF mass spectrometry.

In the fumonisin study, 60% of samples from participants contained detectable levels of urinary FB1. Importantly, median levels of FB1 in NS treatment groups were significantly lower ($p < 0.01$) than the median FB1 levels in the placebo group. A possible mechanism for FB1 sorption to NS is protonation of the amino group at C2 on the molecule. The charged FB1 may facilitate a cationic exchange reaction at negatively charged surfaces of the clay similar to the interaction(s) of cationic surfactants or quaternary amines at interlayer surfaces in layer-lattice clays.

Further work is warranted to delineate the mechanism, surface chemistry and kinetics involved in the sorption process of FB1 (and AFB1) onto NS and to confirm the efficacy and safety of NS clay as a multifunctional intervention for aflatoxins and fumonisins in animals and humans.

(This work was supported by the United States Agency for International Development, USAID LAG-G-00-96-90013-00 through Peanut CRSP at the University of Georgia).

Yucatan II

Tuesday June 29, 2010

Session 3: Human mycotoxin diseases: DON and Aflatoxins.

08:30-09:00 HUMAN MYCOTOXICOSIS IN SOUTH AMERICA: THE BERIBERI CASES IN BRAZIL

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Background: Beriberi is a disease caused by a deficiency of thiamine (vitamin B_1) that affects many systems of the body, including muscles, heart, nerves, and digestive system. This word means "I can not, I can not" in Singhalese, which reflects the crippling effect it has on its victims. It is common in Southeast Asia, where white rice is the main food. It can also be seen in chronic alcoholics with an inadequate diet. A substance able to produce a similar disease to beriberi is citreoviridin (CTV), a metabolite mycotoxin of *Penicillium citreonigrum* Dierckx thought to be the cause of yellow rice poisoning, manifested in humans and experimental animals by respiratory and circulatory failure, paralysis, convulsions and death. The Shoshim-Kakke (cardiac beriberi) in Japan, the Keshan in China and Southeast Asian countries, are related to the consumption of molded rice and yellow (Yellow Rice Disease), contaminated with CTV. This mycotoxin and its derivatives, described as □-pirones compounds, are produced from soil fungi, such as *P. citreonigrum* Dierckx (*P. citreoviride* Biourge), *Eupenicillium ocrhosalmoneum* (*P. ochrasalmoneum* Udagawa) DB Scott & Stolk, which rapidly grow during storage and storage after rice and maize harvest. Citreoviridin was first isolated from metabolites of *P. citreonigrum* strains associated with acute berberi cases (Shoshin-kakke). In Japan, yellow rice disease has not been reported since decades that the yellow rice was excluded altogether from the Japanese market (Saito et al, 1971, Ueno, 1972, 1986). Keshan in China has been linked to CTV, which is often found in samples of rice and maize (Li Dean et al, 2004, ShuQui, et al, 2004). Datta & Ghosh (1981) considered CTV as a anti-thiamine factor and argued that CTV is a potent inhibitor of mitochondrial ATPases, as the adenosine-triphosfatase. The addition of thiamine diphosphate (TPP) produced the reversal of the inhibitory effect of ATpases. These findings suggested the relationship between CTV, the induction of beriberi and its antithiamine effect. The literature that describes the change in eating habits, with the inclusion of other sources of thiamine, has reduced the occurrence of Shoshin-kakke, and has eliminated the occurrence of deaths. Since May 2006, there were 1028 cases of beribéri in southwestern of Maranhão State (Brazil) with 32 deaths. These cases were described as beribéri dry (38%), wet (18%), cardiac (40%) and Wernicke-Korsakoff syndrome (4%).

Aim: The aim of this work was to determine *P. citreonigrum* and CTV levels present in rice samples from Maranhão State, Brazil, where an outbreak of beriberi was described and 32 deaths occurred.

Materials and Methods: A total of 545 samples of 21 different kinds of rice were collected. The regional rice production is rudimentary in small farms which uses rice cultivar called *cateto*. The number of samples was determinated (Rosa et al., 2010) according to the number of sampled houses and/or rice processing sites (i.e. 16-25 total processing sites = three sampled sites, 26-50 total processing sites= five sampled sites).Water activity (a_w) determinations were carried out with Aqualab Cx2 (Decagon, Devices, Pullman WA, USA). The sample preparation procedure and quantitative enumeration of fungal propagules (CFU/g) was carried out according to Pitt and Hocking (1997). The fungal colony forming units (CFU/g) methodology was done by serial dilution in peptone water (1%) and aliquots of 0.1 mL from 10^{-2} to 10^{-4} dilutions in Dichloran Rose Bengal Chloranfenicol agar (DRBC) and Dichloran Glycerol 18% agar (DG18) were placed. *Fusarium* spp were isolated from Nash-Snyder agar and then, plates were incubated at 28ºC for 7 days and identification was done in accordance with the appropriate keys. Levels of CTV were determined by HPLC (Schotwel et al., 1988) and measured using normal phase of liquid chromatography. Rice samples were extracted with acetonitrile:water (86:14, v/v) and extracts were concentrated under N_2. The mobile phase was ethyl acetate:n-hexane (80:20, v/v), and the flow was 1.5 ml/min. The detection was made by fluorescence with 388 nm excitation and 480 nm emission. The standard solution of CTV (SIGMA Co., St. Louis-U.S.) in methanol (100 ng/mL) contained approximately 96% purity and after calibration showed a 6:4 ratio (m/m) of the mixture citreoviridin/isocitreoviridin (iCTV). The detection limit was 1 ng/g.

Results and Discussion: Fungal counts in DRBC ranged from 2.5×10^2 to 4.8×10^6 CFU/g and in DG18 from 1.0×10^2 to 1.0×10^7 CFU/g. *Fusarium* species were found at 1.7×10^2 CFU/g average. Analyzed rice samples from tropical climate area exhibited high fungal counts. Moreover, 335 *Aspergillus* strains and their teleomorphs (70.2%) were isolated, followed by *Penicillium* spp and teleomorphs (13.8%), *Cladosporium* spp (8.7%), *Fusarium* spp. (2.9%) and *Mucor* spp (2.2%). *Penicillium citrinum* was prevalent (12.8%), followed by *A. flavus* (10.4%), *Eurotium* sp (9.9%) and *A. niger* aggregate (8.3%). *Penicillium citreonigrum* (4.3%) was isolated from eleven samples, with only one strain not able to produce CTV. Performed HPLC analyses of rice showed five samples contaminated with CTV at levels ranging from 12 to 96.7 ng/g. The iCTV was detected in two of the five positive samples. Four positive samples were from *cateto* rice harvested in 2006, *agulhão* rice harvested in 2007 and two samples of rice bran (128 and 294 ng/g). All positive samples came from the region of beribéri occurrence, in the southwestern State of Maranhao, Brazil. The methodology used in this study is being optimized. However, it proved to be effective to detect citreoviridin/isocitreoviridin with 86% recovery in samples fortified with 100 ng/g.

Conclusions:This is the first occurrence of Cardiac Beriberi caused by CTV intoxication in West and the first human micotoxicoses outbreak described in the Americas. The administration of thiamine to sick patients in the Maranhão State decreased and stopped mortality. Moreover, the vast majority of beriberi cases were reversed. These data point to the immediate adoption of Good Agricultural Practices, appropriate hygienic-sanitary measures to be applied in regional production and consumption of rice as well as health education targeting the population of the region.

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09:00-09:30 A COMPARISON OF DEOXYNIVALENOL INTAKE AND URINARY DEOXYNIVALENOL IN UK ADULTS

Paul Turner*[1], Kay White[1], Victoria Burley[2], Richard Hopton[1,3], Anita Rajendram[4], Julie Fisher[3], Janet Cade[2], Christopher Wild[1,5]

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Background The mycotoxin deoxynivalenol (DON) is a common contaminant of wheat, maize and barley. In animals DON causes gastrointestinal toxicity, immune toxicity and impairs growth. The adverse health effects in people remain poorly explored, in part due to a lack of robust methods to assess individual exposure.

Aims The relationship between DON intake and urinary DON was examined in order to validate the latter as a biomarker of human exposure.

Methods First morning urine samples were collected from 35 UK adults (aged 19-59) on Monday to Friday of two consecutive weeks. Food diaries were completed throughout the survey period for the 'normal' diet (eight days, Sunday to Sunday, n=35), and subsequently either (a) 'partial intervention' in which bread was the only major source of DON (four days, Monday to Thursday, n=25), or (b) 'full intervention' in which there were no major sources of DON (four days, Monday to Thursday, n=10). For the subjects consuming bread only, duplicate portions were collected to measure DON levels and thus enable estimates of DON intake.

Result and Discussion During the normal diet, partial intervention and full intervention, urinary DON was detected in 198/210 subjects (94.2%), with a mean level of 10.1ng DON/mg creatinine (range nd–70.7ng/mg), in 94/98 subjects (95.9%), mean 5.9ng/mg (range nd–28.4ng/mg), and in 17/40 subjects (42.5%), mean 0.5ng/mg (range nd–3.3ng/mg), respectively. During the partial intervention phase average DON intake explained 83.4% of the variance in average urinary DON ($p<0.001$) in models adjusting for age, sex and BMI. The mean DON intake during the normal phase was estimated as 298ng/Kg bw/day using the mean transfer of ingested DON into urine (72.3% (95%CI: 59.1, 85.5%) from the intervention phase. The estimated DON intake exceeded the rTDI for 6/35 (17.1%) individuals on at least one day.

Conclusion These data demonstrate a quantitative correlation between DON exposure and urinary DON, and serve to validate the use of urinary DON as an exposure biomarker in biomonitoring and epidemiological studies.

This study was funded by the UK Food Standards Agency and supported from the NIEHS, USA grant no ES06052

09:30-10:00 DETERMINANTS OF URINARY DEOXYNIVALENOL IN MALE FARMERS FROM NORMANDY, FRANCE

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Background Dietary exposure to deoxynivalenol (DON) from contaminated cereal crops is frequent in Europe, and farm workers who handle grain or silage may be at additional risk.

Aims To assess the determinants of urinary DON in French farm workers.

Methods Initially a more rapid urinary assay for DON (Turner et al., 2008) was investigated. Secondly an assay to measure a de-epoxy metabolite of DON (DOM-1) was established. DOM-1 is formed by gut bacteria following ingestion of DON. It has only been observed in animals to date. Finally, these were applied to a pilot survey of male French farmers (n=76, aged 23-74), from Normandy.

Results and Discussion DON was detected in 75/76 samples (range 0.5-28.8 ng/ml) and DOM-1 in 26/76 samples (range 0.2-2.8ng/ml). DOM-1 has not been observed in human samples before, and its presence possibly represents acquisition of bacteria from farm animals. In multivariate analysis including creatinine as a covariate, bread consumption (p=0.05), other cereal consumption (p=0.02) were associated with urinary 'DON and DOM-1' concentration combined.

Conclusions This is the first exposure biomarker survey for DON in a French population, and the first demonstration of urinary DOM-1 in humans. Further investigations into occupational activity, handling or air-born exposures would be informative.

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10:00-10:30 URINARY DEOXYNIVALENOL AND INTAKE MEASUREMENTS IN A COHORT OF PREGNANT WOMEN FROM BRADFORD, UK

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Background Deoxynivalenol (DON) is a ubiquitous dietary contaminant of cereal crops (wheat and maize) in temperate regions of the world. DON causes growth faltering and immune suppression in animals.

Aims This study provides DON exposure assessment in a subset of women scheduled to have an elective caesarean section taken from a larger mother/infant birth cohort from the UK (Born in Bradford) involving 10,000 mother/infant pairs.

Methods Women aged 16-44 (n=85) provided a urine sample for DON analysis in the last trimester of pregnancy, and completed a food frequency questionnaire (FFQ).

Results and Discussion The urinary DON biomarker was detected in all measured samples (geometric mean: 10.3ng/ml, range: 0.5, 116.7ng/ml). Levels were higher in women classified as South Asian in origin (geometric mean: 15.2ng/ml; 95%CI: 10.7, 21.5ng/ml compared to Caucasians (8.6ng/ml; 95%CI: 6.6, 11.8ng/ml), p=0.02). Estimated DON intake from FFQ data and typical levels of DON contamination of food suggested that this was mainly due to higher levels of exposure from foods in the bread group, particularly daily intake of chapattis in South Asians (mean 2.42 (95%CI: 1.2-3.7) compared Caucasians (0.17 (95%CI: 0-0.37), p<0.001).

Conclusions This is the first biomarker demonstration of DON exposure in pregnant women from the UK. Urinary DON levels were amongst the highest ever recorded. A larger survey within the Born in Bradford cohort is warranted to investigate the potential risk to both infant and the mother, of maternal DON exposure during pregnancy.

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11:00-11:30 CASES OF HUMAN AND ANIMAL AFLATOXIN POISONING IN DEVELOPING COUNTRIES - AN OVERVIEW

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Background: Aflatoxicoses are diseases caused by aflatoxins in livestock, domestic animals and humans throughout the world. Exposure to these mycotoxins is mostly by ingestion but also occurs by the dermal and inhalation routes. The susceptibility of individuals to mycotoxins varies considerably depending on species, age, sex and nutrition. Acute mycotoxicoses can cause serious and sometimes fatal diseases also (Bennett and Klich, 2003).The possibility of mycotoxin intoxication should be considered when a sudden acute disease occurs in a large population when there is no evidence of infection with a known etiological agent and there is no improvement in the clinical picture following treatment (Peraica *et al*, 1999). The global nature of the mycotoxin problem is based on well-documented human mycotoxicoses such as ergotism in Europe, alimentary toxic aleukia in Russia, acute aflatoxicoses in South and East Asia and human primary liver cancer in Africa and South East Asia. Ochratoxin A is suspected to play a role in Balkan endemic nephropathy in Yugoslavia and chronic interstitial nephritis in North Africa (Steyn, 1995). The present paper reviews the disease outbreaks of aflatoxicoses in developing countries with an emphasis on Indian cases, due to the ingestion of contaminated food and feed with aflatoxins.

Cases of human aflatoxicoses reported in India: Aflatoxicosis is primarily a hepatic disease in humans. Consumed chronically aflatoxins may cause liver cancer. Aflatoxins B_1, M_1 and G_1 have been shown to cause various types of cancer in different animal species. Epidemiological studies have demonstrated a cause effect-relation between aflatoxins but only aflatoxin B_1 is considered by the International Agency for Research on Cancer (IARC) as having produced sufficient evidence of carcinogenicity in experimental animals (Eaton and Groopman, 1994). Although humans and animals are susceptible to the effects of acute aflatoxicosis, the chances of human exposure to acute levels of aflatoxin is remote in well-developed countries. In undeveloped countries, human susceptibility can vary with age, health and level and duration of exposure. In 1974, an outbreak of hepatitis due to aflatoxicosis was reported in 200 villages in western India (Banswada and Panchamahals districts of Rajasthan and Gujarat respectively) resulting in 106 deaths. The outbreak lasted 2 months and was confined to a tribal population whose staple food was corn. Analysis of maize samples from the region showed that affected people might have consumed food contaminated with aflatoxins at between 2000-6000 μg/kg daily over a period of 1 month (Krishnamachari *et al*, 1975a, 1975b). Symptoms of the disease included rapidly developing ascites, oedema of the lower limbs, portal hypertensions and a high mortality rate. An independent study (Tandon *et al*, 1977) of the same outbreak confirmed that aflatoxins were the major cause of this disease. This toxic hepatitis was reported in three adjoining districts of north-west India during the period November and December, 1974 affecting both humans and dogs. Hepatic histology was similar to that described by Krishnamachari *et al* (1975a, 1975b) in addition to a marked high fever at the onset of disease. The level of aflatoxin contaminated in samples from affected and unaffected households was only 0.1μg/kg. It was thought that other mycotoxins or an infection might have caused the outbreak. A follow-up study of the Indian outbreak conducted after a period of one year by Bhat and Krishnamachari (1977) found that survivors were fully recovered with no ill effects from the poisoning. Childhood cirrhosis, a clinical condition mainly confined to the Indian sub-continent has been attributed to aflatoxin contamination (Amla *et al*, 1971). In an investigation of a third outbreak (Sreenivasamurthy 1975, 1977)**,** a correlation between aflatoxin contamination and hepatomegaly in children was reported in south Canara district of Karnataka.

Cases of animal aflatoxicoses reported in India: A suspected outbreak of peanut poisoning affecting 24 *Murrah* buffaloes and resulting in the deaths of six buffaloes was reported by Sastry *et al*, (1965). This was the first record of peanut toxicity in India although frequent cases of liver damage in *Murrah* buffaloes have been recorded in certain areas. More than 200,000 broiler chickens died in 1994 in Ranga Reddy district of Andhra Pradesh, India after eating aflatoxin contaminated peanut cake-based feeds (ICRISAT, 2002). Heavy mortality in chicks in Chittoor district of Andhra Pradesh state, India due to aflatoxicosis was reported earlier (Char *et al*, 1982). The peanut cake implicated in the aflatoxicosis was contaminated with aflatoxin at a level of 3590 µg/kg. Another outbreak of aflatoxicosis on commercial poultry farms was reported in the same district with 100% mortality (Choudary and Rao, 1982). Aflatoxins (1,400-3,600 µg/kg) were found in samples of corn and peanut cake fed to the birds during the outbreak. Egg production dropped by 85-40% during an outbreak of aflatoxicosis in poultry in October 1985 in and around Warangal, Andhra Pradesh (Choudary, 1986). Post-mortem examination of dead birds revealed liver lesions of varying severity. Feed samples were found to be contaminated with aflatoxin (600 µg/kg). No mortality was observed after the feed was changed and egg production gradually returned to normal. Occurrence of aflatoxicosis in poultry in Mysore state, India was also reported (Gopal *et al*, 1969). The disease was first recognized at the Government Poultry Breeding Unit, Hebbal, Bangalore in 1966 when 2,219 chicks died in one week. Subsequently, several sporadic incidences were reported on various poultry farms in the state. The disease was predominant in younger stocks, possibly due to higher inclusion of contaminated peanut cake to afford higher protein levels in the ration. Aflatoxin M_1 and M_2 are the hydroxylated metabolites of aflatoxins B_1 and B_2 and are produced in the milk when cows or other ruminants ingest feed contaminated with these mycotoxins. Limited studies are available on aflatoxin M_1 occurrence in milk in India. An investigation was conducted in Andhra Pradesh, India by Yadagiri and Tulpule (1974) on aflatoxin contamination in buffalo milk. Of 50 milk samples analyzed, 27 were contaminated with aflatoxin M_1 in amounts ranging from traces to 4.8 µg/L. Analysis of the peanut cake, which formed 30% of the ration of cattle, indicated the presence of aflatoxin levels ranging from 1 to 3 mg/kg. Other outbreaks of aflatoxicoses in dairy cattle were also reported earlier from Mysore region (Gopal *et al,* 1968) and in *Murrah* buffaloes from Andhra Pradesh (NIN, 1969).

Cases of human aflatoxicoses reported in other developing nations: In other regions of the world acute aflatoxicosis outbreaks have also occurred. Recently in eastern Kenya (Azziz-Baumgartner *et al*, 2005), an aflatoxicosis outbreak Kenya resulted in 317 cases and 125 deaths. Although aflatoxicosis outbreaks have occurred periodically in Africa and Asia, this outbreak resulted in the largest number of fatalities ever documented. A case control study was conducted by Azziz-Baumgartner *et al*, (2005) implicated aflatoxin-contaminated corn. Aflatoxin B_1 was circumstantially associated with the death of a 15 year old African boy in Uganda (Serck-Hanssen, 1970). Forty-six patients with primary hepatoma were studied in Mulago Hospital, Uganda, over a nine month period. This represented 2.1% of all medical admissions. The ape peak was 25 to 45 years and the male to female ration was 2:1. The predominant clinical picture was abdominal pain and hepatomegaly with a hard, frequently tender mass in the right upper quadrant and rapid clinical deterioration and death within several months (Alpert et al., 1969). Aflatoxins were estimated in 480 food samples stored for consumption between harvests and collected from different parts of Uganda in 1966-67. Among these samples, 29.6% contained detectable amounts of aflatoxins and 37.7% contained more than 1 µg kg^{-1}. The frequency of aflatoxin contamination was particularly high in provinces with a high incidence of hepatoma, or where cultural and economic factors favored the ingestion of mouldy foods (Alpert et al., 1971). Aflatoxin B1 (62 to 4409 pg kg^{-1}) was detected in the livers of 20 children in Ghana who died from kwashiorkor. Aflatoxicol (12-99 pg µm g^{-1}) was detected in the livers of 2 children (Apeagyei et al., 1986). In 1967 a report was published presenting the results of a study of an outbreak of apparent poisoning of 26 persons in two Taiwan rural areas (Ling et al., 1967). The victims came from households which had consumed mouldy rice for up to three weeks, they developed edema of the legs and feet, abdominal pain, vomiting and palpable livers but no fever.

The three fatal cases were children between the ages of four and eight years; autopsies were not done and the cause of the death could not be established (Ling et al., 1967). Yeh et al., (1989) examined the roles of HBV and AFB1 in the development of PHC in a cohort of 7917 men aged 25-64 years old in southern Guangxi, China where the incidence of PHC is among the highest in the world. After accumulating 30188 person-years of observation, 149 deaths were observed, 76 (51%) of which were due to PHC. Ninety-one % of PHC deaths were hepatitis B surface antigen positive ($HbsAg^+$) at enrollment into the study in contrast to 23% of all members of the cohort. Three of the four patients who died of liver cirrohosis were also $HbsAg^+$ at enrollment. There was no association between $HbsAg^+$ and other causes of death. In October 1988, 13 Chinese children died of acute hepatic encephalopathy in the northwestern state of Perak in peninsular Malaysia. Symptoms included vomiting, haematemesis, seizures, diarrhoea, fever and abdominal pain. All had liver dysfunction with increased aspartate aminotransferase and alanine aminotransferase levels greater than 100 IU/litre (Lye et al., 1995).

Cases of animal aflatoxicoses in other developing nations: In 24 samples of poisonous groundnuts aflatoxin was present at levels of 2000 to 300000 μg kg^{-1} (mean 66000 μg kg^{-1}). *Aspergillus flavus*, *A. awamori* and 4 named species of *Penicillium* were isolated from the samples. Deaths of 10 pigs, 3 cattle, 2 goats and attribute sheep were investigated. In 8 samples of groundnut hay there was none to 16000 μg kg^{-1} of aflatoxin (mean μg kg^{-1}). Only 3 samples had over 2000 μg kg^{-1}. It was thought unlikely that normal groundnut hay would poison animals (Hurter, 1966). After eating mouldy groundnuts, 4 pigs and 2 goats died within 24 hours. Investigations showed that the mouldy material contained aflatoxin in high concentration produced by the fungus *Aspergillus flavus*. In various experimental animals, except the rat, it caused acute liver necrosis and generalized haemorrhages (Minne et al., 1964). Inappetance, apathy and neurological signs were seen in a flock of sheep near Karhtoum, Sudan, fed on groundnut oilmeal contaminated with aflatoxins (750 μg kg^{-1}). The gross and microscopic lesions were confined to the liver. The biochemical analysis of the serum was consistent with the presence of liver damage. The presence of aflatoxins in the feeds and tissues of dead sheep supports the view that the condition was due to aflatoxin poisoning (Suliman et al., 1987).

Conclusions: Most of the outbreaks of the aflatoxicosis described here are consequences of the ingestion of food that is contaminated with aflatoxins. The strict control of food quality is therefore necessary to avoid such outbreaks. Disease outbreaks due to mycotoxins continue to be problems of significant public health importance in India. In a majority of the cases, such outbreaks may be missed or misdiagnosed due to paucity of information in this regard. Sufficient attention to such disease outbreaks has not been focused in view of remoteness of the areas of such outbreaks (Bhat and Krishnamachari, 1978). Nearly 40% of the population in India live below the poverty line. It is very difficult to imagine their access to complete safe and toxin free food since people are forced to consume less expensive, poor quality food grains because of poor purchasing power. Aflatoxin contamination is therefore, unpredictable which makes it a unique challenge.

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11:30-12:00 PRESENCE OF FREE AND ADDUCTED AFLATOXINS AND HYDROXILATED METABOLITES IN HUMAN HEPATOCARCINOMAS IN MEXICO.

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Background. The human hepatocellular carcinoma (HCC) is the fifth most frequent cancer in the world with 80% of cases occurring in developing countries. HCC is rapidly fatal in almost all cases with a survival period generally less than 1 year from diagnosis. The chronic infection with hepatitis B virus (HBV), hepatitis C virus (HCV) infection, excessive alcohol consumption and a diet with aflatoxins (AF) are considered to be the major risk factors for HCC. HBV and AF exhibit a synergistic interaction in the development of this disease. AF are secondary metabolites produced mainly by the fungi *Aspergillus flavus, A. parasiticus* and *A. nomius*, and they turn out to be natural and dangerous contaminants of several foodstuffs such as cereals, oil seeds and spices where the most important are maize, rice, sorghum, hot peppers and peanuts, that represent a risk for both animal and human health. There are four important AF: AFB_1, AFB_2, AFG_1 and AFG_2, from which AFB_1 is the most toxic, it is considered a potent hepatocarcinogen for animals and a proved carcinogen for humans.

Aims. The aim of this research was to identify and quantify the free AF and their hydrolized metabolites and the adducts AFB_1-N^7-Gua in human livers, control and with HCC in Mexico, to know the human exposition to this food carcinogen in both groups.

Methodology. Fifteen healthy human livers (controls) and 15 HCC livers, were obtained, analyzed and compared from autopsies of the General Hospital of Mexico ("Hospital General de Mexico"), SSA. The extraction of free AF from liver obtained with SepPak LC_{18} and with immunoaffinity columns for total AF. Two μg mL^{-1} of AFB_1-ADN adduct standard were synthesized. The AFB_1-N^7-Gua adduct is not commercially sold by any industry, and it is necessary as reference standard in posterior analysis, it was synthesized with 2 mg mL^{-1} of AFB_1 and 16 mg of calf thymus DNA in the presence of dichloromethane and 3-chloro peroxybenzoic acid and identified at a retention time of 22 minutes. The DNA extraction and purification was made with Gupta (1984) method. The AFB_1-N^7-Gua adducts from each sample were obtained hydrolyzing pure DNA from each sample with chloride 0.15 M and heat to break it into pieces that could be capted and concentrated by the anti-AF antibodies present in the immunoaffinity columns. The quantification of free AF and AFB_1-N^7-Gua adduct were achieved by high-performance liquid chromatography (HPLC).

Results and Discussion. Average total free AF in control samples obtained with Koeltzow y Tanner (1990) method was 37.2 ng g^{-1}, and with Qian & Yang (1984) was 173.2 ng g^{-1}. Average total free AF in HCC livers with Koeltzow & Tanner (1990) was 52.4 ng g^{-1}, and with Qian & Yang (1984) was 179.5 ng g^{-1}. Variance analysis of the AF difference between control and HCC livers by Qian & Yang (1984) found that AFB_2 (P=0.0054*), AFG_1 (P=0.0092*) and AFM_1 (P=0.0001*), had a significant statistical difference, having HCC samples more AF. There were no significant statistical differences with AFM_2 (P=0.3705) AFP_1 (P=0.6) and AFL (P=0.2332) of both treatments. Qiagen technique recovered more free AF in the addition of the three supernatants, 833.95 ng g^{-1} with an average 83.40 ng g^{-1}, more than the recovery of Gupta with a total of 221.45 ng g^{-1} and an average of 22.15 ng g^{-1}.

The recovery percentage of the method Qian & Yang (1984) with LC_{18} SepPak columns for the free AFB_1 extraction was of 95 % with a detection limit of 0.1 ng g^{-1}. The recovery percentage of the four AF mixture (AFB_1, AFB_2, AFG_1 and AFG_2), was 96 % for AFB_1; 96 % for AFB_2; 81 % for AFG_1 and 90% for AFG_2, all with a detection limit of 0.1 ng g^{-1}. Control liver samples presented average concentration (ng g^{-1}) of AFB_1 (0.1), AFB_2 (0.3), AFG_1 (0.2), AFG_2 (17.5), AFM_1 (2.3), AFM_2 (64.1), AFP_1 (87.8) and AFL (0.8). The HCC samples had average concentrations (ng g^{-1}) of AFB_1 (0.2), AFB_2 (1.0), AFG_1 (0.9), AFG_2 (0.5), and the hydroxilated AFM_1 (16.4), AFM_2 (27.3), AFP_1 (104.6) and AFL (1.4).

Conclusions.Free AFG_1, AFM_1, AFM_2 and AFP_1 were found in large amounts, while there were only traces of AFB_1 in both groups of human liver. AFB_1-N^7-Gua adducts of control human liver samples were not detected by HPLC, and a mixture of HCC human liver samples, one gram per sample, presented 4.5 µg of this adduct, this fact confirms the role of AFB_1-N^7-Gua as origin of human HCC.

12:00-12:30 GENOMICS AND MOLECULAR BASIS OF MYCOTOXIN INDUCED HEPATOCELLULAR CARCINOGENESIS AND ITS CONTROL

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Food may be contaminated by natural or man made toxins, including substances shown to be carcinogenic in experimental animals and humans. Naturally occurring carcinogens include mycotoxins particularly aflatoxins, which contribute to causation of liver cancer worldwide especially in Asia and Africa. Contamination of food may occur directly during its production, storage and preparation. A single fungus can produce several mycotoxins and food and feed can be contaminated by several varieties of mycotoxin producing fungi. Naturally occurring of aflatoxins are categoriesed by IARC as Group I carcinogens causing hepatocellular carcinoma in humans. Ochratoxin A, also a mycotoxin has been classed as possible human carcinogen.

Long term oral administration of lower dose of aflatoxin B, (200 μg/kg/ animal / day) induced hepatocellular carcinoma in albino swiss mice (*Mus musculus)* where as feeding of ochratoxin A (50 mg / kg / animal / day) induced hepatorenal carcinoma specially renal ademoma in experimental animals. A large number of haematological and histopathological changes observed in liver and kideney of toxin treated animals. Pre-neoplastic lesions were observed in mycotoxins fed mice. In liver cells, aflatoxin B_1 is metabolized to form an epoxide which binds to the N7 position of specific guanine, lead to the formation of G to T transversion. Mutation induced by aflatoxin B_1 are found in several genes involved in hepatocellular carcinogenesis. Aflatoxin B1 induces a typical mutation at condon 249 in P^{53} gene (AGG to AGT, arginine to serine). This mutation is rarely found in hepatocellular carcinoma in area of low aflatoxin exposure, but occur in upto 60% of hepatocellular carcinoma in regions of very high exposure to aflatoxins. Concurrent administration of aquesous leaf extract of *Vitis vinifera* L and *Cannabis sativa* L prevent the formation of carcinoma in treated animals.

12:30-13:00 THREE-MONTH CLINICAL INTERVENTION TRIAL WITH NOVASIL CLAY IN GHANAIANS: REDUCTION OF URINARY AFLATOXIN M_1 LEVELS AND NO EFFECT ON SERUM MINERALS

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Background: Aflatoxins (AFs), naturally occurring metabolites of Aspergillus fungi, have long been recognized as hazardous contaminants of food. AF exposure is a major risk factor in the etiology of human hepatocellular carcinoma, particularly in regions of Africa and South East Asia (Wogan 1992). The predominant toxin, aflatoxin B_1 (AFB_1), has been categorized as a Group 1 known human carcinogen (IARC 2002). Acute exposure to AFs via the diet can result in disease (aflatoxicosis) and death in humans, as evidenced by numerous reports, including the recent outbreak in Kenya, where AF poisoning was linked to consumption of foods containing levels as high as 8,000 ppb (CDC 2004). Therefore, development of practical and effective intervention strategies for aflatoxicoses is critical for improving human health, especially in high-risk populations. NovaSil (NS), a calcium montmorillonite clay, has been shown to prevent aflatoxicosis in multiple animal species by preferentially binding AFs in the GI tract and reducing toxin bioavailability (Phillips 1999). Given the safety of NS, demonstrated in a variety of animal models and in a short-term human study, it was hypothesized that NS intervention may be safe and effective in reducing biomarkers of exposure in individuals who are frequently exposed to high levels of aflatoxins and at risk for aflatoxicosis.

Aim: The objectives of this portion of the study were to: 1) evaluate the ability of NS to reduce urinary aflatoxin M_1 (AFM_1) levels and 2) assess the potential interference of NS with serum minerals.

Materials and Methods: A randomized, double-blind, placebo controlled phase IIa clinical trial was conducted in 177 healthy Ghanaian volunteers who received NS clay capsules either in 1.5 g/day (low dose, LD), 3.0 g/day (high dose, HD), or placebo (PL) for a period of 3 months. NS clay was obtained from Engelhard Chemical Corporation (Iselin, NJ, USA) and was further examined for potential environmental contaminants, including polychlorinated dibenzo-p-dioxins/furans (PCDDs/ PCDFs) and priority heavy metals to ensure compliance with federal and international standards. AFM_1 levels were measured in urine samples collected from study participants at baseline, 1- and 3-months after intervention and 1-month after the end of the study period. AFM_1 was analyzed by immunoaffinity column purification followed by high performance liquid chromatography (HPLC) with fluorescence detection, using modifications of methodologies described by Groopman et al. 1992. The protocol was validated, and the presence of AFM_1 was confirmed by mass spectrometry. Creatinine concentrations in the urine samples were

measured at St. Joseph's Regional Health Center (Bryan, TX, USA) in order to correct for variations in dilution among individual samples. Concentrations of 15 nutrient and 15 non-nutrient minerals were measured in 58 HD and 62 PL serum samples collected from study participants at baseline and 3-months after intervention. Mercury (Hg) concentrations were determined by cold vapor atomic absorption (CVAA) with stannous chloride as a reductant. Aluminium (Al), boron (B), barium (Ba), beryllium (Be), calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), molybdenum (Mo), sodium (Na), phosphorus (P), sulfur (S), silicon (Si), strontium (Sr), titanium (Ti), vanadium (V), and zinc (Zn) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) with ytterbium (Yb) as an internal standard. Silver (Ag), arsenic (As), cadmium (Cd), chromium (Cr), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), and thallium (Tl) were determined with inductively coupled plasma mass spectrometry (ICP-MS) with As, Cr, Mn, and Se acquired in DRC mode, and bismuth (Bi), gallium (Ga), and rhodium (Rh) as internal standards. In addition to blanks, spiked blanks, duplicate samples, and spiked samples, results were validated by preparing and analyzing standard reference materials (Seronorm, Billingstad, Norway) with each batch of samples.

Results and Discussion: Levels of AFM_1 in urine samples collected at baseline and 1-month after intervention did not show a statistically significant difference between the PL group and LD group or between the PL group and HD group. In urine samples collected after 3-months of the intervention, no statistically significant difference was found between the PL group and LD group; however, a significant decrease (up to 58%) in the median level of AFM_1 was found in the HD group as compared to the median level in the PL group ($P=0.0391$). No significant differences were found in levels of the nutrient and non-nutrient minerals between the HD and PL groups at baseline and 3-months of NS intervention, except for strontium levels. Strontium was significantly increased ($p<0.001$) in the HD group (male = 113.65±28.00 μg l^{-1}; female = 116.40±24.26 μg l^{-1}) compared with the PL group (male = 83.55±39.90 μg l^{-1}; female = 90.47±25.68 μg l^{-1}) following the 3-month intervention.

Conclusions: These results suggest that intervention with NS clay can considerably reduce urine levels of AFM_1. Furthermore, these findings confirm that overall NS acts as a selective enterosorbent that does not affect the serum concentrations of important nutrient minerals in humans. (Supported by USAID LAG-G-00-96-90013-00).

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13:00-13:30 URINARY BIOMARKER FOR FUMONISIN EXPOSURE IN A SOUTH AFRICAN RURAL INTERVENTION STUDY

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Background: Fumonisins are produced by certain *Fusarium spp* and are frequent contaminants of maize worldwide. The most common analogue, fumonisin B_1 (FB_1) is a possible human carcinogen (IARC,2002). To establish the causal link between exposure and human cancer requires accurate measurement of exposure (Wild & Gong, 2009). To this end a urinary F B_1 biomarker was recently developed enabling assessment of individual exposure (Gong et al, 2008).

Aim: Utilizing a urinary FB_1 biomarker to evaluate the effectiveness of hand sorting and washing maize in reducing dietary fumonisin exposure in a maize consuming population.

Materials and Methods: Twenty-two women responsible for preparing and cooking family foods were recruited from rural villages of the Centane Magisterial area in the Eastern Cape, South Africa. The participants were trained in how to remove infected kernels and to wash maize to reduce fumonisins. They subsequently performed the sorting and washing on the maize kernels which were then cooked and consumed by the participants. On two consecutive days before and after the consumption, respectively, samples of cooked maize meal were collected for analysis of FB_1 and total FB contamination; a 24-hour maize consumption record, together with the maize meal FB level were used to calculate the intakes of FB_1 and total FB. Morning urine samples were collected before and after the intervention for FB1 analysis using a liquid chromatography - mass spectrometry (LC-MS) analytical method (Gong et al, 2008).

Results and Discussion: Urinary FB_1 biomarker data were calculated as pg FB_1 per ml urine, as well as pg FB_1 per mg creatinine to correct for variation in urine volume. The geometric mean level in urine samples collected before the intervention was 225 pg/ml urine (95% CI 144-350 pg/ml), or 470 pg/mg creatinine (95% CI 295-750 pg/mg). After intervention, the biomarker level was significantly reduced to 109 pg/ml urine (95% CI 85-138 pg/ml, p=0.02), or 279 pg/mg creatinine (95% CI 202-386 pg/mg, p=0.02), a 52% and 41% reduction, respectively. The intervention reduced total FB intake from 6.7 to 2.5 µg/kg body weight/day, a 63% reduction, a value in good agreement with the biomarker data. There was a strong correlation between the total FB intake and both urinary FB_1 and urinary FB_1 adjusted for creatinine with correlation coefficients of 0.52 (p=0.0005) and 0.50 (p=0.0008), respectively. The strength of correlation between specifically FB1 intake and urinary FB_1 levels were similar to those with the total FB intake. Based on data from this study, it is estimated that around 0.09% (95%CI 0.06% - 0.12%) of the total FB intake was excreted in urine, estimates lower than previously reported in animal studies (Shephard et al, 1994).

Conclusion: The urinary FB_1 biomarker, being well correlated with FB intake, has a future role to play in assessing health risks from fumonisin exposure and is valuable in assessing the effectiveness of intervention strategies to reduce human exposure. In addition, these preliminary data demonstrate the significant impact of simple interventions on overall exposure to this commonly occurring carcinogen.

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Yucatan II

Tuesday June 29, 2010

Session 4: Advances in Fumonisins and biomarkers.

16:00-16:40 SOME RECENT DISCOVERIES ABOUT FUMONISINS

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Fumonisins are well known mycotoxins from *Fusarium verticillioides, F. proliferatum* and other *Fusarium* species. Several new fumonisins and fumonisin-like compounds have been detected by mass spectrometry in cultures of *F. verticillioides*. Although *Alternaria alternata* has previously been reported to produce fumonisin B_1 (FB_1) in culture, no fumonisins were detected in tomato pathotypes.

Recently, fumonisins B_2 and B_4 were found in *Aspergillus niger* isolated from coffee and fumonisin B_2 in *A. niger* from grapes. These fumonisins were themselves detected in coffee beans and grape must, respectively, adding to the list of foodstuffs and feedstuffs other than maize and sorghum in which fumonisins have been found in recent years - rice, wheat, barley, distillers' dried grains with solubles, faro, cowpea, asparagus, figs, peanuts, black tea and medicinal plants.

FB_1 can bind to proteins (PB FB_1) and to other matrix components (TB FB_1) during food processing involving heat. Model reactions of FB_1 with sucrose, methyl α-D-glucopyranoside and amino acid derivatives have shown that binding to polysaccharides and proteins occurs via the tricarballylic acid side chains of FB_1. The occurrence of bound fumonisins in processed corn foods is common and has now been extended to mildly processed products. Bioaccessibility of free FB_1 and TB FB_1 present in corn flakes has been estimated by *in vitro* digestion experiments.

An unrelated type of binding (or association) relates to instability of fumonisins in rice flour, cornstarch, and cornmeal at room temperature; this can affect the immunoaffinity column cleanup procedure in analysis of naturally contaminated starch-containing corn foods for fumonisins. The occurrence of *N*-fatty acylated fumonisin derivatives in retail fried corn foods has also been demonstrated. Intentional binding of fumonisins to cholestyramine has been demonstrated *in vivo* and is a potential means of detoxification of animal feed.

Among recent toxicological studies on fumonisins, it has been reported that, unlike FB_1, hydrolysed FB_1 did not cause neural tube defects in a mouse model.

16:40-17:20 VALIDATION OF FUMONISIN BIOMARKERS FOR STUDYING HUMAN DISEASE RISKS.

Keynote Conference

Jia-Sheng Wang

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The University of Georgia, Athens, Georgia, USA

Fumonisins are ubiquitous contaminants of cereal grains worldwide. Fumonisin B_1 (FB_1) is a carcinogen and a strong tumor promoter in animal models. Dietary exposure to FB_1 has etiologically been linked to human neural tube defect and to elevated risks for esophageal and liver cancers in certain areas of the world. Disruption of sphingolipids metabolism in ceramide synthesis pathway and metabolites of sphingolipids, especially ratio of sphinganine (Sa) and sphingosine (So), as well as free FB_1 in body fluids, were proposed as the potential biomarkers for FB_1 exposure.

To validate these FB_1 biomarkers for studying human diseases risks, over past 10 years, we conducted animal studies in laboratory, cross-sectional studies, and case-control studies in high-risk populations of Huaian and Fusui areas, where are among the highest incidence of esophageal and liver cancers in China. The time-course of FB_1 induced metabolic alternation of sphingolipids was assessed in serum and urine samples of animals treated with either a single-dose or multiple-doses of FB_1 at different time interval. The dose-response relationship between treatment of FB_1 and free FB_1 in urine and feces and Sa/So ratio in urine and serum was also examined. Dose-dependent urinary and fecal excretion of free FB_1 was found and urinary Sa/So and SaP/SoP were more sensitive biomarker for FB_1 administration in animals. Current status of FB_1 contamination, dietary intake of FB_1, and FB_1 biomarkers in blood and urine samples of healthy adults of cross-sectional studies, cancer cases, and age-, gender-, and residency-matched controls were further assessed.

Food samples and food intake frequencies were collected from individual households in different townships of studied areas. The enzyme-linked immunosorbent assay and immunoaffinity-HPLC-fluorescent methods were used for analysis of FB_1 in food and body fluids. Metabolites of sphingolipids were measured by a robust HPLC-fluorescence detection with D-erythrosphingosine (C_{20}) as the internal standard. Food samples collected were highly contaminated with FB_1 with detectable rate ranged from 85% to 93% and the medium was at 3.2 ppm ranged from 0.1 to 25.5 ppm. Daily intake of FB_1 was varied from 25 μg to 1,083 μg/person.

The ratio of Sa/So in urine and serum samples was comparable among study populations. No statistical significance of Sa/So was found between cases and controls in separate esophageal and liver cancer studies, suggesting that sphingolipids based biomarkers may practically not reflect a significant association with long-term human FB_1 exposure. However, urinary free FB_1 was found to be correlated with food exposure in our cross-sectional studies; furthermore, urinary free FB_1 levels were measured in a case-control study including 189 esophageal cancer patients and 567 age-, gender-,

and residency-matched healthy controls. The averaged urinary free FB_1 level in case group is 468.5 ± 1,216.4 pg/mg creatinine, with a median level of 173.2 pg/mg creatinine (range: 1.2-12,140.6); while the averaged level in the matched control group is 204.3 ± 450.9 pg/mg creatinine, with the median level of 57.4 pg/mg creatinine (range: 0.32-5,109.54).

Levels in the case group are significantly higher than levels in the control group ($p<0.0001$). In addition, the detectable rate of free FB_1 is 95.2% (180/189) in the case group,

which is significantly higher than 84.1% (477/567) in the control group ($p<0.0001$). In summary, the high contamination rate of FB_1 in food and high daily intake of FB_1 in high-risk populations suggest a possible contributing role of FB_1 in human esophageal- and hepato-carcinogenesis. Our results also suggest that the urinary free FB_1 level may be a good biomarker of exposure to study relationship between dietary FB_1 exposure and human disease risks in high risk populations. (Works presented were supported by the NIH/NCI grants CA94683, CA90997, and DOD research contract DAAD 13-01-C0053).

17:20-18:00 USE OF MECHANISM-BASED AND EXPOSURE BIOMARKERS TO ASSESS FUMONISIN TOXICITY IN HUMANS, ANIMALS AND PLANTS AND EFFICACY OF PROCESSING METHODS USING MECHANISM-BASED BIOASSAYS

Keynote Conference.

Ronald Riley*,[1]

with Kenneth Voss[1], Nicholas Zitomer[1], Anthony Glenn[1], Tantiana Burns[1], Alfred Merrill, Jr[2]., Olga Torres[3] and Janee Gelineau van Waes[4]

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Background: Fumonisins (FB) are mycotoxins produced by Fusarium verticillioides and several other Fusarium species. They are known causes of animal and plant diseases and suspected causes of human diseases where maize is consumed in large amounts and diets are likely to be deficient in critical nutrients. There is no single diagnostic approach that can identify/pinpoint when a disease outbreak is due to exposure to FB or even when FB could be a possible contributing factor to a disease outbreak of unknown etiology. One major problem is that the dose-response studies necessary to reveal the threshold for changes in mechanism-specific biochemical alterations (mechanism-based biomarkers) have not been statistically correlated with either the thresholds for disease or exposure biomarkers (parent compound in tissues/fluids). The key is to better define the underlying biochemical changes and the thresholds that ultimately lead to undesired consequences (adverse physiological effects). Fortunately for FB the first site of action or more precisely, the proximate cause/key event is known. However, the linkage of the proximate cause to the downstream effects and, most importantly for the adverse effects in target organs is not as well understood.

Aims: The purpose of this talk is to briefly summarize the current state of biomarker development for FBs.

Approach: Combining information about known exposure, clinical indicators and biomarkers could in the future provide a useful approach for identifying disease causation in the most economical and definitive manner.

Results and Discussion: The following is a brief overview of recent and ongoing collaborative studies conducted by scientists in the Toxicology and Mycotoxin Research Unit in collaboration with scientists from other institutions. Human communities potentially exposed to high levels of dietary FBs have been identified in Guatemala. A preliminary exposure assessment has been completed and exposure and mechanism-based biomarkers are being developed (Torres et al., 2007; Riley, 2010). Maize samples from the 2005 and 2007 crops were collected from commercial venders in 10 highland (> 1700 m) and 10 lowland (< 360 m) Departments as part of a collaborative study with the Instituto de Nutricion de Centro America y Panama, ICTA and Duke University. The results show that lowland maize, highly contaminated with FB, is sold in

highland markets in Departments where neural tube defects (NTD) incidence is often very high. Thus, FB exposure in high risk areas will be greatest in groups that obtain their maize from the market place since we have shown that maize that is grown in the highlands contains very low levels of FBs. Exposure for women consuming nixtamalized maize products made from maize purchased from commercial venders in the Central Highlands and Pacific Lowlands of Guatemala was calculated. The results provide evidence that women consuming nixtamalized maize products will often exceed the provisional maximal tolerable daily intake established by the WHO/FAO Joint Expert Committee on Food Additives. The work is important because it shows that, in Guatemala, Lowland maize sold in Highland markets is probably the primary the source of FB exposure in the highlands. This information provides the basis for future studies to establish in humans the potential health risks from FB exposure and more immediately could potentially serve as the basis for implementing management strategies to reduce FB exposure in humans throughout Central America where maize consumption is high. In collaboration with our colleagues in Guatemala we developed a protocol for obtaining and analyzing human urine and blood to determine FB exposure. We identified two Guatemalan communities where FB_1 exposure is quite different. We obtained approval for a human subject's research protocol for sampling within the communities. A human subject's research protocol was also approved for a study in the USA to validate our methods for FB in urine and sphingolipids in blood spots. These studies are underway but thus far have revealed that only FB_1 is excreted in urine, that the amount of FB_1 excreted is less than 0.1% of the dose, and that detectable amounts in the urine decrease very rapidly (half-life less than 48 h after exposure). Developing exposure and mechanistic markers will provide us with a tool to find high risk populations for future studies and will aid us in our goal to reveal the factors (genetic and nutritional) leading to possible increased susceptibility to NTDs in populations where maize consumption is high and diets are likely to be deficient in folate.

Discovery of the ability of sphingoid base 1-phosphate analogs to induce neural tube defects in mice (Gelineau van Waes et al., 2008 and 2009); **-** In collaboration with scientists at the University of Nebraska Medical Center (Currently Creighton University) we discovered a possible role of FB-induced elevation of sphingoid base 1-phosphates, ligands for S1P Receptors ($S1P_{1-5}$) as a risk factor for neural tube defects in mice. The results show that sphingoid base 1-phosphates are elevated in red blood cells, serum and placenta is of mice exposed to FBs during the period of fetal development when neural tube closure occurs. In addition, an analog of sphingoid base 1-phosphates (FTY720) was found to be a potent inducer of NTDs in the mouse model. These results strongly suggest that the lysophospholipid receptors known as S1PR play a key role in FB-induced NTD in mice. This discovery provides a potential additional human risk factor which could be explored, along with folate deficiency, in Guatemalan women and Hispanic women in the USA who are at higher risk for NTD.

Discovery that hydrolyzed FB induces disruption of sphingolipid metabolism but does not induce neural tube defects (NTD) in mouse models (Voss et al., 2009).- Histopathological and sphingolipid studies were done to demonstrate the maternal toxic and sphingolipid metabolic effects underlying the differences in neural tube defect (NTD) induction in LM/Bc mice given 10 mg/kg bw FB_1 (induces NTD) and up to the 20 mg/kg bw hydrolyzed FB_1 (does not induce NTD and is a dose 7-fold greater (molar equivalent basis) than the lowest dose of FB_1 known to cause NTDs in this experimental model). Significant maternal liver lesions were found only in FB_1-treated females. FB_1 (FB_1) also markedly disrupted sphingolipid metabolism including elevation of the novel sphingoid base 1-deoxysphinganine and depletion of complex sphingolipids whereas hydrolyzed FB_1 had only a minimal effects on sphingolipid metabolism. These results provide additional evidence that NTD induction by FB_1 in mice occurs through a mechanism involving disruption of sphingolipid metabolism. Understanding the underlying mechanistic basis for NTD development in response to FB exposure in mice will assist in predicting the risk in humans. They further indicate that conversion of FBs to hydrolyzed FBs by the nixtamalization cooking method reduces the FB-associated human health risks.

Discovery of a new category of sphingolipids with the potential for use as biomarkers in human studies will lead to a better understanding of the mechanistic basis for FB-induced diseases (Zitomer et al., 2009).- Our laboratory and researchers at Georgia Institute of Technology (and previously Emory University) have been working since 1995 to identify a novel sphingoid base known previously as the "mystery peak" and produced by cultured renal cells. We have recently confirmed the identity of the “mystery peak” as 1-deoxysphinganine and showed that it is produced by the condensation of alanine with a fatty acid by the enzyme serine palmitoyltransferase. This discovery and subsequent characterization of 1-deoxysphingoid bases in mammalian cells and tissues is a major advance in lipidomics. Recently it has been reported that accumulation of 1-deoxysphinganine may be involved in the human neurodegenerative disease known as Hereditary Sensory Neuropathy Type 1 (HSN1). The discovery of 1-deoxysphinganine and deoxydihydro-ceramides will help explain the tissue, species and sex specificity of FBs.

Biomarker based bioassays and in vivo toxicity studies show that alkaline cooking (=nixtamalization) reduces bioavailability and toxic potential of FBs in maize (Burns et al., 2008). **-** An in vitro bioassay of maize, masa and tortilla chips using ceramide synthase inhibition as the endpoint demonstrated that nixtamalization reduced toxic potential and that there was a close correlation between FB_1 concentrations and toxicity. An in vivo rat feeding bioassay comparing *Fusarium verticillioides* fermented maize (culture material), nixtamalized culture material and a nixtamalized mixture of culture material and ground maize showed that FB-maize matrix interactions (presumably binding to matrix constituents) occur during nixtamalization and are protective. FB_1 concentration of the cooked culture material-corn mixture was significantly reduced compared to the other items. Nixtamalization partially reduced the renal toxicity of the culture material. In contrast, nixtamalization after mixing the culture material with maize prevented FB bioavailability and toxicity. Preliminary studies also showed that FB-matrix binding occurs during nixtamalization of whole kernel maize. Together, these findings provide additional evidence that nixtamalization reduces FB bioavailability and toxicity. They further suggest that matrix-binding is protective against FB toxicity.

Improved understanding of the mechanistic basis for the sensitivity of rat kidney to FB toxicity (Riley and Voss 2006; Zitomer et al., 2009). The relative sensitivity of male rat kidney and liver is most likely a consequence of differences in the mechanisms responsible for both FB uptake/clearance and sphinganine metabolism. In a feeding study, we showed for the first time that the sensitivity of rat kidney to FB is a result of preferential accumulation of FB by kidney and the fact that kidney accumulates much greater amounts of sphingoid bases and sphingoid base 1-phosphates compared to liver. This finding is important because the rat kidney is the critical target organ used in the FB risk analysis and upon which the recommended FB tolerable daily intakes are based. Until now, the reason that rat kidney is more sensitive than liver to FB toxicity was not known. In collaboration with scientists at Health Canada we detected 1-deoxysphinganine and 1-deoxyceramides in animal tissues and further showed that 1-deoxysphinganine accumulates to very high levels in the livers (but not kidney) of mice fed FBs. Unlike rat kidney, there are only small changes in sphinganine 1-phosphate in either mouse liver or kidney; which may explain the tissue, species and sex specificity of FBs. Understanding the underlying mechanistic basis for organ/species specific toxicity will allow us to better predict the risk in humans.

FBs are poorly translocated from maize roots to aerial parts but the products of FB-induced ceramide synthase inhibition are readily translocated into aerial plant parts (Zitomer et al., 2008 and submitted). – It was discovered that in maize seedlings watered with pure FB_1, pure FB_2 or the combination of B_1 and B_2, FB_1 was preferentially accumulated in root tissue but was not significantly translocated into leaf tissue. Conversely, the FB that entered roots caused significant elevation in sphingoid bases and sphingoid base 1-phosphates in roots and these were translocated into leaf tissues. This finding was different than what was seen when seedlings were grown from seeds inoculated with the fungus that produces FBs. In this case

both FB_1 and B_2 accumulated in roots whereas only FB_1 was appreciably translocated into leaf tissue. Interestingly, maize varieties resistant to F. verticillioides induced leaf lesion development did not accumulate appreciable amounts of FBs or sphingolipid metabolites in the root tissues. The results suggest that the fungal/plant/soil interaction and genetic factors contribute to the ability of FBs to enter the roots and be translocated into leaf tissues. This is important because plant debris can be a significant source of FBs and thus the factors which control FB entry into plant parts could reduce the levels of FBs in field debris.

Conclusions: The use of mechanism-based and exposure biomarkers for FBs have great potential for answering basic questions about disease thresholds in animals and humans, the mechanistic basis for species and sex sensitivity, efficacy of processing methods and for identifying potential strategies for reducing accumulation in plants.

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Zitomer NC, Jones S, Bacon CW, Glenn AE, Baldwin T and Riley RT (submitted) Sphingoid bases and their 1-phosphates, but not fumonisins, are translocated from roots to aerial tissues of maize seedlings watered

Yucatan II

Wednesday June 30, 2010.

Session 5: Ochratoxin A in food, human health and its control.

08:30-09:00 OCHRATOXIN A PRODUCING FUNGI AND OCHRATOXIN CONTENT IN PISTACHIO AND PEANUT FROM MICHOACÁN

Iliana Tafolla-Muñoz[1], I, Ma. Soledad Vázquez-Garcidueñas[1], Gerardo Vázquez-Marrufo[2] , **Virginia Robinson-Fuentes[1]***

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Background: The consumption of nuts is generally recommended in modern diets in order prevent cardiovascular events because of their high content in nutritious components and healthy fatty acids. However, such composition makes them prone to fungal contamination that can lead to mycotoxin contamination (Fidanza, 2002). It is well known that mycotoxins represent an important threat to human health so it is important to ensure that most food products consumed by the population are safe from the mycotoxicological point of view. Pistachio and peanuts are consumed in Mexico as snacks and they are not usually assessed for their mycotoxins content.

Aim: To identify the fungal flora present in pistachio nuts and peanuts available in retail markets in Michoacan in order to determine the presence of mycotoxigenic fungi and if so, the possible contamination of these nuts by mycotoxins such as ochratoxin A.

Materials and Methods: 52 1 Kg samples of each pistachio and peanuts were collected from retail markets in Michoacan State, central Mexico and stored at -20ºC until required. 50 g subsamples were surface sterilized with a 1% sodium hypochlorite solution for one minute and rinsed twice with sterile distilled water and allowed to dry in a laminar flow cabinet. Nuts were plated, 10 per plate, on Potato Dextrose Agar (PDA). The plates were incubated at 28°C for 4 to 7 days. The resulting fungal colonies were subcultured onto PDA prior identification. The numbers of fungi present in each sample were recorded. Fungal identification was performed according to their metabolic patterns in a Biolog® system. Photographs of the microscopic and macroscopic structures of isolated fungi were taken and compared to those contained in Biolog database to aid identification. OTA content was assessed in pistachio samples using HPTLC after SPE extraction.

Results and Discussion: *Identification of fungi* According to the Biolog analysis of the metabolic patterns showed by the fungi, those isolated more frequently from pistachio nuts and peanuts are: *Aspergillus fumigatus*, *Penicillium verrucosum*, *Cladosporium herbarum*, *Aspergillus restrictus* and *Penicillium solitum.* Macroscopic and microscopic photographs were consistent with genera when compared to the photographs database contained in Biolog. It is very well documented that *P. verrucosum* is an Ochratoxin A

(OTA) producing fungus (Cabañas et al, 2008) and, therefore, the OTA content was assessed in pistachio samples. The analysis of pistachio nuts for ochratoxin A demonstrated that 31% were contaminated but only one sample contained 66 μg/kg and the remainder contained < 36 μg/Kg.

Conclusions: The results here obtained are interesting since the Biolog® system is mainly used for bacterial identification and it was possible to use it for fungal identification. On the other hand, fungal flora was not as abundant as other reports have established. The presence of a micotoxigenic fungus in the samples also revealed the presence of the mycotoxin (OTA). Since OTA represents a risk for human health, efforts should be made to ensure that foods do not exceed the Provisional Tolerable Weekly Intake (0.1 μg/Kg; JEFCA, 2001).

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09:00-09:30 EFFECT OF TWO ROASTING PROCESSES ON THE OCHRATOXIN REDUCTION IN COFFEE BEANS *(Coffea arabica)*

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Background: Ochratoxin A (OTA) is a secondary metabolite with proved nephrotoxic, immunotoxic, teratogenic and carcinogenic effects. In coffee, OTA is produced by certain filamentous fungi *Aspergillus carbonarius, A. niger*, *A. ochraceus*, *A. westerdijkiae*. The thermal stability of OTA is of particular interest to the coffee industry[1]. The roasting impact on OTA content in coffee beans and brew has been reported giving contradictory results[3]. Such variability could be related to the different roasting process conditions: the initial mycotoxin level of raw coffee beans, the type de contamination (natural or artificial), and the roasted degree[4]. Few studies have been reported on the OTA thermal stability in the different roasting processes.

Aim: To assess the effect of two roasting techniques on OTA reduction on green coffee (*Coffea arabica*) beans naturally contaminated.

Materials and Methods:
OTA production in green coffee beans: 20 kg-batches of green coffee beans were inoculated with a toxigenic *A. westerdijkiae* spore solution and incubated for 3 and 9 days at 25°C. *Roasting procedure*: Samples 100 g and 200 g of contaminated coffee were roasted in a cylinder (Probat-Werne) and a Fast Fluidiser-Bed (Neuhaus Neotec) pilot roaster at 230 °C (air temperature). Different degrees of roasting – light, medium and dark – were obtained depending on the length of time spent by the coffee in the roaster. Bean swell, weight loss, and color were used to assess the degree of roasting. At a given time, a 10 g sample was removed. *OTA analysis*: Coffee samples were analyzed for OTA[2]. The samples (10 g) were extracted with 100 mL of a 50:50 methanol/sodium bicarbonate (3% w/v) for green coffee, and a 20:80 at 60°C for roasting coffee. The extracts were purified in an immunoaffinity column (Ochraprep®, R-Biopharm, UK). OTA was quantified by HPLC with a fluorescence detector.

Results and Discussion:
After 3 and 9 days of incubation, green coffees containing 5.3 (L1) and 57.2 (L2) μg kg^{-1} of OTA respectively, were obtained. The coffees were subjected to the two types of roasters, the OTA reduction was compared for a same roasting technique. The reduction observed was similar for the two levels and for two processes at the maximal time of roasting: 95.1 % (L2) and 97, 2 % (L1) for cylinder technique, while 81.3 % (L2) and 79.2 % (L1) for fluidized bed method. The kinetics of OTA reduction showed different degradation rates in both processes. In the ln C/Co representation as a function of time it was observed two phases for cylinder technique: slow destruction (k = -0.02)

followed by rapid destruction (k =-1.12), while for fluidized bed method, one phase (k = -0.29) was observed. Similar results have already been reported using the cylinder method[5]. However, when the kinetic of thermal decomposition was superposed between processes, a considerable similitude between the kinetics was noted. The decomposition OTA during roasting process observed the same type of sigmoid curve (y=a+b/(1+exp(-(x-c)/d), that is characterized by the thermal decomposition of organic compounds in precursor compounds.
Three different roasting points were set according to the L* color coordinate. The OTA reduction was compared for the different roasting degrees: light, medium and dark. The OTA reduction levels were 63 % and 33 % at medium roasting point, and 88 % and 68 % at dark roasting point for the cylinder and the fluidized bed techniques, respectively, the cylinder was then the most efficient technical process for OTA reduction (88 %).
The thermal diffusion process study in the coffee beans during roasting could probably explain the efficiency of the roasting cylinder technique which was more efficient for OTA reduction. In the case of the fluidized bed, the heat diffusion time was reduced, only the external layer of the coffee beans was concerned by the thermal processing, and then OTA reduction was less important. The time in the roaster to reach the same coffee roasting level can explain also the difference.

Conclusion: This study supplied important information on the thermal stability of OTA in the roasting coffee process. In these experiments, the best efficient process is the cylinder roaster which a 93 % OTA level reduction in coffee was observed. However, the disappearance of OTA comes along with the appearance of new products whose identification and toxic evaluation establish the next stages of this work.

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09:30-10:00 EFFECT OF PLANT EXTRACTS ON OCHRATOXIN A PRODUCTION BY *ASPERGILLUS CARBONARIUS*

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Background: Ochratoxin A (OTA) is a mycotoxin, which has been described as a potent nephrotoxin, carcinogen and teratogen, with immunotoxic properties in rats and possibly in humans. *Aspergillus carbonarius* is predominantly responsible for the production of Ochratoxin A (OTA) in grapes, wine, and dried vine fruits (Bragulat et al., 2001; Abarca et al., 2003), especially because a high percentage of its isolates are able to produce the toxin (Cabañes et al., 2002; Romero et al., 2005). Chemicals are used, but the negative consumer perception of chemical preservatives drives attention towards natural alternatives. Resistance of fungal strains to traditional fungicides, the limitation of the number of fungicides allowed for postharvest application, and increasing public concern regarding contamination of perishables with fungicidal residues have increased the need for the development of new safe and biodegradable alternatives. Antimicrobial and antioxidative properties of the essential oils and extracts from many plants have recently been of great interest.

Aim: The aim of this study was to investigate the in vitro effect of plant extracts on OTA production by *Aspergillus carbonarius*.

Materials and Methods: The plants used were eucalyptus (*Eucalyptus globulus*) and marigold (*Calendula officinalis*). Previous studies have demonstrated that ethanolic and chloroformic extracts of these plants were inhibitory for the growth of several toxigenic food spoilage fungi. Plant extracts were prepared by mixing 50g of each plant with 250 ml of the solvent. The mixture was mechanically homogenised at 300 rpm for 1h. The extracts were filtered and evaporated to dryness at 45ºC. Dry extracts were dissolved in ethanol and sterilized by filtration. The *A. carbonarius* strain, isolated from dried vine fruit, was maintained on Potato Dextrose Agar. Spore suspensions were prepared and diluted in a sterile Tween 80 0.05% solution to a concentration of 10^6 spores/ml. Petri plates of Malt Extract Agar (MEA) and Czapek Yeast Extract Agar (CYA) containing the corresponding plant extract were inoculated with 2 μl of the spore suspension. The concentrations of extracts evaluated were 500, 250, 100, 50 and 10 μg/ml media. Controls were prepared by adding the same concentration of ethanol. The plates were incubated 7 days at 25ºC. OTA production was determined by TLC applying the “plug” method described by Filtenborg & Frisvad (1980).

Results and Discussion: OTA production was reduced to 50% by the eucalyptus ethanolic and chloroformic extracts at the maximum concentration evaluated (500 mg/ml) on MEA. However, suboptimal concentrations caused an increment in the toxin production. On CYA, no inhibition, but an increment was observed by eucalyptus

extracts at any of the levels studied. The addition of 500 mg/ml of ethanolic extract on CYA produced an OTA accumulation four times higher than the control. Neither of the marigold extracts was effective to inhibit the mycotoxin production on both media. The ethanolic extract on MEA increased in a 500% the amount of OTA produced compared to the control.

Conclusions: Considerable attention has been given to the development of natural antifungals based on plant extracts or essential oils. Even though their effect on growth of many fungal species has been studied, there are few data on the influence of these substances on mycotoxin production by toxigenic strains. The results of the present work show that although some plant extracts are effective to reduce the growth rate of the fungus, they could stimulate toxin production, especially when applied at suboptimal doses. The use of natural antimicrobial compounds is important not only in the preservation of food but also in the control of human and plant diseases of microbial origin.

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10:00-10:30 CHEMICAL AND STRUCTURAL MODIFICATION OF CLAY AND ZEOLITE FOR ISOTHERMAL ADSORPTION OF OCHRATOXIN A.

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Background: The mycotoxins are secondary metabolites produced by fungi that reduce the growth and reproductive parameters in animals, particularly in farms concern species. Mycotoxin ochratoxin A is a pseudopeptide formed with phenylalanine and isocoumaric acid, which has a nefrotoxic effect, mainly in poultry production. Nowadays, the main alternative to reduce the toxic effect of feeds and raw materials contaminated used in the poultry production are the adsorbents, however, the inorganic aluminosilicates has been shows a low adsorption of mycotoxis such ochratoxin A. To increase the adsorption of ochratoxin A, has been reported the chemical and structural modification of aluminosilicates such clays and zeolites using surfactants as ammonium salts, however, the protocol for the optimal cationic exchange of surfactant in the active surface and the effect of chemical and structural modification on the isothermal adsorption of ochratoxin A requires more studies.

Aim: To determine the effect of chemical and structural modification of clay and zeolite on the isothermal adsorption of ochratoxin A.

Material and Methods: In order to obtain the modified materials, representative samples of clay and zeolite were treated with cationic surfactant octadecyldimethyl benzyl ammonium in two ways, dry and wet protocol. The modified materials were analyzed by scanning electron microscopy and RX fluorescence to confirm the chemical and structural modification. The *in vitro* isothermal adsorption of ochratoxin A was carried out by equilibrium assays and the residual ochratoxin A was quantified by reverse phase HPLC using fluorescence detector. The parameter of equilibrium constant (K) and sites number (N) were obtained using Langmuir model by the linear resolution of Lineweaver-Burk.

Results and discussion: The results show that the chemical modification by surfactant, increases the adsorption of ochratoxin A in both, clay and zeolite. The clay change it's adsorption from 2.47 to 35.29 $\mu molg^{-1}$ in the wet process and to 724 $\mu molg^{-1}$ in dry process. In other hand, the zeolite increases the adsorption of ochratoxin A from 49.31 to 67.60 $\mu molg^{-1}$ in the wet protocol and reach the 1687 $\mu molg^{-1}$ in dry process. The results shows that the dry protocol shows the higher increase in the adsorption of ochratoxin A, additionally, the dynamic incorporation of surfactant by the dry process has the advantage versus wet process, since is not required the elimination of the water used in the chemical modification.

Conclusion: The chemical and structural modification of clay and zeolite using the wet and dry protocols increase the adsorption of ochratoxin A,

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11:00-11:30 SYNERGYSTIC EFFECT OF CITRININ, FUMONISIN AND OCHRATOXIN A ON CELL VIABILITY AND GENOTOXICITY: ROLE IN HUMAN NEPHROPATHY AND UROTHELIAL TRACT TUMOURS

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Analyses of several food and feed items show the simultaneous presence of several mycotoxins, notably fumonisins (FB), citrinin (CIT) and ochratoxin A (OTA). All the three toxins induce nephrotoxicity. The aim of this study was to determine the cytotoxic and genotoxic combined effects of either CIT and OTA or FB and OTA. The studies were conducted (i) in cells culture and (ii) in vivo on rat fed with ground wheat enriched with OTA and/or CIT; and/or FB. When OTA and CIT, or OTA and FB are simultaneously present, the decrease of viability (measured by MTT test) of human kidney cells (HK2) induced by OTA is considerably enhanced. Expression of biotransformation enzymes (CYP, COX, LIPOX) in HK2 is differently modulated depending of the treatments: CIT alone, OTA alone or both together. Induction of COX2 and LIPOX by CIT increased genotoxicity of OTA measured by DNA adducts formation detected by P32 post-labeling. DNA adducts patterns of rat kidney after a 3-weeks feeding by either CIT alone or OTA alone or both together, are similar to those obtained on cell cultures.

The main OTA DNA-adduct, found in human tumours, identified as C8 dG-OTA is increased by simultaneous presence of CIT and OTA. In the same way, in in vivo studies on rat and pig fed simultaneously by OT and FB in feed we observed increased OTA specific DNA adducts including C-C8dG OTA adduct and the both OTHQ related adduct. Both toxins (OTA and FB) modulate the arachidonic acid cascade and induce c-jun. These specific adducts are found in human urothelial tumours, notably in Balkan regions. This data is particularly interesting as we previously shown that families suffering BEN eat food more frequently contaminated by OTA and CIT.

In cell as in blood and kidney of human and rat we isolated OTA and several OTA derivatives identified by HPLC ms/ms, including quinone OTA (OTHQ), GSH-OTA, GSH-OTHQ, DC-OTHQ. The data indicate clearly that exposure to low concentration of mycotoxin which is considered as safe when they are present together can lead to dramatic effect. Until now, regulation does not take into account co-contamination.

Granted by the 'Région Midi-Pyrénées, program Food safety 2003-2008', ARC 2005-2007; Ligue National Française contre le cancer, 2006; EU 'Ota risk assessment' 2002-2005.

11:30-12:00 EXPOSURE OF INFANT[1]S TO THE MYCOTOXIN OCHRATOXIN A THROUGH HUMAN MILK

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Ochratoxin A (OTA) is a mycotoxin produced by some species of the genera *Aspergillus* and *Penicillium* with a widespread occurrence in food and feed [1]. The main route of exposure to OTA is through the intake of contaminated food and levels of the mycotoxin can be assessed in biological fluids, i.e. plasma, urine and human milk [2, 3]. Based on the nephrotoxicity of OTA in several species and its possible carcinogenic properties, limits for food and feed have been set, and also tolerable daily intakes (TDI) values have been proposed. Since human milk is the ideal form of nutrition for infants, the aim of this study was to assess the exposure of infants to OTA with human milk.

In a small cohort nine mother-child pairs were asked to provide milk and urine from their babies up to six days after the delivery. Liquid-liquid extraction with chloroform was used for sample clean-up, and analysis was performed using HPLC with fluorescence detection and/or tandem mass spectrometry detection (Limit of Quantification 50 ng/L) [3].

OTA was present in 100% of human milk samples, with a mean level of 117.4 ± 40 ng/L. Additionally, all infant urine samples were tested positive for OTA and metabolites, confirming the exposure of breast fed infants to OTA. Calculation of OTA-intakes with human milk showed that, even at low OTA levels, the TDI set for adults (5 ng/kg-bw/day) can be exceeded. Thus, exposure of infant to OTA may pose a possible health risk for the developing infant.

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Acknowledgment

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12:00-12:30 ISOLATION AND CHARACTERIZATION BY LC MS/MS OF C8- 2'-DEOXYGUANOSINE OTA-ADDUCT FROM KIDNEY DNA OF RAT AND HUMAN EXPOSED TO OTA. CORRELATION WITH OTA METABOLITES IN BIOLOGIC FLUIDS

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Ochratoxin A is a nephrotoxic and carcinogenic mycotoxin, involved in Balkan endemic nephropathy (BEN) and associated urinary tract cancers. Conflicting results have been obtained regarding the genotoxicity of OTA and its ability to react directly with DNA upon oxidative bioactivation to yield covalent DNA adducts (1, 2). Using high sensitive method of DNA adduct postlabeling, we previously demonstrated by comigration with authentic standard the presence of covalent OTA DNA adducts (3). The aim of this paper was the LC/ms/ms identification of covalent OTA adduct in *in vivo* samples.

For this purpose, the first step was to form OTA guanine adducts by *in vitro* incubation of DNA from salmon testis in presence of OTA and activation. The purified DNA has been hydrolysed in the conditions used for postlabeling. Large amount of the OTA DNA adducts after phosphorylation have been collected and purified. The diphospho dG OTA has been converted into dG-OTA nucleoside, and then analysed by LC ms/ms in view to define the sensitivity of the method. In a second time we purified OTA-DNA adducts from kidney of Lewis and Dark Agouti rat fed OTA (subchronic study and carcinogenic study).

One adduct has been identified as C-C8 dG OTA. These DNA adducts are found in kidney tissue from human suffering Balkan endemic nephropathy and urinary tract tumours as well as in rat fed OTA. Like other chlorinated phenols, OTA undergoes an oxidative dechlorination process to generate a quinone (OTQ)/hydroquinone (OTHQ) redox couple that may play a role in OTA-mediated genotoxicity. To determine whether the OTQ/OTHQ redox couple of OTA contributes to genotoxicity, the DNA adduction properties, as evidenced by the 32P-postlabeling technique, of the hydroquinone analog (OTHQ) have been compared to OTA in the absence and presence of metabolic activation (pig kidney microsomes) and within human kidney cells. OTHQ generates DNA adduct in the absence of metabolic activation. While OTA does not interact with DNA in the absence of metabolism but the OTQ-mediated DNA adduct noted with OTHQ are also observed with OTA following activation with pig kidney microsomes. Comparison of DNA adduction by OTHQ and OTA in human cell lines shows that OTQ-mediated adduct form in a dose- and time-dependent manner. The adduct form at a faster rate with OTHQ, which is consistent with more facile generation of OTQ from its hydroquinone precursor.

In another hand, we identified by ms/ms the OTA derivatives in blood and urine of rat and human exposed to OTA. Ochratoxin B (OTB), open-ring ochratoxin A (OP-OA), 4hydroxylated OTA, 10 hydroxylated OTA, OTalpha, OTbeta, two different OTHQ metabolites (quinone form) and GSH-OTA derivatives could be identified by nano-ESI-IT-MS. As for DNA adduct, the repartition of the metabolites are dependent of the sex and could be related to genetic polymorphism.

Altogether these data clearly shown that OTA is a complete carcinogen, and determination of OTA DNA adduct is a relevant biomarker of exposure.

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12:30-13:20 TOWARDS UNDERSTANDING SIGNIFICANCE OF OCHRATOXIN A IN HUMAN HEALTH

Keynote conference

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It will soon be half-a-century since the first exploration of toxic *Aspergillus ochraceus* in South Africa led to isolation of ochratoxin A (OTA) and its characterisation to become one of the founding fathers of mycotoxicology. Subsequent recognition of its natural involvement in seasonal outbreaks of porcine nephropathy in Denmark, of considerable economic importance though mediated by a *Penicillium* mould spoiling poorly-stored cereals in northern latitudes, raised some concerns for human health. These concerns were then accentuated in the 1990's after a US NTP study showed OTA to be the most potent renal carcinogen for the male rat. The International Agency for Research on Cancer then quite reasonably classified it as a possible human carcinogen, although neither before nor since has it been shown conclusively to have caused any human morbidity. Subsequently, some food safety regulatory authorities have placed statutory limits on OTA in foodstuffs and food products of an order similar to those for aflatoxin B_1, which is well-recognised as an actual human carcinogen. Concurrently, a large literature on natural occurrence, usually in very small amounts, in human foodstuffs has followed availability of increasingly sophisticated analytical methodology, though with variable validation of accuracy and comparability between laboratories. In contrast to the rather high OTA concentration, sometimes clinically nephrotoxic in commercial pig production and unwittingly associated with poor storage of animal feed in times past, human foodstuffs usually have very low OTA content, much less than is necessary to manifest morbidity in long-term experiments in animals. The mis-match for OTA between some experimental animal toxicological data and the absence of any human clinical data is a challenge for toxicological research.

Contributary to the present dilemma is the reasonable ethical attempt in several parts of the world to minimise whole animal experimentation. However, such is not comfortably replaced, particularly concerning nephropathy, by tissue-cultured cells because the functional complexity of rat kidney involves ~ 50,000 independent filtration

systems, each differentiated throughout its length for various physiological functions. Producing rat renal tumours experimentally is tedious and expensive. However, economy in the cost of OTA can be made by limiting the period of continuous contaminated dietary exposure to 9 months during the first year of life. Nevertheless, it is still necessary to keep rats for nearly 2 years in order to find tumours as they develop. There is still no substitute for systematic sampling, probably from about 18 months of age and never yet employed, in order to discover early neoplastic lesions which are preferable for immunohistochemical study before necrosis in maturing tumour changes protein content.

In recent years, excessive research focus has been devoted to the question of whether or not OTA forms covalent adducts with DNA, as a potential mechanism for causing the genetic damage implicit in development of carcinoma. All this has been predicated on an arbitrary regulatory attitude to chemical carcinogens according to whether the carcinogenesis is caused *via* direct binding to DNA or involves some other mechanism. Recent findings which support the direct genotoxicity of OTA will be described. The precise mechanism should be only of academic interest because, if there are competing mechanisms, the actual one may never be known. It is much more important to know how genetic changes are made by OTA in kidney, to become detectable within rat renal carcinomas, and whether they match those in human urinary tract tumours, most of which are of unknown cause. My archive of tumours from recent OTA lifetime experiments in rats is providing a unique source of material for molecular genetic analysis on frozen material and immunohistochemical probing of wax-embedded tissues, as a basis for comparison with human material. Recent findings will be described and the implications discussed. In addition, DNA ploidy distribution in rat renal adenomas and carcinomas will be described and compared with the situation in a range of human urinary tract tumours. The objective of these studies is to help decide whether the rat is a good model for humans in the debate about significance of dietary OTA in human health and the appropriateness of regulatory measures.

Session 6: Genomic analysis of Mycotoxins.

16:30-17:20 GENOMIC ANALYSIS PROVIDES NEW INSIGHTS INTO MYCOTOXIN PRODUCTION BY *ASPERGILLUS FLAVUS*

Keynote Conference

Gary A. Payne*

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Background: *Aspergillus flavus* is capable of producing several known mycotoxins. The best characterized are aflatoxins, cyclopiazonic acid (CPA), and aflatrem. The analysis of the *A. flavus* genome sequence and the use of whole genome DNA microarrays have increased our understanding of the potential of *A. flavus* to produce known and uncharacterized mycotoxins (1). Genomic analysis of *A. flavus* has revealed an expansive number of genes predicted to encode a diverse array of secondary metabolites.

Aims: This study assessed the ability of *A. flavus* to produce aflatoxins, cyclopiazonic acid (CPA), and aflatrem as well as other potentially toxic secondary metabolites.

Materials and Methods: Genes for secondary metabolism in fungi are often arranged into specific clusters dispersed throughout the genome (2). A bioinformatics analysis of the genome sequence of *Aspergillus flavus* (www.aspergillusflavus.org) using the Secondary Metabolite Unknown Regions Finder (SMURF; http://www.jcvi.org/smurf) allowed the prediction of 55 gene clusters. This algorithm searches for multifunctional enzymes including nonribosomal peptide synthetases (NRPSs) for nonribosomal peptides, polyketide synthases (PKSs) for polyketides, hybrid NRPS-PKS enzymes for hybrids, and prenyltransferases (PTRs) for terpenoids. All these enzymes have been shown to be involved in secondary metabolism (2,3). The biosynthesis of aflatoxins requires a PKS, aflatrem requires a NRPS, and CPA biosynthesis involves a hybrid NRPS-PKS. In the next step of the analyses, the program searches the surrounding genes for motifs of genes associated with secondary metabolism. We used whole genome Affymetrix DNA microarrays (www.aspergillusflavus.org) to follow the expression of these predicted clusters under 28 experimental conditions, including during the infection of maize seeds (4).

Results: The 55 predicted clusters for secondary metabolism were subjected to hierarchical clustering analysis to group those clusters that displayed similar expression patterns under the 28 conditions examined. This resulted in the separation of expression profiles into four clades, A-D. Based on gene expression patterns, the aflatoxin cluster and the CPA cluster were placed into clade A with six other predicted clusters. This

clade was characterized as having moderate to high levels of gene expression in most of the 28 conditions examined, and contained clusters that are predicted to encode secondary metabolites with diverse function including polyketides, non-ribosomal peptides, pigments, and siderophores. Interestingly, genes in the clusters for aflatoxin and CPA were among the most highly expressed secondary metabolite genes during infection of maize seeds. CPA was produced under all conditions favorable for aflatoxin production, as well as several conditions that did not support high concentrations of aflatoxin. In general, CPA production appeared to be less sensitive to environmental conditions than aflatoxin production. The other three clades (B-D) contained clusters whose genes were lowly expressed across almost all experimental conditions. Within clade D was cluster 15, one of the clusters necessary for the synthesis of aflatrem. Aflatrem is produced by two biosynthetic clusters (5). Expression cluster 15 genes required LeaA, a global regulator of secondary metabolism (3).

Conclusions: Genome analysis of *A. flavus* and whole genome transcriptional profiling under several environmental conditions showed the potential for *A. flavus* to produce a broad range of secondary metabolites, some of which are known mycotoxins. Two mycotoxins, aflatoxin and CPA, were produced under a wide range of conditions, and CPA was produced in all conditions conducive for aflatoxin production. Further, CPA was produced under a wider range of environmental conditions than aflatoxin. Thus maize contaminated with aflatoxin is also prone to contamination with CPA if colonized by a strain of *A. flavus* that produces CPA. We observed only low expression of the genes for aflatrem, indicating that contamination of food and feed with this mycotoxin is less likely. While we observed the expression of many clusters predicted to produce secondary metabolites in our study, expression of most of these clusters was low. This may indicate that some of the clusters are silent as has been observed in other fungi (6) or that we did not grow the fungus under conditions conducive for their transcription. Regardless, given the potential for mycotoxigenic fungi to produce secondary metabolites, we must always be alert for the presence of a new toxin in our food sources.

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17:20-17:50 GENES AND ENZYMES OF THE PATHWAY FOR FUMONISIN DEGRADATION OF *SPHINGOPYXIS SP*. MTA144

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Background: Biodegradation of fumonisins may be a feasible approach for detoxification of feed and food. Microbes that can break down fumonisins have previously been described. We isolated a fumonisin degrading strain, *Sphingopyxis sp*. MTA144, from a soil sample.

Aims: The aim of this work was to study the enzymes encoded in the *fum* gene cluster of *Sphingopyxis sp*. MTA144 with respect to their possible application for enzymatic fumonisin detoxification of feed and food.

Materials and Methods: The genome region containing the *fum* gene cluster was sequenced. The *fumD*, *fumH* and *fumI* genes were cloned and expressed in *Pichia pastoris* or *E. coli*. The recombinant enzymes were purified and characterised.

Results and Discussion: Genes associated with fumonisin catabolism were located in a cluster spanning 16 kb of the genome of *Sphingopyxis sp.* MTA144. The genes were found to be organised in two operons controlled by a bidirectional promoter, presumably regulated by two transcriptional regulators, FumB and FumC. Three transmembrane permeases or transporters were found, encoded by *fumA*, *fumG* and *fumJ*. The *fumD* gene, encoding a fumonisin carboxylesterase, was expressed in *Pichia pastoris* and *E. coli*, and found to catalyse the hydrolytic cleavage of both tricarballylic acid side chains from fumonisin B1 (FB_1). The recombinant FumI enzyme, an aminotransferase, recognised hydrolysed fumonisin B_1 (HFB_1), but not intact FB_1, as substrate. The preferred acceptor of the amine group from C2 of HFB_1 was pyruvate. The presumed reaction products are 2-keto HFB_1 and alanine. Substrates of the enzyme encoded by the *fumH* gene, an alcohol dehydrogenase, were both HFB_1 and 2-keto HFB1. The enzyme showed a strong preference for one of the five available hydroxyl groups. Structure determination of the reaction products by NMR is in progress. Kinetic parameters of the enzymes were determined, and activities were correlated with temperature, pH and co-substrate concentration. The Michaelis constants K_M were low enough for the enzymes to be active in the range of fumonisin concentrations that are typically found in contaminated maize.

Conclusions: An application of recombinant enzymes derived from the *fum* gene cluster of *Sphingopyxis sp.* MTA144 for detoxification of fumonisins seems feasible.

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17:50-18:20 POPULATION GENETIC STUDY OF Gibberella zeae (*Fusarium graminearum*) ISOLATED FROM WHEAT IN URUGUAY.

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Background: In Uruguay, *F. graminearum* is the most common species that infects wheat and is responsible for Fusarium head blight and contamination of grain with deoxynivalenol (DON). The development of measures to control this disease makes it necessary to know the population genetic structure of *F. graminearum* to understand the epidemiology and evolutionary potential and to improve strategies to control this fungal pathogen.

Aim: The aim of this study was to determine the genetic structure of populations of *F. graminearum* isolated from wheat in Uruguay.

Materials and Methods: The presence of *Fusarium* spp. was evaluated in 80 samples of wheat harvest during 2003/2004 from different parts of Uruguay. Of a total of 144 strains identified as *F. graminearum*, 61 strains were evaluated by AFLP markers using the methodology proposed by Vos et al. (1995) and modified by Leslie & Summerell (2006). Three primer-pair combinations with two selective nucleotides on each primer were used: EcoRI + AA / MseI + AT, EcoRI + CC/ MseI + CG and EcoRI + TG / MseI + TT. The EcoRI primer was labeled with γ33P for detection of bands by autoradiography.
We included strains identified as belonging to *F. graminearum* lineages 1-7 (O'Donnell et al. 2000) as standards on each gel. We manually scored the presence or absence of polymorphic AFLP bands in each of the strains tested and recorded the data in a binary, present/absent format. The genetic similarity was estimated using the SAS software, version 6.11 (SAS Institute, Cary, NC) taking into account all pairs of the strains tested according to the Dice coefficient (Dice 1945). Dendograms were prepared by using the UPGMA algorithm in NTSYS Sahn version 2.01 (Rohlf, 1990). The AFLP data were subjected to bootstrap analysis with 1000 replications with PAUP program version 4.0 (Swofford, 1998) to determine if the was significant genetoic substructure or clustering among isolates as they were resolved by the AFLPs.

Results and Discussion: A total of 151 AFLP bands were identifided in the 200-600 pb range from the 61 analysed isolates when using the three primer pair combinations. The analysis of profiles generated by AFLP revealed the presence of 151 loci from the 61 strains. The primer combination EcoRI + CC and MseI + CG amplified bands of 45, while the combination EcoRI and MseI + TG + TT and EcoRI + AA and MseI + AT amplified bands 79 and 27, respectively. The genetic similarity between isolates of *F. graminearum* analyzed was 72.5% (range = 47.7 - 93.5%). The genetic similarity coefficients indicate that any of the isolate belonging to the same species are greater

than 0.60 (60%). In the present study we included strains identified as belonging to *F. graminearum* lineages 1-7 in order to obtain the standard AFLP profiles for subsequent comparison with our strains. All isolates analyses had AFLP profiles typical of F. graminearum lineage 7. Knowing the lineage of F. graminearum that prevalent in Uruguay provides information on the origin of our population, as well as the potential route of entry of this pathogen. This knowledge could prevent the inadvertent introduction of foreign populations.

Conclusions:

- The average genetic similarity among isolates of *F. graminearum* was 72.5%.
- All strains of *F. graminearun* belong to lineage 7.

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Yucatan II

Thursday July 1, 2010

Session 7: FAO Forum/SLAM
Food safety risk assessment of chemical contaminants.

08:30–12:00 APPLYING RISK ANALYSIS TO MYCOTOXIN CONTROL AND THE CODEX ALIMENTARIUS STANDARD SETTING PROCESS

Keynote Conference

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The presentation will demonstrate the use of risk analysis as the basic framework for applying a modern strategy for prevention and control of mycotoxins and in the standard setting process.

A number of governments in developing countries are already taking steps to improve and strengthen their systems for food safety management and for addressing mycotoxin problems. Several are moving away from the traditional approach focused on end-product control towards a preventive and science-based approach.

Risk analysis is an important part of a science-based approach to food safety. It provides a means to strengthen the ability of traditional food safety systems to meet current challenges by providing a framework to effectively manage, assess and communicate risks in cooperation with the diverse stakeholders involved. Risk analysis has been defined by the Codex Alimentarius as a process consisting of three components: risk assessment, risk management and risk communication. The three components of the risk analysis framework are interdependent and none can occur independently of the other. Interaction between the components is essential if the risk analysis is to be effective.

A science-based approach to food safety is not a completely new concept. It is related to processes such as good agricultural practices, good hygiene practices, good manufacturing practices and HACCP, which are already used in many countries. What is new is the use of risk analysis as a framework to view and respond to food safety problems in a systematic, structured and scientific way to enhance the quality of decision-making throughout the food chain, including in the elaboration of standards such as those of the Codex Alimentarius. A science-based approach strengthens the capacity of traditional food safety systems to meet current challenges and improve the availability of safe food. Scientific evidence can be used to minimise the occurrence of food-borne hazards, including mycotoxin contamination, to reduce and manage risk, and to improve the outcomes of decision-making. This approach enhances the ability of food safety regulators to: identify hazards; characterise the nature and extent of those hazards; assess exposure to the identified hazards; and estimate the likelihood of the

resulting risks and potential impact on human health, and also to set standards accordingly.

Risk analysis provides food safety regulators with the information and evidence they need for effective decision-making. The process often begins with risk management, which defines the problem, articulates the goals of the risk analysis and defines the questions to be answered by the risk assessment. The science-based tasks of 'measuring' and 'describing' the nature of the risk being analysed (i.e. risk characterization) are performed during the risk assessment.

Risk management and assessment are performed within an open and transparent environment based on communication and dialogue. Risk communication encompasses an interactive exchange of information and opinions among risk managers, risk assessors, the risk analysis team, consumers and other stakeholders. The process often culminates with the implementation and continuous monitoring of a course of action by risk managers. Mycotoxins are natural contaminants and pose a particularly difficult issue for the risk assessor. Natural contaminants of food are usually considered to be unavoidable and are regulated differently than avoidable synthetic chemicals. Because they are not avoidable, small amounts can be legally allowed if it can be shown that the permitted levels are not injurious to human or animal health. Natural chemical contaminants that are produced by biological organisms are usually considered independently of the producing organism with regards to the hazard identification and characterization. Nonetheless, the hazard posed by the producing organism can often be of use for hazard identification before the chemical contaminant is characterized. In addition, the producing organism is an important factor in risk management since control strategies targeting the producing organism may prove to be the most effective way to reduce the levels of the contaminant in food.

Understanding the environmental and physiological factors that control the production of the natural contaminant is critical in predicting under what conditions exposure will be high and in developing control strategies. Because natural contaminants often occur in basic commodities and may be found in export commodities, the health risk posed by exposure to the contaminant must be considered relative to the possible adverse impact on food availability and other economic impacts in the setting of standards.

Together with risk assessment and risk communication, **risk management** is an essential component of risk analysis. Risk management plays a key role at the beginning of the risk analysis process in identifying food safety problems and considering the best ways to manage them. The consideration of different policy alternatives is a critical part of risk management. This requires a focus on the scientific aspects of the risk (i.e. the detail and the outcome of the risk assessment) as well as any associated legal, ethical, environmental, social, economic and political factors that are important to people. Risk management therefore must be carried out in consultation with interested stakeholders and in synergy, during the entire process, with **risk communication** activities. The comprehensive assessment of all the available management options that result from this process ensure that decision-makers make informed decisions on the most appropriate prevention and control options.

Countries need to develop their own risk management strategy and prevention and control procedures for mycotoxins and harmonize their standards to Codex.

CAC. 2003. Draft Working Principles for Risk Analysis for Application in the Framework of the Codex Alimentarius. Appendix IV. ALINORM 03/33a

09:30-10:00 RISK ASSESSMENT FOR THE B- AND C-SERIES FUMONISINS IN MAIZE AND MAIZE-BASED BEVERAGES IN RURAL AFRICAN COMMUNITIES

Keynote conference

Gordon S. Shephard*[1]

with Liana van der Westhuizen[1], Hester-Mari Burger[1], Jaco van Zyl[1], John P. Rheeder[1], Dirk J. van Schalkwyk[2]

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Background: The fumonisin mycotoxins are produced in maize by *Fusarium verticillioides* and *F. proliferatum*. They cause leukoencephalomalacia in horses and pulmonary oedema in swine and are hepato- and nephrocarcinogenic in rodents. The main concerns for human health are their association with oesophageal cancer and neural tube defects. Exposure to fumonisins in human populations is mainly through consumption of maize and maize-based food products. In many African populations, maize is a dietary staple and frequently consumed at levels of 400-500 g/person per day. The most abundant fumonisins are those of the B-series (FB_1, FB_2 and FB_3). Little is known of the natural occurrence of the C-series (FC_1, FC_3 and FC_4) and their possible contribution to fumonisin exposure.

Aim: To undertake a risk assessment for exposure to the B-series fumonisins in 2 rural African districts (Centane and Bizana in the former Transkei region of South Africa) using maize meal consumption data and to quantify the additional contribution to fumonisin exposure posed by the C-series fumonisins and also the consumption of maize-based beverages.

Materials and Methods: Field workers visited individual households in Centane (n=68) and Bizana (n=93) and interviewed the householder responsible for food preparation with respect to the type of dishes prepared, number of meals cooked per day, the number of meals that were maize-based, the number of people participating in the meals and fate of left over food. Weighed food records were taken for each member of the household. Participants were divided into children (1-9 years; assumed mean body weight 20 kg), adolescents (10-17 years; assumed mean body weight 50 kg) and adults (18-65 years; assumed mean body weight 60 kg). The exposure assessment was based on individual maize consumption figures and a mean of total fumonisin (FB_1 + FB_2) contamination in home-grown maize from the two districts (1142 µg/kg in Centane and 542 µg/kg in Bizana). The risk was characterized in terms of the provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg body weight per day proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The relative contribution of C-series fumonisins to the PDIs was assessed by the relative level of contamination of B- and C-series fumonisins as determined by their LC-MS/MS quantification in home-grown

maize collected over one harvest. Additional fumonisin exposures from the consumption of a non-alcoholic lactic acid fermented maize-based beverage (marhewu) and of maize-based beer (umqombothi) were assessed.

Results and Discussion: Children and adolescents had mean maize consumptions of 246 and 368 g/person per day without a significant difference ($p>0.05$) between the two districts. Adults had significantly different ($p<0.05$) mean maize consumptions in the Bizana and Centane districts (379 and 456 g/person per day, respectively). The exposure assessment based on combined FB contamination levels indicated that mean probable daily intakes (PDIs) for all three population groups were significantly ($p<0.05$) higher in Centane than in Bizana. Mean PDIs for infants, adolescents and adults in Centane were 14.14, 8.33 and 8.67 µg/kg body weight per day and in Bizana were 6.60, 4.05 and 3.43 µg/kg body weight per day, respectively.

These means are all above the PMTDI of 2 µg/kg body weight per day set by JECFA. In Bizana, 77% of the study population had PDI values above the PMTDI, whereas all participants in Centane were above this value. The highest PDI values were determined in children, being a function of their lower body weights. Determination of fumonisins by LC-MS/MS analysis of home-grown maize collected in Centane and Bizana indicated the co-occurrence with the B-series fumonisins of the C-series analogues, FC_1, FC_4 and, to a lesser extent, FC_3. A significant ($p<0.05$) linear correlation was observed between total B-series ($FB_1+FB_2+FB_3$) levels and total C-series levels ($R^2=0.972$) in which the total FC contamination levels were 5% of the total FB levels. By implication, if FC were to be considered as part of the risk assessment, PDI values for fumonisins would be 5% greater than calculated based on FB contamination levels. The above exposure assessment was conducted on the basis of maize food intake, whereas rural African communities also use maize for various maize-based drinks, particularly marhewu and umqombothi in these communities. Marhewu is a popular beverage with a mean intake of 474 mL/day per person. However, its fumonisin contamination is relatively low (mean 20 µg/L), which implies a PDI contribution of less than 0.2 µg/kg body weight per day in adults. On the contrary, maize beer (umqombothi) is consumed irregularly by only part of the community.

Mean total fumonisin B levels in Transkeian umqombothi were found to be 369 µg/L, while mean consumption levels were found to be highest in men at 112 mL/day, increasing their PDI by 0.7 µg/kg body weight per day. However, beer drinking is frequently a sporadic event resulting in acute high fumonisin exposures.

Conclusions: Rural African communities using maize as a dietary staple are widely exposed to fumonisins at levels exceeding the PMTDI due to their reliance on home-grown maize and high daily maize consumption levels. The additional practice of consuming maize-based beverages, either non-alcoholic on a regular basis or maize beer at irregular intervals may increase this food safety risk.

10:00-10:30 RISK ASSESSMENT AND MYCOTOXIN LEGISLATION IN BRAZIL.

Keynote Conference

Myrna Sabino

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Brazil is a tropical country where the climate favours the growth of various species of mycotoxin-producing fungi. Agricultural products and related products offered for export have been rejected by importing countries.

Among the countries of Latin America, Brazil is the most motivated and structured in mycotoxins and Brazilian research responds to the needs of the country, reflecting international concerns and recent developments in this area.
Incidence of mycotoxins and analytical methodology has continued to receive the most attention however there is a considerable increase in research of mycotoxins on prevention, control and processing effects.

A big group (researchers from many Institutes and Universities) is working in technological innovation: Food Safety production of Immuno-Reagents for Quality Control in Food and water.

The mycotoxins of major risk in Brazil are aflatoxins in peanuts, Brazil Nuts and fumonisins in corn, spite of there are many programmes on Ochratoxin A in coffee, Don in wheat, AFM1 and ochratoxin A in human milk, diet total and biomarkers.

In Brazil, the Ministry of Health has established limits for aflatoxins, B,G and M1 in industrialized products destinated for humans. The Ministry of Agriculture,has established limits for aflatoxins in Livestock, Supply for animal feeds, and agricultural products, among other commodities (food and feeds).

At present the Ministry of Health have been discussing a new legislation suggesting reduction in the maximum legal limits in many mycotoxins in products for foods for human consumption. These new limits are similar to EC.

Our main objective is to prevent and control adverse effects of mycotoxins.
Both the public and private sectors in Brazil have been making a concerted effort to control mycotoxins in foods and feeds consumed by humans and animals.

10:30-11:00 EUROPEAN UNION: RISK BASED REGULATIONS SAMPLING AND IMPORT CONDITIONS FOR MYCOTOXINS

Marina Miraglia
with Carlo Brera

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Since the adoption of the white paper on food safety (2000), the EU has rather coherently, explicitly and exhaustively taken up a policy for food safety mainly based on the needs of assuring a high level of food, animal and plant health through coherent farm-to-table measures and a science-based risk management. Additional basic needs include efficient functioning of the internal market, effective control systems, and relations with third countries and international organizations.
In order to implement those targets, horizontal and vertical legislative instruments have been issued.

Regulation(EC) 178/2002 represents the basis of the food law since it reaffirms the principles of risk analysis, institutes EFSA (European Agency for Risk Assessment) and provides the general terms for the Precautionary Principle and for traceability.
Also the EU Official Control System is rather well structured in order to assure the compliance with feed and food law. The system, regulated by the horizontal EC regulation 882/2004, requires to the Member States national control plan to be carried out regularly , on a risk basis and with appropriate frequency.

In addition, Community and National Reference laboratories are established. The Italian National Reference Laboratory (NLR) for mycotoxins is the Italian National Institute for Health.

As for contaminants the EU policy is, as stated in EC Regulation 315/93/EEC, to exclude from the market food with unacceptable level of contaminants, that should be kept as low as reasonably be achieved. In addition the EU policy is to fix maximum tolerable levels for certain contaminants.
Mycotoxins have received increasing attention since 90ties. Actually the first maximum tolerable level laid down was in 1976 for AFB1 in feed, mainly in order to limit the level of AFM1 in milk.

For the time being a rather exhaustive legislation package for mycotoxins has been laid down , even though additional provisions for additional toxins and matrices are in the pipeline. Maximum levels have been fixed for the main mycotoxins (aflatoxins, ochratoxin A, patulin, zearalenon, deoxynivalenol, fumonisins) in the more risky food matrices (cereals, nuts, coffee, fruit juice, wine, spices, liquorice). Special limits are also fixed in food intended for infants and children.

Special rules at import are imposed for certain food from certain third countries, the list of those food and countries being continuously updated on the basis of the new knowledge.

Finally the EU greatly accounts for prevention policy for mycotoxins. Guidelines for prevention have been issued for *Fusarium* toxins and for patulin; in addition in the European research framework emphasis and funds have been devoted to the development of research projects aimed at studying prevention measures for mycotoxins.

11:00-11:30 CLIMATE CHANGE: THE EFFECT OF CLIMATE CHANGE ON MYCOTOXIN CONTAMINATION OF FOOD: AN EMERGING RISK.

Keynote Conference

Marina Miraglia*
with Barbara De Santis and Carlo Brera (Italy)

Climate change is envisaged as a priority among the emerging risks for human health. During the past few decades sound international scientific literature on the issue has been published. Among the most important and accredited reports published, the UN's International Panel on Climate Change in its Fourth Report (IPCC, 2007; Solomon et al., 2007) include many projections, statements and conclusions, relevant for human health, often quoted on the basis of their likelihood.

The report states that: "Warming of the climate system is unequivocal, as is now evident from observations of increases in global average air and ocean temperatures, widespread melting of snow and ice and rising global average sea level" and that "Some systems, sectors and regions are likely to be especially affected by climate change", the last statement stressing the wide differences in climate changes that will be encountered by geoclimatic "blocks" worldwide geographic area.

Agricultural production systems are complex and extremely susceptible to climatic change, the effect and the susceptibility being however widely different depending on the on the regional and local ecosystem interactions and patterns together with individual ability to respond to different climatic pressures. When considering agriculture and climate change in a global perspective, each agricultural ecosystem could experience positive or negative impact depending on the peculiar climatic change and, moreover, due to the globalization of trade, effects and consequences of climate change on local agricultural systems may virtually have an impact on regions of the globe far away.

It is expected that climate change may influence in many respects the world's food supply system, and food safety with significant influence also on food security due to increased yields at higher latitudes and to decrease at lower latitudes.

Toxigenic fungi attack on plants is one of the more risky climate dependent biotic plant disease, and it is also influenced by non-infectious factors, such as insect damage, other pests attack and bioavailability of (micro) nutrients: this factors are that are in turn driven, therefore, susceptible to climatic conditions. Toxigenic fungi may lead to the production of mycotoxins, highly toxic chemical substances; and climate represents the key agro-ecosystem driving force of the fungi colonization and diffusion. Also the mycotoxin biosynthesis is climate dependent and influenced by peculiar agro-ecosystem conditions.

This contribution aims at addressing the impact of climate change on one of the most crucial issue of food safety, namely mycotoxin contamination.

Yucatán III

Tuesday June 29, 2010

Session 8: Mycotoxins in food and commodities.

08:30-09:00 MYCOTOXINS IN FOOD FOR DIRECT CONSUMPTION IN CHILE

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Background: ***Mycotoxins are substances produced*** from fungal secondary metabolic processes that can be naturally found in various agricultural harvests. These metabolites can contaminate food, feed, or the commodities used for their elaboration, producing a group of diseases known as mycotoxicosis, toxic syndromes which can affect both humans and animals. Diverse matrixes, i.e. peanuts, corn, cotton, soy, cereals and its by-products, different nuts and fruit juice, milks and milk products, meat, leaver, etc can be affected by them. In Chile, the legislation has only set amount limits for Aflatoxins (5 ppb), Aflatoxin M1 (0.05 ppb) and Zearalenone (200 ppb).

Aim: Determine the presence and quantify the quantity of different mycotoxins: Aflatoxins, Patulin, Zearalenone, Ochratoxin A, Nivalenol, Deoxynivalenol and T2 toxin in food for direct consumption in Chile.

Material and Methods: 305 analyses were performed. 155 samples, collected from supermarkets from the metropolitan region and other regions by their respective Regional Ministerial Secretary, were selected. The samples analysed consisted of nuts fruits, various fruit juices, and maize, weath and rice products, like flour and breakfast cereals. The extraction method used for Aflatoxins was based in the use of multifunctional columns. For Patulin, liquid extraction was performed. For Nivalenol, Deoxynivalenol and T2 toxin, SAX ionic exchange columns were performed. For Zearalenone and Ochratoxin A, inmunoaffinity columns were used. The analytical methods used were the ones proposed by the Japanese expert, Dr. Hisashi Takeda, according to the “Food Safety Enforcement Program” project between the Chilean Ministry of Health, the Japanese International Cooperation Agency (JICA) and the Public Health Institute of Chile. Mycotoxical quantification was performed using high performance liquid chromatography (HPLC), except for T2 toxin, which was quantified using LC/MSMS.

Results and Discussion: For Aflatoxins, in all the analyzed samples (79) which were nut fruits, maize, rice and weath products, only one was positive, nevertheless, the quantity found was far superior to the allowed by Chilean legislation (33.3 ppb imported japanese type peanuts). For Patulin, from the 40 juice fruits, 19 were positive, been one superior to the limit of 50 ppb established by the Codex Alimentarius (175 ppb Apple juice of internal production). For the T2 toxin, from 34 analyzed maize, rice and weath

products samples, none of them showed detectable results. For Nivalenol and Deoxynivalenol, from 24 analyzed samples for each mycotoxin, none of them was positive for Nivalenol, and one was positive for Deoxynivalenol. The mycotoxin found in this sample was below the allowed limit for this mycotoxin according to Codex Alimentarius (94 ppb breakfast cereal). For Zearalenone, from a total of 37 samples of rice, maize and weath, 4 samples were positive, been the results between 4,8 to 86,1 ppb, below the Chilean legislation limit of 200 ppb. Finally, for 88 samples of maize, wheat and rice products analyzed for Ochratoxin A, 20 samples were positive; from them, only a rice sample was above the allowed Codex Alimentarius limit of 5 ppb. Those samples who present values above national or international regulations, was confirmed by LC/MSMS.

Conclusion: From the total of analysis performed (305), 45 samples were positive, and from them, 3 were above the international limits established by Codex Alimentarius. Some of these mycotoxins who presents values superior to the allowed by this regulation, don't have allowed limits in the Chilean legislation, this reflects the need to consider include on it.

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1.3 A rapid multiresidual determination of type A and type B trichothecenes in wheat flour by HPLC-ESI-MS. Food Additives and Contaminants, March 2005, 22(3): 251-258

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09:00-09:30 AISLAMIENTO DE ESPECIES DE *Fusarium* Y DETECCIÓN DE FUSARIOTOXINAS EN ALIMENTOS PARA CONSUMO HUMANO EN LA CIUDAD DE PANAMA

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RESUMEN

Se realizó un estudio en 170 muestras entre cereales, harinas y plátanos (35 maíz en granos, 35 arroz, 30 avena en hojuelas, 55 muestras de harina de trigo y 15 de plátanos) obtenidas en lugares comerciales de la Ciudad de Panamá entre 2005 al 2008. Las muestras fueron sembradas en medio de cultivo Csapek-Dox con suplemento de peptona (CDSP) y Papa Glucosa-Dicloran (P.G.D). Los medios de cultivos fueron incubados por 10 días en aerobiosis. Las colonias compatibles con ***Fusarium*** fueron aisladas en Agar de Malta y clasificadas en especies, por características taxonómicas. De las 170 muestras estudiadas 37 muestras (21.7%) presentaron contaminación por ***Fusarium.*** De las 37 muestras positivas para ***Fusarium***, 26 (70.2 %) se aislaron del maíz, 10 (27%) de los plátanos y 1 (2.7 %) de la harina de trigo***.*** Las especies aisladas fueron: ***F. verticillioides, F. moniliforme, F. semitectum, F. nivale, F. sporotrichioides y F. oxysporum.*** Las 170 muestras analizadas por la técnica de Cromatografía de Capa Fina (TLC), para la detección y cuantificación de ZEA, DON y MON. Un total de 10 muestras (5.8 %) resultaron positivas para fusariotoxinas. Seis muestras de harina resultaron positivas para DON, 3 muestras de maíz para zearalenona y 1 muestras de plátano para MON. Las concentraciones DON en harina de trigo fueron de 12 µg/g (12 ppm) a 70 µg/g (70 ppm). Las de Zearalenona en las muestras de maíz fueron entre 35 µg /g (35 ppm) a 90 µg /Kg (90 ppb) y la de MON en la muestras de plátanos fue de 120 µg /Kg (120 ppb). N o se detectó niveles de DAS ni T-2*.*

INTRODUCCIÓN

El género ***Fusarium*** comprende un grupo de hongos filamentoso, pertenecientes a la clase deuteromicetes que tiene una distribución universal (ubicuos) y con gran importancia económica ya que son habituales fitopatógenos. Su amplia distribución se atribuye a su capacidad para crecer en gran número de substratos y a su eficaz mecanismo de dispersión; el viento y la lluvia juegan un importante papel en su diseminación. Especies de este género se encuentra en el suelo y en la microflora normal de alimentos como maíz, trigo, cebada, soya, arroz, papa, plátano, banano, etc. También los miembros de este género son causantes de reacciones alérgicas e infecciones en humanos. Puede entrar al cuerpo mediante inhalación de esporas, ingestión, vía cutánea o inoculación traumática. Las infecciones por el género ***Fusarium*** se incluyen dentro de las hialohifomicosis, esto es, las causadas por hongos oportunistas que presentan hifas hialinas septadas. Además de causar afecciones en animales y humanos puede causar micotoxicosis ya que el género ***Fusarium*** contiene importante especies productoras de micotoxinas como son entre otras: ***F. moniliforme***, ***F, poae, F. sporotrichioides, F. oxysporum, F. solani, F. semitectum, F.***

graminearum, y ***F. nivale***. Algunas especies producen micotoxinas en los cereales y que pueden afectar a la salud de personas y animales si estas entran al organismo por la ingesta principalmente. Entre las principales micotoxinas producidas por especies de Fusarium tenemos: Zearalenona, Toxina T-2, Fumonisinas, toxina HT-2, Deoxinivalenol (DON o vomitoxina), diacetoxiscirpenol (DAS), Moniliformina (MON).

El *objetivo* del presente estudio fue el de aislar e identificar especies del género ***Fusarium*** y determinar la concentración de las fusariotoxinas en las muestras analizadas de cereales en granos, harinas de trigo y plátanos maduros.

MATERIALES Y MÉTODOS:

Estudio Micológico:
Se estudiaron 170 muestras de cereales, harinas y plátanos (35 maíz en granos, 35 arroz, 30 avena en hojuelas, 55 muestras de harina de trigo y 15 de plátanos) obtenidas en lugares comerciales de la Ciudad de Panamá. Estas muestras se sembraron en medio de cultivo Csapek-Dox con suplemento de peptona (CDSP) y Papa Glucosa-Dicloran (P.G.D) y fueron incubadas por siete días en aerobiosis. Las colonias que presentaron morfología compatible con ***Fusarium*** fueron aisladas en Agar de Malta y clasificadas en especies, por características taxonómicas.

Estudio Micotoxicológico:
Las 170 muestras fueron analizadas por la técnica de Cromatografía de Capa Fina (A.O.A.C, 995), para la detección y cuantificación de ZEA, DON y MON. Esta técnica empleada produjo la recuperación de 90% ± 2% ZEA (maíz) y 89% ± 3% DON (trigo) y límites de detección de 20 mg / kg y 15 mg / kg, respectivamente. Para el DAS y T-2 (en maíz) la recuperación fue de 94% ± 3%.*y* de 96% ± 3% correspondientemente.

La técnica de Cromatografía en Capa Fina recomendada por la AOAC
AOAC Official Methods Analysis (1995)

Se analizaron las 170 muestras por Cromatografía en Capa Fina para la detección y cuantificación de ZEA, DON y MON.

Para de detección y cuantificación de DON:

El método siguiente permite cuantificar la presencia de desoxinivalenol en trigo (AOAC 1995).

Extracción. Se añadió 200 mL de acetonitrilo-agua (84+16) a 50 g de harina de trigo y agitó enérgicamente durante 30 minutos. Filtramos y recogimos 20 mL en un vaso.
Purificación. Se preparó una columna con 1,5 g de una mezcla de tierra de diatomeas previamente lavada con ácido, carbón activado y alúmina neutra activada (3+7+5). Aplicamos succión y tapar con lana de vidrio. Agregamos 20 mL del filtrado y cuando alcanzó el ápice del relleno, añadimos los 10 mL de acetonitrilo-agua (86+14) con los que se enjuagó el vaso, continuando la succión hasta que el flujo se detenga. Recogimos los eluatos.
Concentración. Evaporamos el solvente y retomamos en 3 mL de acetato de etilo. Pasamos a un tubo con tapa. Enjuagamos con tres porciones de 1,5 mL de acetato de etilo y agregamos al tubo. Evaporamos a sequedad el contenido del tubo.

Cromatografía. Disolvimos el extracto en 100 mL de cloroformo-acetonitrilo (4+1) y aplicamos alícuotas de 5 y 10 mL junto a 1, 2, 5, 10 y 20 mL del testigo (20 ng desoxinivalenol/mL) sobre la placa de gel de sílice G-60. Desarrollamos con cloroformo-acetona-isopropanol (8+1+1).
Evaporamos bajo campana de extracción de vapores durante 10 minutos.
Revelado. Rociamos con solución hidroalcohólica (1+1) de cloruro de aluminio hidratado al 20%. Observamos bajo luz UV para detectar interferencias. Calentamos la placa en estufa a 120ºC durante 7 minutos y observamos la mancha celeste de desoxinivalenol a un Rf aproximado de 0,6

La Detección de Zearalenona se realizó mediantes la técnica de Thomas and Truckess.
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RESULTADOS:

De las 170 muestras estudiadas 37 muestras (21.7%) presentaron contaminación por ***Fusarium.*** De las 37 muestras positivas para ***Fusarium,*** 26 (70.2 %) se aislaron del maíz, 10 (27%) de los plátanos y 1(2.7 %) de la harina de trigo***.*** Entre las especies aisladas fueron: ***F. graminearum, F. verticillioides, F. moniliforme, F. semitectum, F. nivale, F. sporotrichioides y F. oxysporum.***
En el maíz se asilaron cepas de ***F. graminearum*** (9), ***F. sporotrichioides*** (7), ***F. semitectum*** (6), ***F. moniliforme*** (4).
En el plátano se aislaron cepas de: ***F. oxysporum*** (9) y ***F. verticillioides*** (1).
En la muestra de harina se aisló ***F. graminearum (1).***

Las 170 muestras analizadas por la técnica de Cromatografía de Capa Fina, para la detección y cuantificación de ZEA, DON y MON. Un total de 10 muestras (5.8 %) resultaron positivas para fusariotoxinas: 6 muestras de harina para DON, 3 muestras de maíz para zearalenona y 1 muestras de plátano con MON. No se detectó niveles de DAS ni T-2.

Las concentraciones de micotoxinas resultaron en:
Harina de trigo: 12 µg /g (12 ppm) a 70 µg /g (70 ppm)
Maíz: Zearalenona: 35 µg /g (35 ppm) a 90 µg /Kg (90 ppb)
Plátano: 120 µg /Kg (120 ppb)

CONCLUSIONES

- De un total de 170 muestras de cereales, harinas de trigo y plátanos analizadas, se aislaron especies de género ***Fusarium*** en 37 (21.7%) muestras.
- De las 37 muestras con presencia de especies del género ***Fusarium***
  26 (70.2 %) se aislaron del maíz, 10 (27%) de los plátanos y 1(2.7 %) de la harina de trigo.
- Entre las especies de ***Fusarium*** aisladas fueron: ***F. graminearum, F. verticillioides, F. moniliforme, F. semitectum, F. nivale, F. sporotrichioides y F. oxysporum.***
- En las muestras de maíz se asilaron 9 cepas de ***F. graminearum,*** 7 de ***F. sporotrichioides***, 6 de ***F. semitectum*** y 4 de ***F. moniliforme.***
  En las muestras de plátano (maduro) se aislaron cepas 9 de ***F. oxysporum*** y una de ***F. verticillioides.*** En la muestra de harina de trigo se aisló una cepa de ***F. graminearum.***

- Las 170 muestras analizadas por la técnica de Cromatografía de Capa Fina, para la detección y cuantificación de ZEA, DON y MON. Un total de 10 muestras (5.8 %) resultaron positivas para fusariotoxinas
- 6 muestras de harina para DON, 3 muestras de maíz para zearalenona y 1 muestras de plátano con MON.
- No se detectó niveles de DAS ni T-2.
- Las concentraciones DON en harina de trigo fueron de 12 µg /g (12 ppm) a 70 µg /g (70 ppm).
- Las concentraciones de Zearalenona en las muestras de maíz fueron entre 35 µg /g (35 ppm) a 90 µg /Kg (90 ppb).
- La concentración de MON en la muestra de plátano fue de 120 µg /Kg (120 ppb).

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09:30-10:00 *FUSARIUM* EMERGING MYCOTOXINS IN BREAKFAST AND INFANTS CEREALS FROM MOROCCO: CASES OF ENNIATINS (A, A1, B, B1), FUSAPROLIFERIN AND BEAUVERICIN

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Background: *Fusarium* are a group of fungi associated with grain diseases such as *Fusarium* head blight and foot rot. According to recent knowledge related to fungi, in the field of mycotoxicology, *Fusarium* species produce a new group of bioactive compounds called emerging mycotoxins (enniatins, fusaproliferin and beauvericin and moniliformin) that are not widely and deeply investigated.

Aim: To quantify emerging mycotoxins: enniatins ENs (ENA, ENA1, ENB and ENB1), beauvericin (BEA) and fusaproliferin (FUS) in cereals products from Morocco.

Materials and Methods: 68 samples of cereals products (breakfast cereals and infant cereals) purchased from supermarkets in Morocco were surveyed for the presence of ENs, BEA and FUS. Samples were extracted with a mixture of water/acetonitrile (85/15, v/v) by using an Ultra-turrax homogenizer. Mycotoxins were then identified and quantified with a liquid chromatography (LC) with diode array detector (DAD). Positive samples were confirmed with an LC-MS/MS.

Results and Discussion: Results showed that the frequencies of contamination of total samples with total ENs, FUS and BEA were 32.3, 10.2 and 5.8%, respectively. ENA1 was the most mycotoxin found (37.53-688.36 mg/kg). ENB1 levels varied between 0.61 and 794.84 mg/kg. ENB levels varied from 1.05 and 89.12 mg/kg. ENA contaminated two samples (2.9%) with contamination levels of 10.12 and 29.17 mg/kg. FUS contamination levels ranged between 0.54 and 7.46 mg/kg. BEA was found in four samples (5.8%) and levels varied between 0.6 and 10.63 mg/kg.

Conclusion: Since no regulations limits are worldwide set for these toxins in foods, Special attention is needed relating to their contamination levels. The high contamination levels found in some cereals products could be of a negative impact especially for children and babies health in the country.

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10:00- 10:30 CONTAMINATION WITH MOLD AND AFLATOXINS IN SOME SPICES AND TEAS ON THE MACEDONIAN MARKETS

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Background: *Aspergillus and Penicillium* probably are economically most important fungi. They often are the main reason for spoiling the food, althought some of them are used as starter culture for production of food. Anyway, these fungi gain more attention after 1960, when aflatoxins are found. In the next years it will be found a hundreds such toxic metabolites, especially from *Aspergillus* and *Penicillium* species. Aflatoxin B1 is the most hepatocarcinogenic supstance; also others mycotoxins are responsible for different toxicaemias, so it is important to take a great attention in food production and control.

Aim: The aim of this paper was to determine the presence of *Aspergillus* and *Penicillium* species in different spices and teas, which dominate on the Macedonian markets. All isolates were identified to the species level, and they were characterized for some physiological and biochemical attributes. For some of them it was conducted test for production of mycotoxins.

Materials and methods: It was analyzed 96 samples from different spices and teas which dominate on the Macedonian markets. They were in commercial packages, and only a few were from the open markets. Isolation of *Aspergillus* and *Penicillium* species were according to First international workshop for standardization of methods for mycological examination of food (King et al., 1986) and Second international workshop for standardization of methods for mycological examination of food (Samson et al., 1990). Determination of macro- and micromorphological characteristics was according to Pitt (1991), and determination of profiles of intracellular and extracellular secondary metabolites was according to Filtenborg et al., 1983.

Results and discussion: From our results, we can say that mean value for the number of fungi for all samples, as $\log_{10} g^{-1}$, is changing according to medium and incubation temperature, and it is from $\log_{10} 3.89\ g^{-1}$ on DBRH on 35^{o}C to $\log_{10} 4.63\ g^{-1}$ on DG18 on 25^{o}C.

In this paper it was identified 57 taxons, 20 from *Aspergillus*, one from *Emericella*, 5 from *Eurotium* and 31 from *Penicillium*.

Contamination with aflatoxicogenic fungi was found in 21 samples od spices and 2 samples from tea.

Conclusion: In conclusion, we can say:

- The largest contamination was found in bay leaf (log_{10} 6,17 g^{-1}, DG18 and 25^{o}C), and the smalles in paprika (log_{10} 2,0 g^{-1}, DG18 and 25^{o}C);
- The largest number of fungi in tea was found in camomilla (log_{10} 6,9 g^{-1}, DG18 and 25^{o}C), and the smallest in Indian tea (log_{10} 2,5 g^{-1}, DG18 and 25^{o}C);

- The most common contaminants (relative frequency > 0.1) in our samples were: *Aspergillus niger, Aspergillus fumigatus, Eurotium repens, Aspergillus flavus, Penicillium chrysogenum, Emericella nidulans, Aspergillus versicolor, Eurotium amstelodami, Eurotium rubrum, Aspergillus sydowii, Aspergillus ochraceus, Penicillium lividum, Penicillium purpurogenum, Aspergillus terreus, eurotium chevalieri* and *Penicillium aurantiogriseum*.

- From 40 A. flavus, only 11 (27,5%) were capable for production of aflatoxins in pure cultures, which was determined with TLC;

- All isolates from *A. nomius* and *A. parasiticus* were capable for production of aflatoxins in pure cultures.

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11:00-11:40 THE ROLE OF NATURAL MYCOFLORA ON ASSESMENT OF MYCOTOXIN PRODUCTION IN DRIED FIGS

Keynote conference

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Background: Dried fig, very nutritional and a healthy food, is one of the dried fruits sensitive to mycotoxin production (Heperkan, 2006). In this study the relationship between mycoflora and four different types of mycotoxin such as fumonisin (Karbancioglu-Guler, F. and Heperkan, 2009) ochratoxin A (Karbancioglu-Guler, F. and Heperkan, 2008), cyclopiazonic acid (CPA) and aflatoxins in dried figs were discussed. Fumonisins produced by several *Fusarium* species especially by *F. verticilloides* and *F. proliferatum* and natural contaminants of corn and corn products all over the world (Logrieco et al., 1998; Scaff and Scussel, 2004; Moretti et al., 2010). Among the fumonisin derivatives, FB_1 is the most common one and constitutes about 70-80% of the total fumonisin content of *F. verticilloides* cultures and naturally contaminated foods (Rheeder et al., 2002). Fumonisins have been classified as a possible human carcinogen (Group 2B) by International Agency for Research on Cancer (IARC, 1993). It has been found that there may be a positive correlation between human esophageal cancer rates and occurrence of *F. verticilloides* and fumonisin in diet in Southern Africa and China (Castellá et al., 1999).

Ochratoxin (OTA) A is a toxic secondary metabolite, naturally produced by three main species of mould, *Aspergillus ochraceus, Penicillium verrucosum* and *Aspergillus* section Nigri especially *A. carbonarius* (Aish, et al.,2004). With regard to the animal studies and epidemiological studies on humans, OTA has been classified as a possible human carcinogen (Group 2B) by IARC.

Cyclopiazonic acid is produced by *Penicillium* species such as *Penicillium camemberti P. urticae*, *P. commune* and *Aspergillus* species such as *A. flavus*, *A. versicolor*, *A. oryzae*, and *A. tamarii* (Dorner et al., 1983; Vinokurova et al., 2007). CPA is thought to be responsible for the “Turkey X” disease together with aflatoxin, in the 1960 outbreak that killed 100.000 poultry (Vinokurova et al., 2007). Aflatoxin is produced by most members of *Aspergillus* section *Flavi* such as *Aspergillus flavus*, *A. parasiticus* and *A. nomius*.

Aim: Mold mycoflora in naturally dried figs were investigated in samples obtained after drying. The relationship between mycoflora and mycotoxin contamination were discussed in this five year’s study.

Materials and Methods: A total of 115 samples of dreid figs were collected from orchards during the drying stage in Turkey. Fig samples were taken from 7 different districts for two consecutive years. Fungi were identified using morphological, phenotypic and molecular characteristics where possible. Ochratoxin A, fumonisin and

aflatoxins were determined by high performance liquid chromatography (HPLC). Cyclopiazonic acid were determined by and thin-layer chromatography (TLC) respectively.

Results and Discussion: Samples were heavily contaminated with members of Aspergillus section Nigri and Fusarium spp. Members of *Aspergillus* section Flavi and *Penicillium* spp. were lower than the two groups of mould above in dried figs. Fifty-five (47.2%) of the 115 samples were found to contain detectable levels of ochratoxin A, ranging from 0.12 to 15.31 µg/kg. Fumonisin B_1 contamination were determined 86 of the 115 samples (74.7%) at detectable levels. Cyclopiazonic acid and aflatoxin contamination were investigated in 48 *Aspergillus* section *Flavi* positive dried fig samples. Higher CPA incidences than AF have been determined in dried fig samples.

Conclusion: Ochratoxin A, fumonisin, aflatoxin and cyclopiazonic acid were determined in dried figs. To the best of our knowledge, CPA and fumonisin were determined for the first time in our study in dried figs.

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12:00-12:30 EFFECT OF MYCOTOXINS ON FOOD SAFETY

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Food safety has become a priority for both developed and developing countries. It is compromised due to the introduction of hazards during production, processing, storage, transport or final preparation for consumers. For food derived from animals, the hazard may originate from a number of sources including the consumption by food production animals of contaminated feed.
The problem of mycotoxins do not end in feed or reduced animal performance. Many are transferred into meat, visceral organs, milk and eggs. Their concentration in food is usually lower than the levels present in the feed consumed by the animals and unlikely to cause acute toxicity in humans.

In our experiments we studied influence of feeding of meat obtained from infected poultry fed zearalenone contaminated diet in mammals (rats). Four groups of rats (10 rats in each group) were formed: 1- control group (basal diet + meat from healthy quails), 2 - meat from quails infected with escherichiosis (E.coli O4 LD_{50}), 2 –meat from quails fed zearalenone contaminated diet (0,05±0,013 mg/kg of live weight), 3 – meat of quails infected with escherichiosis (E.coli O4 LD_{50}) and fed zearalenone contaminated diet (0,05±0,013 mg/kg of live weight). We didn't observe any changes in behavior and heath status of rats during experiment. But we established that the blood parameters of experimental rats were changed. We found reduced white and fed blood cells count and protein concentration. During histological examination it was established that experimental rats had pathological changers in liver: hemorrhage, granular dystrophy, fat decomposition, development of necrosis and in testis: basal membrane damage and necrosis of interstitial cells. After necropsy we didn't isolate E.coli from liver and meat of experimental rats. Results of our experiment suggest that feeding of meat from E. coli infected quails fed zearalenone contaminated diet can change blood parameters of rats and can eventually decrease the health status of mammals.

Increasing standards of food safety standards across the world are placing more emphasis on the control of mycotoxins in food and animal feed. Mycotoxin contamination is a global problem that will continue to attract more atten tion particularly in relation to human health. Due to their ubiquitous nature, no one part of the food production chain can totally protect or prevent the problems associated with mycotoxins. The use of mycotoxin adsorbents is an established practice in many parts of the world.

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12:30-13:00 DAILY INTAKE OF CARCINOGENIC MYCOTOXINS INCLUDING AFLATOXIN, OCHRATOXIN A AND FUMONISIN BASED ON ANALYSIS OF SEVERAL STAPPLE

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A number of cereals and other crops are susceptible to fungal attack either in the field or during storage. These fungi may produce mycotoxins. Several of them are very stable and could be found in final products such breakfast cereal or meal. Some of mycotoxins have carcinogenic properties. They are regulated by WHO, and EU. We have analysed several cereals (rice, wheat, maize) from different origin (France, Vietnam, Moldavia, Czech Republic, Morocco) but also olives; coffee; breakfast cereals.

We also analysed during one month the total diet of three Serbian families.

The contamination of rice by these mycotoxins was at alarming rate; especially AFB1 and can reach 30 µg/kg. Amount of these toxins in rice was greatly affected by the season of the year, particularly the rainy season which proved to be the major risk factor to the presence of AFB1 and CIT. OTA, AFB and FB were alsos detected in maize and wheat to level over the acceptable limit put by EU OTA has been detected in 75% of the breakfast cereal ranging from LOQ (<0.2 µg/kg) to 12.7µg/kg.

This high level was detected in a sample containing dry fruit and fibres. Most of them are also contaminated by CIT in the range of 1.5 to 42µg/kg. FB1 has been detected in almost all samples, ranging between 10 to 1110µg/kg. FB1 has been detected not only in corn flakes, but also in samples containing oat or rice. All samples of ground coffee contain OTA ranging from trace (< LOQ, 5 samples) to 11.9 µg/kg. Nine samples were over 1.5µg/kg. Some samples contain also OTB (dechlorinated OTA). The amount of OTA passing in the beverage ranged between 20-140%. Higher recovery of OTA in beverage as expected is due to two types of interference (i) presence of OTB which cross-reacts with OTA-antibodies (ii) alkalinisation of OTA converted in open ring OTA (OP-OA) which is no more recognized by antibodies.

We observed also that more the coffee was roasted, more isomerization of OTA occurred. Based on a typical menu including some of these ingredients and using the average mycotoxin's amount for calculation, we observed that the tolerable daily intake (TDI) was respectively 39-fold; 7-fold and 3 fold higher than the virtual safety dose (VSD) established for AFB1, OTA and FB. In the both families suffering Balkan endemic nephropathy (BEN), the TDI of OTA was regularly over the VSD and their food contains simultaneously Citrinin which is known to enhance carcinogenicity of OTA. In contrast, in the family non-affected by BEN, the OTA intake is low, and the food is not contaminated by CIT.

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Yucatán III

Wednesday June 30, 2010

Session 9: Physiology, antibiosis, toxicity and storage problems

08:30-09:00 PHYSIOLOGICAL RELATIONSHIP AND EFFECTS OF FOOD GRADE ANTIOXIDANTS AND ENVIRONMENTAL FACTORS ON GROWTH, SCLEROTIA, AFLATOXINS AND *nor*-1 EXPRESSION BY *Aspergillus parasiticus* RCP08300

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Background: *Aspergillus flavus* Link and *A. parasiticus* Speare are the most important aflatoxin producing fungi which are predominantly responsible for the occurrence of aflatoxins in peanuts produced in Argentina (Passone *et al.* 2009). Aflatoxins are potent carcinogenic and teratogenic metabolites (IARC 1993). These fungi also produce specialized structures called sclerotia that may be formed in response to adverse conditions or as a normal step in the fungi life cycle (Moore 1995). Different mixtures of antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) showed to be effective fungitoxicants by inhibiting aflatoxigenic fungi and their toxin accumulation in peanuts (Passone *et al*. 2009). The biosynthesis of aflatoxin appears to be regulated by several interlinked mechanisms that include transcriptional regulatory elements and physiological factors affecting fungal metabolism (Sweeney *et al*. 2000). Among regulating genes, *nor*-1 encodes a 31 kDa NADPH-dependent ketoreductase which is involved in conversion of norsolorinic acid to averantin (Trail *et al.* 1994).

Aim: To examine factors affecting growth, sclerotial characteristics, aflatoxin accumulation and *nor*-1 expression by *A. parasiticus* RCP08300 at different water activities (a_W), temperatures and sub-optimal antioxidant concentrations.

Materials and Methods: The aflatoxin producer isolate, *A. parasiticus* RCP08300, was used in this study. This strain was isolated from stored peanut (Passone *et al*. 2008). The effects of BHA and BHT mixtures (1 + 1 mmol l^{-1} and 5 + 5 mmol l^{-1}), a_W (0.98, 0.95, 0.93 and 0.89) and temperatures (28 and 20 °C) on 3% peanut meal extract agar on growth (Marín *et al*. 1995), sclerotial characteristics (Nesci & Etcheverry 2009), aflatoxin accumulation (Geisen 1996) and *nor*-1 transcript level by *A. parasiticus* were examined.

Results and Discussion: Growth rate and aflatoxin production were inhibited by BHA-PP mixture (1 + 1 mmol l^{-1}). Although sclerotia production and *nor*-1 expression was stimulated by this treatment, sclerotia dry weight and volume were reduced by 62.3 and 31.2%, respectively. The higher dose of mixture applied significantly reduced all parameters tested in *A. parasiticus* strain.
The genotypic information linked to growth parameters, sclerotial characteristics and mycotoxin production in relation to stress factors provide a valuable tool for investigating the ecophysiological basis of aflatoxin gene expression and ultimately may lead to more effective control strategies for this important mycotoxigenic pathogen.

Conclusions: Data show that all parameters tested in *A. parasiticus* have been significantly influenced by interacting stress factors.

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09:00-09:30 DETECTION AND QUANTIFICATION OF *Aspergillus* SECTION *Flavi* IN STORED PEANUTS BY REAL-TIME PCR OF *nor*-1 AND EFFECTS OF STORAGE CONDITIONS ON AFLATOXINS PRODUCTION

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Background: Peanut is an important food commodity in Argentina with an annual production exceeding 6 x 10^5 tons (SAGPyA 2008). Argentinean peanuts are frequently contaminated by *A. flavus* and *A. parasiticus* and this infection can occur during growth, harvesting, transportation or storage (Passone *et al*. 2008). The development of a rapid, sensitive method for detection and differentiation of potential aflatoxigenic species in foods is needed to estimate any potential health risk associated (Valasek & Repa 2005). Information derived from these test would allow informed decisions about storage life of the product and the need for specific mycotoxin analysis. In this direction, DNA-based detection methods like RT-PCR appear more sensitive and specific. Early detection of aflatoxin-producing species is critical to prevent mycotoxin entering the food chain and aflatoxin concentration can be correlated with the density levels of aflatoxigenic species detected on naturally contaminated samples (Shapira *et al*. 1996).

Aim: To apply a rapid, sensitive and specific assay, not inhibited by matrix effects, to detect and quantify *Aspergillus* section *Flavi spp.* in stored peanuts. The assays were performed in combination with an efficient and economic fungal DNA extraction procedure, tested in peanuts stored and conditioned at different water activities during a 4-month period. The effects of fungal density and storage conditions on aflatoxin produced were also investigated.

Materials and Methods: One-thousand kilograms of in-pod peanuts destined for human consumption were artificially dried up to 0.92 $\pm$ 0.01, 0.88 $\pm$ 0.01, 0.84 $\pm$ 0.01 and 0.76 $\pm$ 0.02 a_W, distributed in four flexible containers and used to carry out the study from July to November 2008. A real-time PCR (RT-PCR) system directed against the *nor*-1 gene of the aflatoxin biosynthetic pathway as target sequence was applied to monitor and quantify *Aspergillus* section *Flavi* population in peanuts. The quantification of fungal genomic DNA in naturally contaminated peanut samples was performed using TaqMan fluorescent probe technology, the primers and the internal probe used in the reaction were those proposed by Mayer *et al*. (2003). A standard curve relating *nor*-1 copy numbers to colony forming units (cfu) was constructed. Counts of species of *Aspergillus* section *Flavi* from unknown samples obtained by molecular and conventional count (CC) methodologies were compared. Aflatoxin contents from 100 peanut kernels samples were determined following the methodology proposed by Trucksess *et al*. (1994).

Results and Discussion: Sensitivity tests demonstrated that DNA amounts accounting for a single conidium of *A. parasiticus* RCP08300 can be detected. A correlation between cfu data obtained by RT-PCR and CC methods was observed (r = 0.613; p < 0.0001). However, the cfu values obtained by RT-PCR were usually higher (0.5-1 log units) than those obtained by conventional counts (CC). A decrease of fungal density was observed throughout the storage period, regardless of the quantification methodology applied. Total aflatoxin levels ranging from 1.1 to 200.4 ng/g were registered in peanuts conditioned at the higher a_W values (0.94-0.84 a_W).

Conclusions: The RT-PCR assay developed appears as a promising tool in the prediction of potential aflatoxigenic risk in stored peanuts, even in case of low-level infections, and suitable for rapid, automated and high throughput analysis.

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09:30-10:00 DETERMINATION OF THE OPTIMAL CONDITIONS FOR DEOXYNIVALENOL AND ZEARALENONE PRODUCTION BY TWO STRAINS OF *FUSARIUM GRAMINEARUM*

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Introduction: The uncontrolled development of moulds that contaminate vegetal foods results in the build up of toxic metabolites. Risk assessment of these natural food contaminants requires experimental intoxications in order to characterize the toxicity of these substances and to determine the dozes with no effect and the dozes which the animals can tolerate.
If mycotoxin toxicity is well characterized on laboratory animals, their effects on farm animals are less documented, mainly on the chronic intoxications. Farm animals are more exposed to these contaminants given their conditions of rearing and the feeding regime rich in cereal grains. The study of the chronic mycotoxin toxicity in these animal species allows determining the effects without endangering animal health, effects that may have important economic repercussions on animal production and on the quality of animal foods.
The accomplishment of such studies requires large amounts of toxins (dozens of grams) and the price of the commercial pure mycotoxins is expensive. An alternative strategy would be to use naturally contaminated foods or raw materials, but in this case there is the danger for poly-contamination (several mycotoxins), and the results are difficult to interpret because the observed effects can be the result of the synergic action of several mycotoxins and because it is practically impossible to reproduce the experimental protocol. The only feasible way is the experimental, laboratory production of mycotoxins.
Taking into consideration the fact that the studies of fungal and mycotoxic contamination of the cereal grains from Romania revealed the constant presence of *Fusarium* strains, of deoxynivalenol (DON) and zearalenone (ZEA), sometimes in concentrations exceeding the limits impose by the European regulations, we aimed to determine the optimal conditions of production for these two mycotoxins. To this purpose we have studied the impact of some environmental parameters (temperature, duration of culture and substrate) on mycotoxin production.

Purpose: Determine the optimal conditions for deoxynivalenol and zearalenone production.

.**Material and methods:** We selected two *Fusarium graminearum* strains: *F. graminearum F6G10* producing deoxynivalenol and *F. graminearum DMSZ 1095* producing zearalenone. The fungal culture was obtained by growing the two strains on a medium with malt and incubation at 25^{o}C for 7 days. The resulting cultures were used as inoculum; 3, 1 cm^2 squares have been placed on the testing mediums (wheat, coarse ground corn and barley) sterilized in autoclave. The mycotoxins have been extracted from cultures with a solution of acetonitrile : water. The extraction yield was evaluated using samples of wheat and corn artificially contaminated with known amounts of

mycotoxins. Mycotoxin purification was done comparatively by purification columns: the purification of DON was achieved in column with coal : Al_2O_3 : celite (07:05:03), column with coal : Al_2O_3 (0,75:0,75) and in MycoSep column; the purification of ZEN was done in columns of C18 and in column with coal : Al_2O_3 : celite. The purified extracts have been dried in nitrogen flow and retaken in acetonitrile:water, then diluted or concentrated in order to be compatible with the calibration curve. DON and ZEN quantification was done by thin layer chromatography and HPLC.

Results and discussions: *Influence of the substrate on the production of DON and ZEA.* DON has been produced in the largest amount on rice (2776 µg/g) after 5 weeks of incubation at 25^{o}C; the largest amount of ZEN was produced on corn (1783 µg/g), after 6 weeks of incubation at 25^{o}C. The production of ZEN on wheat was insignificant and therefore it was removed from the study.
Influence of the temperature and period of incubation. To evaluate the influence of the temperature and period of incubation, the producing strains have been cultivated on optimal mediums (barley for DON and corn for ZEN) and incubated for 8 weeks at several temperatures (15, 20, 25, 30 and 35^{o}C). Production kinetics showed two stages for DON: the amount of DON in the medium increased for 5 weeks, then decreased progressively; the production of DON peaked at the temperature of 25^{o}C during the fifth week of incubation. The production of DON was lower, but in quite large amounts at 15^{o}C, but synthesis was inhibited at 30-35^{o}C. The kinetics of ZEA production is comparable with that of DON, characterized by an increase of the mycotoxin concentration during the first 5-6 weeks followed by a rapid decrease of the amount of ZEA in the culture medium. Temperature influenced the production of ZEA, similarly with the production of DON; the highest production was at 20-25^{o}C, while the temperatures of 30 and 35^{o}C inhibited the synthesis of ZEA. Under optimal conditions of medium, the two strains have synthesized sizeable amounts of mycotoxins: almost 5000 mg DON/kg culture medium and 1700 mg ZEA/ kg culture medium. These concentrations are enough to study the effects of these mycotoxins on the farm animals. The extracts contain impurities which make quantification difficult and whose biological activity might interfere with the toxic effect of the investigated mycotoxins. This is why they had to be purified. Several purification columns have been tested. The yield of purification for DON was 72-83% after passage through the coal:Al_2O_3:celite (07:05:03) column and 76-89% for the coal : Al_2O_3 (0,75:0,75) column, while the yield for purification in Mycosep column was just of 65-67%. The best yield of purification for ZEA was achieved in the C18 column (91,07±4%), while it was of just 55-56% for the coal:Al_2O_3:celite (07:05:03) column.

Conclusions: The cultivation of the strongly toxicogenic strains of *Fusarium graminearum* for 5-6 weeks at 20-25^{O}C on rice (DON) and corn (ZEA) allows the production of amounts enough to produce experimental intoxications. The optimal hydro-thermal conditions for these syntheses are frequently met in Europe and correspond to the climacteric conditions from spring and early summer in Romania, which explains the constant presence of these toxic compounds in the cereal products in our country.

10:00-10:30 **THE EVALUATION OF STORAGING TECHNIQUES ON QUALITY CHARACTERISTICS OF TURKISH RED CHILLI PEPPER AT SEMI COMMERCIAL MANUFACTURING**

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The aim of this study, hermetic and vacuum storage methods were assessed under commercial scale in comparison with traditional method in the warehouse, evaluating the quality indices of Turkish red chilli peppers (RCPs). One tone of flaksed and mechanically dried RCP with maximum 10±1% of moisture content were stored for seven months under a low pressure of 80-100 mm Hg, sealed hermetic conditions and tradational storage method (in opened piles of barns; referred as a control). Basic quality parameters related to microbiological counts, aflatoxin amounts (B_1 and total), pungency properties, color levels, moisture contents, organoleptic qualifications were detected before and after 6 months storage.

Mercantile scale experiments displayed that the best quality RCPs occured from vacuum storage with very low losses in quality indicators (capsaicin, colour, aflatoxin). On the other hand, hermetic storing resulted in high level losses of colour, while microbial growth and aflatoxin occuring were inhibited, and the pungency of RCP was protected. This examination successfully supported the feasibility of commercial application of hermetic and vacuum storage technology for long-term storing of RCP at the first practice in the world.

Vacuum technology was proven to be an effective, chemical-free and economical method to disinfest commodities of insect, to inhibit the development of moulds, aflatoxin occurence and to prevent the quality damage of RCP due to the oxidative and fermentative processes. In conclusion, this semi-scale commercial trial indicates the sealed flexible vacuum-hermetic storage technolgy introduced potentially singificant advantages over tradationally storage methods in ability to enhance preservation of quality characteristics such as colour, pungency, and aflatoxin of RCP for long-period storage.

11:00-11:40 MYCOTOXINS IN ARGENTINA WHICH IS THE HORIZON?

Keynote Conference

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Mycotoxins are toxic secondary metabolites of fungi belonging essentially to the *Aspergillus*, *Penicillium, Fusarium* and *Alternaria* genera. They can be produced on a wide range of agricultural commodities including cereals and oilseeds under a diverse range of situations worldwide. Maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.), soybean (*Glycine max* L. Merr) are important commodities used as staple food, food ingredients and animal feed. The accumulation of mycotoxins in foods and feeds represents a major threat to human and animal health as they are responsible for many different toxic effects including the induction of cancer, digestive, blood and nerve defects, and immunotoxicity. It has been estimated that one quarter of the world's food crops, including many basic foods, are affected by mycotoxin producing fungi (WHO, 2001).

Fungal and mycotoxin contamination can begin in the field and under certain conditions increase during harvest, drying and storage. Due to their thermal stability mycotoxins can remain after food processing. Mycotoxins can never be completely removed from the food supply; therefore there is a need to ensure these metabolites are reduced to an acceptable level.

Different strategies have been evaluated to reduce the entry of mycotoxins in the food and feed chains. Prevention strategies are based on using the HACCP approach and to identify the critical control points.

The application of HACCP to the control of mycotoxins takes a proactive approach to mycotoxin control, an approach of "**prevention is better than cure**". This attitude is appropriate since the presence of mycotoxins in a finished product is a result of event and circumstances affecting commodities much earlier in the food production chain. The HACCP normally operate within the framework of a Total Quality Management (TQM) system such as ISO 9000. HACCP type principles can be applied to the earliest stages of food production, at harvest, and the stages that follow on, including storage and processing. It is possible to take an holistic view "from field to plate and from plough to plate (Magan and Olsen, 2004).

Argentina is an important producer of cereals and oilseeds, its production is devoted to both the domestic and external markets. Previous reports on the situation of mycotoxins in Argentina showed that the mycotoxins of concern in the area are aflatoxins, fumonisins, trichothecenes (mainly DON), but other toxins are also relevant like ochratoxin A and zearalenone (Chulze, 2004, Piñeiro, 2004, Rodriguez Amaya, 2000). Studies on mycotoxins have been done in Argentine since the 1970s, at that time dealing mainly with aflatoxin in peanuts. Different research groups from public universities, research institutes and professionals have studied potential measures to

control the causal fungi and the occurrence of mycotoxins in different agricultural commodities including cereals, oily seeds and fruits. The ecophysiology and genetics of toxigenic fungi, chemical and biocontrol of toxigenic fungi and mycotoxins production, fate of mycotoxins during food processing, methodology for mycotoxins as well as toxicological studies (Chulze, 2006). Several national and international meetings devoted to mycotoxins have been organized and also the subject has been discussed in other more general meetings, related to Plant Pathology, Microbiology, Mycology, Veterinary and Food Sciences and Technology.

In 1992 the Latin American Society of Mycotoxicology (SLAM) was created during the VIII International IUPAC Symposium on Mycotoxins and Phycotoxins held in Mexico. The Society combined efforts to contribute to the prevention and control of mycotoxin in Latin America and the Caribbean region. Several Argentinean researchers are members of the SLAM, and the Society allows us to discuss and publish results of the region research, to organize workshops, seminars, congress. The III Latin American Congress on Mycotoxicology (SLAM) was held in Córdoba, Argentina during 2000.

Research projects on mycotoxins supported by the European Commission were carried out in the last five years for example the Mycotox project (2003-2006) that addresses the methodological and analytical issues associated with the establishment of a Food Quality Management System for controlling mycotoxins in cereal chains, coordinated by CIRAD, France. Argentinean researchers were involved in this project. During 2004 another project, the Myco-globe Specific Support Action in the VI EU Framework programme, coordinated by Dr. Angelo Visconti, ISPA, Italy. Argentinean partners were also beneficiaries of the project. In the frame of the project a conference on “Advances in research on toxigenic fungi and mycotoxins in South America ensuring food and feed safety in a Mycoglobe context” was held in Córdoba, Argentina in 2006. with the participation of researchers and speakers from 16 countries around the world. The Mycoglobe project allowed exchange information and results with countries from different continents within the wider framework of a global information system on mycotoxins and toxigenic fungi.

Considering the cost of the research in the area of mycotoxicology it is necessary to optimize resources and to work in a coordinate way in order to joint efforts to reach in a shorter time better solutions to reduce the impact of mycotoxins in the food and feed chains. From 2003 a group with interest on mycotoxins including researchers and professionals from public universities, research institutes and the Agri-Food Quality, National Agri-Food Health and Quality Service (SENASA) began to discuss different aspects in the area and the need to joint efforts and work in coordinated way. During 2006 was formalized the governmental decision for the implementation of national research programs on Food Safety with the inclusion of mycotoxins in order to improve food control systems, to ensure consumer protection and promote the international or regional food trades. An open call to submit proposals in the way of integrated research projects under the instrument Projects in Strategic Areas (PAE) was formalized by The Ministry of Science, Technology and . Productive Innovation from Argentina

As a result of this call, through an interdisciplinary approach by a coordinated and joint effort among different institutions including national organisations of control, research

laboratories from national institutes and public universities, and with the guarantee of the private sector, an integrated research project has been lunched. The main goal of the proposal is intended to develop a sustainable system to prevent and control mycotoxins. The main objectives of the project are:

-To extend and optimize the analytical capacity of the laboratories, to harmonize methodology according to ISO 17025

.-To establish sampling plans and to carry out studies on mycotoxin occurrence at a national level.

-To develop forecast models to generate alerts for the main mycotoxins mainly for wheat and maize.

-To generate technological information that will allow control of mycotoxin accumulation (resistant germplasms, natural pesticides, biological control, detoxification)

-To develop alerts for mycotoxicosis and to be able to establish bases for a protocol of mycotoxin regulation in feedstuffs

.-To generate a data base on natural occurrence of mycotoxins, survey of food intake for risk analysis studies.

-To carry out training at all the levels of the food and feed chains (including researchers, technicians, decision makers).

-To communicate the results through meetings, brochures, publications. Also the implementation of the project will improved the cooperation with developed countries to share experiences in order to reduce the impact of mycotoxins.

Also Argentinean researchers are active members of the Latinoamerican Society of Mycotoxicology and The International Society of Mycotoxicology.

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11:40-12:10 ANTIMICROBIAL ACTIVITY OF DIFFERENT ANAEROBIC FUNGI ON SELECTED ATCC STRAINS OF MICROORGANISMS

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Background: Anaerobic fungi inhabit certain regions of the alimentary tract of many herbivorous mammals (Orpin & Joblin, 1988; Teunissen *et al.,* 1991). The importance of anaerobic fungi in herbivore digestion remains unclear, but their capacity to produce large amounts of fibredigesting enzymes (Williams & Orpin, 1987) and their ability to digest up to half of the dry weight of plant tissue *in uitro* (Lowe *et al.,* 1987) suggest that they contribute significantly to plant fibre digestion in herbivores.

Aim: In this paper we want to examine antimicrobial activity of different anaerobic fungi on selected ATCC strains of microorganisms. As test microorganisms we used: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404.

Materials and methods: Experiments were conducted with fermentation supernatants (FS) from cultures of anaerobic fungi. We used dilution and diffusion method with BioMerieux discs.

Results and discussion: Only three isolates showed antimicrobial activity: isolate OEM, from deer, isolate DKR, from domestic cow, and DKZ, from domestic goat. Results from these experiments showed distinct differences in antimicrobial activity of anaerobic fungi against test microorganisms. Among bacteria, most susceptible was *E. coli*, with MIC 50 μl, against sporogenic and asporogenic Gr-positive bacteria. FS from OEM and DKZ showed bigger efficacy against yeast, and FS from DKR showed bigger efficacy against mold.

Conclusion: In conclusion, we can say that these isolayes of anaerobic fungi showed distinct differences in antimicrobial activity against test microorganisms. However, it requires more experiments to obtain more reliable results about antimicrobial activity of anaerobic fungi.

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12:40-13:10 ANTAGONIC ACTIVITY OF *ASPERGILLUS NIGER* OVER *ASPERGILLUS PARASITICUS* ATCC16990

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Chemical and/or biological strategies have been sought to inhibit growth of *Aspergillus parasiticus* or *Aspergillus flavus* as a means to decrease aflatoxin contamination in diverse substrates. The use of aflatoxicogenic *A. flavus* applied to cotton fields to reduce inoculum -- and as a consequence to reduce aflatoxin contamination in cotton seed-- proven to be successful. However this technique has limitations for its application on stored grains and seeds. Therefore the objective of this work was to analyze both physically and chemically the behavior of *A. niger* over *A. parasiticus* ATCC 16990 *in vitro*. The physical aspect was analyzed via confrontation on solid culture media; the chemical aspect was performed utilizing the metabolites produced by cultured *A. niger*. Potato Dextrose Agar and broth were used as a culture medium and microbiological techniques were applied to obtain the spore suspensions. The inhibition zone was measured in mm, and the effect of secreted metabolites was determined by HPLC quantification of aflatoxins and fungal growth. Only one of five strains of *A. niger* analyzed inhibited *A. parasiticus* growth. The metabolites of this strain inhibited growth up to 50 % and aflatoxin synthesis up to 99 %. The nature of the compound, as well as the mechanism(s) of action, is currently under study.

12:40-13:10 TOXICITY DETERMINATION OF ZEARALENONE IN *Drosophila melanogaster* Oregon-flare AND Flare STRAINS.

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Background: Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin produced by several species of *Fusarium*. ZEN has caused an earlier onset of puberty in children, endometrial adenocarcinomas, hyperplasia and breast cancer in women. It induces apoptosis, DNA fragmentation, micronuclei production, chromosome aberrations and DNA adduct formation. In mammalian cells, ZEN binds to, and activates the estrogen receptors, increasing the estrogen-dependent transcription of genes responsible of activation of procarcinogens like *cyp´s 450* these ability to genes and promote undesirable estrogenic effect. Additionally the metabolisms of estrogenic compounds like ZEN alters the transcription of Cyp 450 involved in oxidative metabolism increasing the potential genotoxicity and cancer risk.

Aim: To determine the involvement of CYP450 enzyme complex in ZEN in the *Drosophila melanogaster.*

Material and Methods: In order to determine the participation of CYP450 enzymes, we using two different third instars larvae, *flare*^{3}and O*regon-flare*^{3} wich express the cy450 genes differentially (inducible and high levels of CYPs, respectively). Strains were exposed to ZEN (5 to 500 µM) during approximately ten days, then survival percentage was determined.

Results and discussion: The concentration range of ZEN used in toxic essays raised from 5 to 500 µM, the survival percentage shown differences between strain. The CL_{20} value was 260µM for the O*regon-flare*^{3} while 155.25µM for the *flare*^{3} strain. These differences could reveal the involvement of CYP450 enzymatic system in the metabolism of ZEN given that in *Oregon-flare*^{3} strain the *Cyp 450* genes are upper expressed. Thus, the CYP activity may transform the mycotoxin decreasing its toxic effect.

Conclusion: ZEN has a toxic effect in *D. melanogaster*, subtoxic concentration was determined as 260 µM and 155. 25 µM for the O*regon-flare*^{3} and *flare*^{3} strain respectively. It looks like that in *Drosophila melanogaster* the CYP450 complex is involved in the protective response against ZEN.

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Session 10: Mycotoxins in Latin America

17:00-17:30 SPECIES COMPOSITION OF BLACK ASPERGILLI ON VINEYARD SOILS IN ARGENTINA

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Background: Ochratoxin A (OTA) producer fungi live as saprophytes in the superficial layer of the vineyard soil which is the main inoculum source for wine grapes. It has been demostrated that the movement of the air carries the spores from the soil to the grape surface, thus the risk of contamination with OTA in wines might be related to the presence of toxigenic strains in the soil. The information about species composition of black *Aspergillus* strains occurring on vineyards soil is limited; moreover the accurate identification of *Aspergillus* species in the section *Nigri* is important because both the fungi presence and the toxin profiles of each species define potential risk for OTA contamination.

Aim: The aim of this work was to evaluate the black *Aspergillus* inoculum potential in vineyard soils from different wine-producing regions of Argentina using classic and molecular methodology to identify the strains; to determine the toxigenic capacity of the strains isolated, and to relate the frequency of isolation of the *Aspergillus* section *Nigri* species with the environmental conditions.

Materials and Methods: The soil sampling was done in the wine-producing regions of Mendoza, San Juan, Neuquén, Rio Negro and La Rioja provinces, during 2008's harvest season. The total fungal and *Aspergillus* section *Nigri* counts were determined using surface spreading onto Dichloran Rose Bengal Chloramphenicol (DRBC) culture medium. The isolated strains belonging to the genera *Aspergillus* section *Nigri* were grown in malt extract agar (MEA), for identification by morphological characteristics, and Czapek yeast extract agar, for determining the toxigenic ability of the strains. The toxin production was screening using TLC and confirmed by HPLC. The strains were identified by molecular methods based on PCR, using specific primers. The meteorological data was kindly provided by the Instituto Nacional de Tecnología Agropecuaria (INTA). The statistical analysis conducted was ANOVA followed by a multiple comparison analyses (Fisher Method LSD) (p<0.05). The Pearson correlation coefficient was used to evaluate the incidence of the isolates and the meteorological and geographical conditions of the region. Data analysis was conducted using SigmaStat software for Windows v2.03 (SPSS Inc., USA).

Results and Discussion: All the soil samples studied from different regions showed *Aspergillus* section *Nigri* incidence. Vineyards from the Rio Negro province (General Enrique Godoy and General Roca) showed the highest incidence of *Aspergillus* section *Nigri* with levels of 2.9 x 10^4 and 2.3 x 10^4cfu/g, respectively. In other locations the incidence ranged from 9.1 x 10^2 to 4.8 x 10^3cfu/g (mean = 2.8x10^3). From a total of 147 strains isolated belonging to *Aspergillus* section *Nigri*, 37% were classified as uniseriated black *Aspergillus* and 61% as biseriated, of which only 2% were *A. carbonarius*. All the strains isolated belonging to *Aspergillus* section *Nigri* were evaluated for OTA production. The 50% of the biseriated strains were OTA producers with levels from 0.5 to 18.6 ng/g for those belonged to *A. niger* aggregate, and up to 30 ng/g for *A. carbonarius*. Several studies support that the levels of OTA produced by *A. carbonarius* are very variable (Serra *et al*. 2005; Perrone *et al*. 2006) and some *A. carbonarius* strains are not OTA producers. The identification of the strains isolated was confirmed using a PCR method with specific primers.
A negative correlation coefficient between the incidence of *Aspergillus* section *Nigri* and the altitude was observed (CC -0.79). No correlation was found between rain levels and *Aspergillus* section *Nigri* incidence. This agrees with results reported by Mitchell *et al.* (2003) and Bellí *et al*. (2004) who consider the rain influence as minor or secondary factor in determining the occurrence of *Aspergillus* Section Nigri.

Conclusion: In general, all the strains isolated belonging to *Aspergillus* section *Nigri* showed low capacity to produce OTA. The main ochratoxigenic species, *A. carbonarius*, was isolated in low frequency. Biodiversity and distribution of *Aspergillus* section *Nigri* species depend on the environmental factors. We can conclude that temperature and altitude are the main factors influencing the presence of OTA producers, while rain levels are less important. From the results found we could infer low levels of OTA in wine in Argentina.

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17:30-18:00 *Claviceps paspali* TOXINS IDENTIFICATION

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Background: *Claviceps paspali*, is an *Ascomycete* which cause a highly specific parasitic disease to genus *Paspalum* forage. The fungus has been isolated from this substrate and studied its life cycle in previous studies by the authors of this paper. In months from March to May -culminating the asexual phase of their cycle- the fungus make a sclerotome on the spikes of plants, which when ingested by cattle, produces a toxic syndrome, characterized by tremors and ataxia, due to the presence tremorgenic mycotoxins.

Aim: The objective of this work was to isolate and identify toxins in the sclerotia of *Claviceps paspali* isolated from *Paspalum spp*.

Materials and methods: The sclerotia were harvested from highly contaminated spikes, which were left to dry, seed husks were eliminated and eventually, seeds were finely ground. The extraction was performed with chloroform for 24 hours, filtered and evaporated the solvent in rotary evaporator. The concentrate was dissolved in hexane-methanol-water and placed in a decanting to separate the lower fraction (methanol-water) with toxins. It was evaporated to dryness, obtaining the extract. Thin layer chromatography (TLC) was performed, to evaluate the number of substances and their Rf. A chromatography column was designed in order to isolate fractions present in the 100 obtained samples. Then, they were tested on TLC, proton nuclear magnetic resonance (H^{+1} NMR) spectra, and carbon nuclear magnetic resonance (C^{13}NMR), allowing the identification of various toxins. Also two patterns (paspaline and paspalinine) were compared with plaque extract TLC, verifying their presence in it.

Results and Discussion: A number of 6 different toxins were isolated: paspaline, paspalicine, paspalinine, paspalitrems A, B and C, which were determined by thin layer chromatography and studied by Nuclear Magnetic Resonance.
Literature attributes to a hydroxyl group in carbon C19, the tremorgenic activity. Paspaline and paspalicine, lacking a hydroxyl group in carbon number 19, do not show biological tremorgenic activity in animals. However, they are considered precursors of the other, since they are most abundant in immature sclerotia.

Conclusions:
It is very importan to evaluate the presence of this micotoxins in the late stage of asexual phase of the *Claviceps paspali* cycle, due to the fact that micotoxins produced in this stage are responsible for the tremorgenic syndrome in animals. If not possible, it is advisable to remove the animals from grazing, before sclerotia maduration

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18:00-18:30 FUMONISINS IN CORN-BASED FOODS INTENDED FOR CONSUMPTION IN LATIN AMERICA: A QUALITATIVE SYSTEMATIC REVIEW.

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Background: Fumonisins are mycotoxins characterized by their natural contaminants of corn crops, its high resistance to food processing and diversity of toxic effects in humans, including cancer. The main source of intake of fumonisins is given through the consumption of processed foods with contaminated corn. In Central America, Mexico and parts of the Andean zone, corn is a staple food of the population, especially in rural areas where it is grown for domestic use. The wide variety of foods prepared from corn allows its consumption is common in all ages. Mexico has linked the consumption of fumonisins contaminated foods with problems in neural tube development during pregnancy stage.

Objective: The aim of this study was to determine through a qualitative systematic review from scientific literature, which is the percentage of Latin American corn derived foods contaminated with fumonisinas and its level of fumonisins measured by using High-Performance Liquid Chromatography (HPLC) methodology

Materials and Methods: A systematic review from literature was performed. It´s covering databases were: Cochrane Register of Controlled Trials (CENTRAL), PubMed, SciELO, Ebsco, Science Direct, Springer Link, and Lilacs. The following descriptors were used: "fumonisins", "corn" and "America". Original papers in English, Spanish and Portuguese were selected. The studies conducted on food derived from corn and Latin American origin were included. The studies in food intended for animal consumption or the employment different methods to HPLC were excluded. The last search was conducted in January 2010. Twenty potentially relevant original papers was obtained, which were subjected to critical appraisal by using adapted guide JAMA evidence®: "Evidence About Diagnostic Tests Appraising." Each study was assessed independently by two investigators.

Results and Discussion: Fumonisins B1 (FB1): 73% (577/787), fumonisin B2 (FB2): 64% (453/712) and fumonisin B3 (FB3): 49% (119/242) were found on foods respectively. The highest fumonisin levels from foods (> 1 mg / kg) were in: Creamed corn, "fubá", "cornmeal, and corn flour, all of them from Brazil, foods with lower toxins levels were the snacks. Most of the foods analyzed results were averaged for fumonisins B1 and B2 above 0.2 mg / kg. In fact to according EC legislation 2007 of the European Community, it´s trading for infants and young children should be prevented. None of the 40 samples of starch (Brazil, Colombia, Uruguay) presented contamination by fumonisins.
Conclusion: The presence of fumonisins in food derived from corn is a common factor in Latin American countries, most contaminated foods were flour and cornmeal (polenta, infant cereals and other) these products are often prepared at home by simple water or milk addition. Were showed high exposure to fumonisins in the rural areas and children from Latin America, that join to the lack of monitoring and control policies in this region, leaving this vulnerable population to the effects of fumonisin. It is essential that Latin American countries establish public policies to

prevent the risk of mycotoxin contamination in cereals including storage, processing and regulation of domestic trade in food.

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Yucatan IV

Tuesday June 29, 2010.

Session 11: Methodology

08:30-09:00 RADIOACTIVITY AND AFLATOXIN IN BRAZIL NUTS

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Background: Brazil nut (*Bertholletia excelsa*) is as a nutritive source of calories and antioxidant compounds. It has radioactive elements due to the Brazil nut tree high capacity of uptaken Radium from soil, for example. It is native from the Amazon region and despite of the chemical and nutritional properties, the association of Brazil nuts with the aflatoxins (AF) contamination has been reported. As the AF are classified as carcinogens, their presence in Brazil nuts has been studied, in order to prevent the consumers' health and to enforce the international commerce regulations.

Aim: To quantify the aflatoxin and radioactivity in Brazil nuts and to determine the association of their limits.

Materials and Methods: Different sizes of shelled Brazil nuts were obtained from a Brazil nut factory in the 2009 harvest. The samples were analyzed for: a) Aflatoxin: by LC MS/MS (Xavier & Scussel, 2008) with Detection Limit of: 0,180 µg/kg for total AF; b) Radioactivity: ^{226}Ra and ^{228}Ra activities were measured by gamma spectrometry, using a hyper-pure germanium detector with 67% of relative efficiency; c) Moisture Content: by gravimetric method (AOAC, 2005).

Results and discussion: The average of activities values was: 50.10 ± 0.66 Bq.kg-1 to ^{226}Ra and 42.25 ± 1.10 Bq.kg-1 to ^{228}Ra. The values of effective doses due to the Brazil nut ingestion were calculated based on the values of consume in some Brazil geographic regions. All doses were under the radioactivity limit established by the UNSCEAR (2000). The aflatoxin range was from 1.4 to 3.7 µg/kg, and 100% of the

samples were under the limit of 4.0 µg/kg. Regarding the moisture content the range was 1.5 to 2.9 g%. It is necessary to emphasize the samples were dried during the process and the levels were under the recommendation of 15% for moisture content (*Codex alimentarius*, 2006).

Conclusion: Despite of the presence of AF and the high radioactivity in Brazil nuts as compared to other nuts, the levels were under the limits of regulation or recommendation. The association between the radioactivities and AF has to be studied deeply, concerning other variables such as other radionuclides or compounds, as well as the risk assessment and consumption.

Acknowledgments: To the Foundation for Support the Research of the Amazon Estate (FAPEAM), for support this research.

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09:00-09:30 IDENTIFICATION OF *FUSARIUM* METABOLITES BY A LC-HR-MS METABOLOMICS APPROACH USING STABLE ISOTOPE LABELING.

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Background: Fungi are able to produce a huge variety of different secondary metabolites, like terpenes, non-ribosomal products, alkaloids, polyketides and many more, which can act for instance as mycotoxins or antibiotics. Still, many novel compounds are found in fungal strains today or already known substances are discovered to be produced by strains formerly unknown to produce them (e.g. fumonisins by *Aspergillus niger*, Frisvad et al. 2007). Within an interdisciplinary project we aim to further understand the pathogenicity of *Fusarium graminearum* strains towards important crops like maize or wheat. As such we see it important to characterize the entire set of metabolites, produced at certain conditions, to get a comprehensive picture. While mycotoxins are an important group of compounds, there are further compounds that have already been shown to be virulence factors (e.g. siderophores, reviewed by Haas et al. 2008) of plant diseases. The lack of standards for potentially thousands of compounds requires high resolution mass spectrometry to generate accurate masses. Even with sophisticated analytical equipment and various software assistance it remains a difficult task to unambiguously assign signals to the fungi and identify the produced compounds.

Aim: To identify non-volatile secondary metabolites of *Fusarium graminearum*.

Materials and Methods: Defined numbers of *Fusarium graminearum* PH-1 spores were transferred into liquid minimal media containing single ^{12}C or ^{13}C carbon sources, respectively. 5 replicates were produced per time point. After 1, 2 and 3 weeks of growing, the cultures were diluted with the same volume of methanol, centrifuged and frozen at -80°C until measurement. After reversed phase chromatography, a Thermo LTQ-Orbitrap HR-MS was used to monitor the m/z values from 100-1000. Liquid media containing ^{12}C carbon sources, ^{13}C carbon sources and a 1:1 (v:v) mixture of the inoculated media were analyzed.

Results and Discussion: Excellent mass accuracy <2 ppm was achieved for the majority of the identified compounds. While the total number of peaks (substances) in the measured ^{12}C or ^{13}C media were similar, only substances with a typical ^{13}C isotope pattern (resulting from partially incomplete labeling) were assigned to arise from fungal metabolism. As there is no difference in retention time between labeled and unlabeled compounds, in the mixture both isotope patterns could be found for fungal metabolites in the same spectra. The difference in masses is equivalent to the number of carbon atoms in the compounds, further helping to generate unique molecular formulas. Molecular formulas were generated using “Seven Golden Rules” for heuristic filtering of molecular

formulas obtained by accurate mass spectrometry (Kind and Fiehn, 2007) and searched against in Antibase 2007, a fungal metabolite database to propose possible structures.

Conclusion: Many system related compounds (e.g. from the used lab ware, column, etc.) could be found in mass spectra, which are not arising from fungal metabolism. Even with background filtering it is not completely possible to get rid of these influences. Stable isotope labeling is a valuable tool to unambiguously identify substances originating from the cultured fungus. As other compounds can be produced during different growing conditions, it is important to carefully control these conditions during biological studies.

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09:30-10:00 EVALUATION OF MYCOTOXINS IN BRAZIL-NUTS AND ROASTED COFFEE BY LC-MS/MS(QqQ)

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Background: As fungi can grow in raw materials and processed food, either still during plant growth, after harvest and/or during storage, the toxins that may contaminate them can be produced by different strains and several of them be present in the same sample. Therefore, it is important that the methodology used is able to detect all of them, or at least the most important/toxic ones, together in the same analytical run. The main multi-toxin method that gathers compounds produced by both, field and storage fungi, developed to date, which is very accurate, sensitive and self confirmatory is liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

Aims: To survey Brazil nuts (*Bertholletia excelsa*) and ground coffee *(Coffea arabica)* (roasted and packed in different countries) for mycotoxins contamination applying a multi-toxin method based on mass spectrometry. Also to evaluate the possibility of different toxins, other than ochratoxin A (OTA) and aflatoxins (AFLs), contamination in those food substrates.

Material and Methods: *Brazil nuts*: dried (processed) in-shell and shelled nuts packs of 250 and 500 g. Moisture content (7.3 and 6.9 %, respectively). Total number of samples: 17. *Roasted ground coffee:* packs of 250 and 500 g of different brands and countries (Brazil, Colombia, Finland, Italy, Sweden and The Netherlands). Total number of samples: 24. Type of packaging: under vacuum (19) and in-air (6) (moisture content 3.4 %). *Toxins evaluated:* AFLs (AFB_1; AFB_2; AFG_1; AFG_2), OTA, antibiotic Y (ANT Y), beauvericin (BEA), enniantins (A, A_1, B, B_1), fumonisins (FB_1 and FB_2), mycophenol acid (MPA), trichotecenes (neosolaniol-NEO) and zearalenone (ZON) *Equipment:* Alliance HPLC, 2695 Separations module equipped with a quaternary pump, degasser, auto sampler and a 10 μl loop. ODS-EP column (150 mm length, 2.1 mm id, 5.0 μm particle size), preceded by a guard-column (5 mm length, 2.1 mm id, 5.0 μm particle size) of the same sorbent. MS/MS spectrometer MicroMass Quattro triple-quadrupole (QqQ), Micromass Ltd (Manchester, UK) equipped with ESI probe. *Multi-toxin method:* LC-MS/MS, with electrospray ionization in the $[M+H]^+$ and $[M+H]^-$ mode (Kokkonen and Jestoi, 2008).

Results and Discussion: Only a few papers have published shelled Brazil nuts toxin contamination and those were at lower levels when compared to the in-shell nuts (Pacheco and Scussel, 2009). In the present work, no mycotoxin was detected in any of the dry in-shell Brazil nut or coffee samples up to the LOQ, method despite of the sample types, packaging country brands. It was not possible to track the coffee grain country of origin except for the Brazilian and Colombian coffee packs. Data showed the high quality of the shelled Brazil nuts and coffee products commercialized in European countries inclusive the Latin American brands. The values of LOQ obtained were

excellent, as already reported in the literature for LC-MS/MS multitoxin methods, showing the adequacy of that type of detector and it's importance to the mycotoxin field.

Conclusions: Samples analysed presented to be very safe and acomplishing to the international regulation mycotoxin wise. Utilizing high quality row material (coffee grains or shelled nuts) can unsure high quality and safe final products for consumption.

Acknowledgements: authors thank to Capes - Coordenacao de Aperfeicoamento Pessoal de Nivel Superior - the Brazilian sponsors - for providing a post-doctoral grant for VMS at EVIRA.

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4. World Mycotoxin Journal, v. 2, p. 295-304.

10:00-10:40 DEVELOPMENT OF AN UPLC-Q-TOF/MS METHOD FOR DETERMINATION OF DON AND ITS CONJUGATED GLUCOSIDE FORM IN WHEAT AND OATS

Keynote Conference

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Background: Deoxynivalenol (DON) can be found in conjugated forms in cereals. It is produced by the plants when infected by toxigenic *Fusarium* strains in order to protect themselves against the harmful effects of the toxin. This detoxification process involves several reactions such as hydrolysis, reduction and oxidation of toxins - Phase I and/or glucosylation, sulfation, acetylation and methylation - Phase II (Wallnofer et al, 1987, Ruhland et al, 2006). The main DON conjugates that have been detected in plants are the hydrosoluble glucosyde form.

Some extraction procedures for DON and DON-3-glucoside (DON-3-Glc) have been reported only for maize, wheat and their food products. They can be extracted from wheat or maize with a mixture of acetonitrile-water and purified by using MycoSep 230 column. The technique most suitable for investigating known for conjugated mycotoxins in cereals and feed ingredients to date is liquid chromatography (LC) combined with tandem mass spectrometry (MS/MS) (Berthiller et al, 2005, Sulyok et al, 2007, Zachariasova et al, 2008). However, techniques utilizing quadrupole time-of-flight mass spectrometry (Q-TOF) can detect any (known and unknown) analyte present in the sample, enables accurate mass measurement and gives very important data, useful for structural identification of unknown compounds. Furthermore, ultra performance LC (UPLC) with Q-TOF/MS gives fast data acquisition rates, with the aid of automatic peak finding and mass spectra production (Diaz-Cruz and Barcelo, 2007, Farre et al, 2007). This technique has high resolution (10 to 1000) and gives the draw-backing of 3Q technology / low mass resolution. Analytical methods for DON conjugate in cereal matrices are scarce and the presence of DON conjugates in feed matrices can be missed with the current methodology, causing unexpected high toxicity symptoms in animals.

Aim: To develop a method able to detect and identify simultaneously DON and its conjugate DON-3-Glc in oats and wheat by UPLC-Q-oa-TOF/MS, a faster acquisition rate and automatic peak finding method.

Materials and Methods: Standards of DON from Sigma (St. Louis, USA) and DON-3-Glc from Biopure (Vienna, Austria). Wheat and oats (blank and naturally DON-contaminated) from Norway, obtained in the markets of Helsinki, Finland. Clean-up Mycosep columns: 227 and 230, Romer Labs Dianostic GmbH (Herzogenburg, Austria)

and MassLynx (MetaboLynx and QuanLynx) software, Waters Corp. The leucine-enkephaline solution at concentration of 0.7 μg mL^{-1} was prepared in acetonitrile:water -0.1% formic acid (50:50). Sodium formate prepared with 0.05M NaOH in 2-propanol:water -0.5% formic acid (90:10).

High resolution full scan TOF mass spectra and real time reference mass correction was applied to analyse DON and its glycoside conjugate from wheat and oats matrices. The separation was carried out in an UPLC C_{18} column (100 mm, 2.1 mm id, 1.7 um) with gradient mobile phase composed of acetonitrile: water (0.1% formic acid) in a total run time of 4 minutes. The extraction solvent for DON and DON-3-Glc was acetonitrile:water (84:16) and the clean-up was carried out in Mycosep column. Detection of toxins was performed in exact mass chromatograms with a mass window threshold of 0.5 Da.

Results and Discussion: Calibration curve was linear from 0.5 to 12.0 mg kg^{-1} for both compounds. The method values of LOD utilizing the wheat and oats matrices were 0.25 and 1.0 and the LOQ of 1.0 and 2.0 mg kg^{-1}. Their mean recoveries from the wheat and oats samples were 89 % and 81 %, respectively. The levels obtained from the positive naturally contaminated samples ranged from 1.9 to 27.4 mg kg^{-1} for DON and 2.0 to 15.2 for DON-3-Glc, respectively and the conjugate form was also detected in theDON negative (Blank) samples. Utilizing a Metabolynx software browser some other DON metabolites were also screened and identified.

Although DON and DON-3-Glc analysis was the aim of this study, also other metabolites were investigated utilizing the software Metabolynx Browser. With that it was possible to identify other conjugates. The conjugates detected were quite diversified and were produced by the sulphation, hydroxylation or glucosylation metabolite reaction in the plant (Scussel et al, 2008).

The exact mass is a very important tool to confirm the detected unknown components in the sample matrices. For this purpose it was used an elemental composition calculator. The i-FitTM works on the basis of exact mass and matches with the elemental composition suggested with the isotopic pattern for the metabolites of interest. Typically the lowest i-FitTM value, the most likely is the correct answer, this is used together with the exact mass measurement obtained.

Conclusions: By means of UPLC-Q-oa-TOF/MS it was possible to identify and quantify DON and its mono-glucosyde metabolite in the naturally contaminated samples and also in the non-contaminated oats and wheat. Q-TOF/MS with Metabolynx was an excellent method for screening the presence of the known and unknown conjugates as it can detect all substances present in a sample. Further steps will be carried out in order to confirm the conjugates detected with MetaboLynx and QuanLynx with focus on their i-Fit possibilities. It will be also important to make available reference standards of those conjugates to enable their use in routine quantification analysis.

Acknowledgements: authors thank to Capes - Coordenacao de Aperfeicoamento Pessoal de Nivel Superior - the Brazilian sponsors - for providing a post-doctoral grant for VMS at EVIRA.

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11:10-11:40 MALDI-TOF ICMS AS A MODERN APPROACH TO IDENTIFY POTENTIAL AFLATOXIGENIC FUNGI

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Background: The *Aspergillus* section *Flavi* is among the best studied fungi, having different commercial applications, but also causing biodeterioration of commodities and food spoilage. Fungi from this Section are also responsible for the production of highly toxic secondary metabolites – the aflatoxins. They are morphologically and genetically very similar, and can be difficult to differentiate by both cultural and molecular biology methods. Besides that, new species are continuously being described in this Section. A reliable identification typically implies the analyses of a variety of morphological, biochemical and molecular traits. Recently, Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Intact Cell Mass Spectrometry (MALDI-TOF ICMS) has been used to generate spectra of protein masses in a range of 2,000 to 20,000 Da that are a *taxa* specific fingerprinting. This technique has already shown high potentialities to discriminate very closely related *taxa* and, it has been used as a new tool in the polyphasic approach to identify potential aflatoxigenic fungi.

Aim: This work aims to validate the MALDI-TOF ICMS technique on *Aspergillus* Section *Flavi* identification and authentication. As a matter of consequence, obtained results by spectral analysis were compared to those obtained by morphological, biochemical and molecular biology methods.

Materials and Methods: 1. Morphological analysis: fungi were cultured on three different media (Malt Extract Agar [MEA], Czapek Yeast Autolysate [CYA] and CYA supplemented with 20% saccharose [CY20S]); 2. Biochemical analysis: Aflatoxins and Cyclopiazonic Acid analyses were performed by HPLC; 3. Molecular biology analysis: Partial calmodulin gene was sequenced; 4. MALDI-TOF ICMS analysis: spectra of protein masses, on 2,5-dihydroxybenzoic acid (DHB) in a range of 2,000 to 20,000 Da, were obtained using Shimadzu Axima-LNR equipment and treated for fungal identification using SARAMIS^{TM} Package.

Results and Discussion: 1. A good agreement between methods on species level identification was obtained; 2. Molecular biology and spectral data analyses generated similar dendrograms with concomitant strains clustering; 3. Biochemical data analysis generated also a dendrogram which is compared with the previous ones; 4. Under the experimental conditions used spectral analyses were able to identify potential aflatoxigenic species.

Conclusion: MALDI-TOF ICMS has shown a very good resolution on the identification of *Aspergillus* Section *Flavi* species. Results obtained with MALDI-TOF ICMS were similar to those obtained by DNA sequence analysis, with the advantage of being (a) rapid, (b) inexpensive in terms of labour and consumables, and (c) reliable when compared with other biological techniques. Using MALDI-TOF ICMS the results showed a great potential to the fungal identification and it is another additional step for our polyphasic fungal identification approach. However, even with the polyphasic approach fungal identifications remain in some situations time-consuming and decisions regarding what represents a species tend to be subjective.

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11:40-12:10 DETERMINATION OF AFLATOXIN IN CASSAVA CHIPS: VALIDATION OF NEW ANALYTICAL METHOD IN CASSAVA FLOUR

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Abstract

A new HPLC – PHRED (photochemical reactor for enhanced detection) method for the analysis of aflatoxin in cassava flour was developed and validated. Samples were spiked with a mixture of each aflatoxin at 5, 10, and 20 µg/kg mixed with either 1 g or 5 g of NaCl and extracted with methanol/water (80/20, v/v) by shaking for 10 min or 30 min. Cleanup using Aflatest® immunoaffinity columns was then done after which the samples were analyzed by reverse phase HPLC with fluorescence detector and post-column derivatization using PHRED under isocratic conditions. The method was validated for recovery, linearity, and precision at the three concentrations. Recoveries ranged from 52.0 -70.0%, 69.0-85% and 80.0-89.0% at 5.0, 10.0, and 20.0 µg/kg, respectively. It would appear that the amount salt (NaCl) and the shaking time are critical in this method and showed optimal performance when 1 g of salt was used and shaking was for 10 min. There was good linearity and precision which allowed for baseline separation from interferences e.g. coumarins.

12:10-12:40 MULTIMYCOXIN ANALYSIS WITH LC-MS/MS: TOY OR TOOL?

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Background: Approximately 300-400 compounds are currently recognized as mycotoxins. Despite that, most analytical methods focus on those fungal metabolites that are addressed by legislation and some of their derivatives, which makes up approximately two dozens of compounds. In contrast to that, occurrence data on the remaining metabolites ranges from scarce to non-existent. From that, one might receive the impression that analytical routine methods are sufficient for the surveillance of health hazards posed by fungal infection. This would imply that the determination of a broader range of fungal metabolites is only of minor interest for food analysis and that the main application area of LC-MS/MS based multi-mycotoxin determination is chemotaxonomy.

Aim: The aim of this presentation is to show the valuable information LC-MS/MS based multi-methods can provide in addition to conventional analytical methods. At the same time, the methodological limitations will be covered as well in order to demonstrate that this approach is no universal remedy either.

Materials and Methods: Detection and quantification was performed with a QTrap 4000 LC-MS/MS System coupled to an 1100 Series HPLC System. Chromatographic separation was on a Gemini® C_{18}-column, 150 x 4.6 mm i.d., 5 µm particle size. Elution was carried out using an acidified water/methanol gradient containing 5 mM ammonium acetate. ESI-MS/MS was performed in the scheduled multiple reaction monitoring mode both in positive and negative polarities in two separate chromatographic runs per sample by scanning two fragmentation reactions per analyte (Vishvanath et al. 2009). Enhanced Product Ions scans were carried out using the third quadrupol as linear ion trap.

Sample preparation was reduced to a single extraction step using an acidified acetonitrile/water mixture, followed by analysis of the diluted raw extract.

Results and Discussion: The latest generation of fast-scanning tandem mass spectrometers enables to create a quantitative LC-MS/MS protocol for the simultaneous determination of more than hundred fungal metabolites in a single chromatographic run. The instruments´ sensitivity, selectivity and robustness allows the determination of low µg/kg concentrations of the target analytes even in crude extracts of complex matrices (such as nuts, raisins, DDGS or house dust), although effective counteractive measures (such as matrix-matched calibration or isotopically labelled standards) are required to deal with the suppression of the analytical signal caused by co-eluting matrix constituents.

The application of the method to real world samples revealed that routine methods are not able to extract the full information on the mycotoxin pattern in contaminated samples. In grains samples, emerging mycotoxins (such as the enniatins), phytotoxins (such as the Alternaria metabolite tentoxin) and marker metabolites (such as aurofusarin) were found to be more prevalent than the *Fusarium* mycotoxins addressed by legislation. As considers the latter, metabolic products such as zearalenols or deoxynivalenol-3-glucoside may exhibit concentrations that are in the same order of magnitude as the parent toxins.

The complexity of the pattern of mycotoxins that was observed in the investigated samples (e.g. aflatoxins, ochratoxin A and various *Alternaria* metabolites were found in a peanut sample from Turkey) emphasizes the need to simultaneously screen for different classes of mycotoxins. Unusual analyte/matrix combinations, such as aflatoxin M1 in peanuts or fumonisin B2 (but no fumonisin B1) in bread were observed as well, but these results require an additional product ion scan for confirmation.

The flexibility of the “dilute and shoot” approach enables to transfer to method to completely different matrices, such as building materials from damp indoor environments, without any modification. The contribution of airborne mycotoxins to adverse health effects reported by inhabitants of damp buildings is still a matter of intense debate, manly due to methodological gaps. The application of the developed method to 14 samples from damp buildings revealed the presence of 20 different fungal metabolites at concentrations of up to 130 mg kg^{-1}. Most of these metabolites have never been identified before in real world samples, although they are known to be produced by indoor relevant fungi (Vishwanath et al., 2009).

Conclusion: LC-MS/MS based multi-mycotoxin analysis is not meant to replace well-established single target methods for mycotoxins addressed by legislation, since it cannot always compete in terms of accuracy and sensitivity that may be obtained by a tailor-made sample preparation protocol that is often involved in those methods.

However, the comprehensiveness of occurrence data that may be obtained helps to extract the full information from infected samples and to reveal the prevalence of fungal contaminants that have not been sufficiently addressed by analytical methods so far.

Therefore, this analytical approach can considered to be a valuable tool for food safety and for mycotoxicology in general.

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12:40-13:10 A SENSITIVE RAPID ELISA TEST FOR THE DETECTION AND QUANTITATION OF T-2 AND HT-2 TOXINS IN GRAIN COMMODITIES

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Background*:* T-2 and HT-2 toxins are trichothecene mycotoxins produced by several species of *Fusarium* molds. As T-2 toxin is readily metabolized to HT-2 toxin, and the toxins have been shown to produce numerous adverse effects on many animals, these two mycotoxins are frequently evaluated together.

Animals affected by the toxins include swine, dairy cattle, poultry, dogs, cats and horses. Effects of the toxins include digestive disorders, hemorrhage, edema, oral lesions, dermatitis, and blood disorders. Damage caused by the toxins to the digestive track is irreversible. In the most severe cases, these toxins will cause death. T-2 toxin is the principal causal toxin in the human disease alimentary toxic aleukia.

Poultry studies have shown T-2 intoxication has led to a reduction in weight gain and other problems such as beak lesions, poor feathering, motor function impairment and increased susceptibility to *Salmonella* spp.

The best protection against these mycotoxins is monitoring for their presence in feeds and foods. That means testing all along the pathway from initial harvest of grains to the finished product.

Aim*:* To develop an easy to use, accurate analytical method to detect and quantitate the two toxins T-2 and HT-2 in food commodities.

Materials and Methods*:* A rabbit polyclonal antibodies-based Enzyme Linked Immunosorbent Assay (ELISA) for the detection and quantification of T-2 and HT-2 toxins in agriculture commodities was developed. The assay is a direct competitive ELISA in a micro-well format. Samples were extracted by shaking 5 g ground sample with 25 ml of 70% methanol-water (1:5) for 3 min. Extracts were diluted 1:1 with water and then used in the ELISA test. Free T-2 or HT-2 toxins in the samples and controls

are allowed to compete with enzyme-labeled HT-2 toxin (conjugate) for the antibody binding sites. After a wash step substrate is added, this reacts with the bound conjugate to produce blue color. More blue color means less T-2/HT-2 Toxins. The test is read in a microwell reader to yield optical densities. The optical densities of the controls form the standard curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of T-2/HT-2 Toxins.

Results: A very sensitive polyclonal antibodies were produced from rabbits after immunizing with T-2 /HT2 conjugated to carrier protein. The detection limit of the assay is less than 1 ng/ml (less than 10 ppb) of individual T-2 and HT-2 or a mix of both mycotoxins. The test can detect T-2 or HT-2 at 100% of either or a mixture of them in corn, wheat, barley, rye and oats. Concentration of T-2 or HT-2 required for 50% binding inhibition is 6.8 ng/ml (68 ppb). The antibody used is very specific for T-2 and HT-2. The antibody has no cross reactivity with other trichothecenes such as T2-tetraol, deoxynivalenol (DON), 3-acetyl-DON, 15-acetyl-DON, fusarenon-x, zearalenone or nivalenol. The mean recovery of T-2, HT-2 or a mixture of both in corn, wheat, barley and oats determined by this method was 87% which is acceptable recovery.The assay correlates very well with LC/MS results of naturally contaminated samples (r = 0.995).

Conclusion: T-2/HT-2 Toxins test is a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) that can be used for the quantitative analysis of T-2/HT-2 toxins in such commodities as corn, barley, oats, rye, soy and wheat. This is the first test that could detect both T-2 and HT-2 equally.

Yucatán IV

Wedneday June 30, 2010

Session 12: Mycotoxin Control.

08:30-09:00 *Lactobacillus rhamnosus* AND *Lactobacillus fermentum* WITH POTENCIAL ANTIFUNGAL ACTIVITY AGAINST *Aspergillus* SPECIES

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Background: Lactobacilli are considered to benefit the health of the consumer when ingested as probiotics. These are commonly defined as viable microorganisms that exhibit a beneficial effect on the health of the host when they are ingested. There is a increased interest to find lactic acid bacteria with favourable health effects on human and animals. The inhibitory actions of Lactobacillus spp. are based on both competition for nutrients and the production of various compounds, such as organic acids, hydrogen peroxide, bacteriocins. Lactic acid and phenylacetic acid in combination have shown antifungal properties. A variety of inhibitory mechanisms are utilized by strains of lactobacilli to inhibit fungi. One is the production of a mixture of organic acids that inhibit *Aspergillus* species. Previous studies performed for us showed that these strains presented the most characteristics needed to consider them as effective probiotic strains, such as resistance to various antibiotics used in several human and animal infections, autoaggregation, surface hidrophobicity, co-aggregation and adherence were studied (Pascual, 2004) .

Aims: To evaluate the antifungal activity of *L. fermentum* strain L23 and *L. rhamnosus* strain L60 against toxigenic species of *Aspergillus flavus.*

Materials and Methods: *L. fermentum* strain L23 and *L. rhamnosus* strain L60 were selected from among 100 strains of *Lactobacillus* on the basis of its bacteriocinogenic ability. The bacteria were grown in De Man/Rogosa/Sharpe (MRS) broth or agar at 37°C under a 5% CO_2 atmosphere for 24 h. Antimicrobial activity of L60 and L23 on Gram negative and positive bacteria was carried out by cross streak test (Pascual, 2004). L60 and L23 were assayed for aflatoxin-producing *Aspergillus flavus* inhibition by the modified agar overlay method (Magnusson and Schnurer 2001). Here, MRS agar plates on which L60 and L23 were inoculated as 2 cm long lines and incubated at 37°C for 48 h in optimal conditions. Plates were overlaid with soft agar preparation of MEA (malt extract agar) containing 1 x 10^2 fungal spores ml^{-1} determined by counting on a Neubauer haemocytometer. The plates were then incubated aerobically at 25°C for five days. They were examined for clear inhibition zones around the bacterial streaks and the clear fungal inhibition zones were scored as -, d, +, ++. A plate with the fungal

growth without L60 or L23 develop was used as a positive control. Another surface-spread agar method modified by our group was used. From two overnight cultures of L60 and L23 the MRS plates were 3/4 sown of surface and incubated in optimal conditions at 37 °C for 48 h. Subsequently, in the free surface on the MRS plates a block of agar containing *A. flavus* grown in MEA was inoculated. Plates were then incubated aerobically at 25°C for five days in darkness. A plate with the block of agar containing *A. flavus* fungal was used as a positive control. A screening by TLC (Thin Layer Chromatography) on the aflatoxin-producing A. flavus to determine variations on AFB1 production was performed.

Results and Discussion: *L. fermentum* L23 and *L. rhamnosus* L60 are both producers of secondary metabolite actives, such as organic acid, bacteriocins and in the case of L60, hydrogen peroxide. The bacteriocin production was previously characterized and purified by Pascual *et al*, (2008a,b). Both strains shown a wide spectrum of antimicrobial activity evaluated on the growth of Gram positive and negative bacteria, being some of them pathogens in human and animal infections and phytopathogenic microorganisms. These results are similar to those of Strus *et al* (2002). Furthermore, both L23 and L60 presented antifungal activity on the development of yeasts (*Candida* species) and filament fungus as *Aspergillus flavus* strains, which are aflatoxin producers. In the interaction studies, L23 and L60 inhibited the 100 % of the *Aspergillus flavus* strain assayed (n=10). Similar results were found by Onilude *et al* (2005), who used different strains of LAB. The fungal growth of these aflatoxigenic strains showed inhibition of sporulation and reduced the production of esclerotia because of the antimicrobial influence exerted by lactobacilli. Furthermore the analysis of the mycotoxin production from L60 or L23 showed a decrease on the AFB_1 production when compared with the control.

Conclusion: The lactobacilli studied in this work showed a high probiotic potential with a large inhibitory activity on aflatoxicogenic fungus. These results are important having in mind that these aflatoxicogenic *A. flavus* strains are natural contaminants of feed used for animal production which could be effectively controlled by L60 and L23.

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09:00-09:30 AFLATOXIN B_1 ADSORPTION BY YEAST CELL WALL FROM COMMERCIAL ORIGIN®

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Background. Among more than 300 mycotoxins described as yet, aflatoxin B_1 (AFB_1) and the group of fumonisins (FBs) are the toxins of major concern in tropical and sub-tropical regions. Aflatoxins are toxic secondary metabolites produced by species of *Aspergillus* genus, mainly *A. flavus* and *A. parasiticus* and its effects are carcinogenic, mutagenic, teratogenic and hepatoxic. Aflatoxin B_1 is one of the most potent known hepatocarcinogens. Mycotoxin contamination of feed is a serious problem because they reduce feed consumption, decrease growth rate and reduce immunity. Besides the health aspects, which involves the presence of toxins or toxic metabolic products in food and / or by-products such as meat intended for human consumption, its presence in animal feed has also important economic connotations causing losses by increasing mortality and production (Rosa et al., 2001, 2006). One of the most effective methods to control risks of mycotoxins in animal husbandry is based on the use of specific materials that adsorb mycotoxins. These substances adsorb the toxins in the gastrointestinal tract to form insoluble complexes that are eliminated in the feces. Thus, the toxic effects are diminished by reducing the bioavailability of mycotoxins. In particular, the use yeast cell β-glucans and mannans- walls (PL), mainly composed of oligosaccharides. They are usually introduced as a food additive in the animal production industry since the 90s.

Objective. To evaluate the efficacy of a commercial yeast cell wall to adsorb AFB_1.

Materials and methods. Adsorbent: a yeast wall of commercial origin (Safmannan ® - Saf do Brasil - Agricultural Division) was resuspended in buffer at pH 2. **pH 2 buffer solution:** 50 mL of a solution of potassium chloride (KCl) 0.2 M was added 13 mL of a solution of hydrochloric acid (HCl) 0.2 M. **Aflatoxin B_1 Solution:** 10 mg of AFB_1 (Sigma) were resuspended in methanol (MeOH) to obtain a solution of 2 mg/mL. From this, the toxin was diluted to obtain the required concentrations for each test. **Adsorption test:** to determine the potential adsorption, saturation isotherms were previously made with different concentrations of the mycotoxin (250, 200, 100, 50, 10, 5, 2, 1 and 0, 1 mg/ml). Then, adsorption isotherms were made between the PL (2 mg/ml) with AFB_1 (15,346, 10:33, 7:34, 2:08 and 4.83 mg/ml). Tests were assayed at pH 2 and 6, in triplicate. All mycotoxin concentrations were evaluated using high performance liquid chromatography (HPLC).

Results and Discussion. Figures 1 and 2 show the adsorption isotherms at pH 2 and 6, respectively. Visual inspection shows a S-type isotherm, following a Hill model that explains the cooperative adsorption isotherm between the toxin and the adsorbent. The mathematical expression of the adjustment equation is □=□$_{max}$ [ZEA]^n/k_D^n+[ZEA]n where □□is the mass of adsorbed ZEA by g of PL, n the number of sites and k_D constant adsorption site.

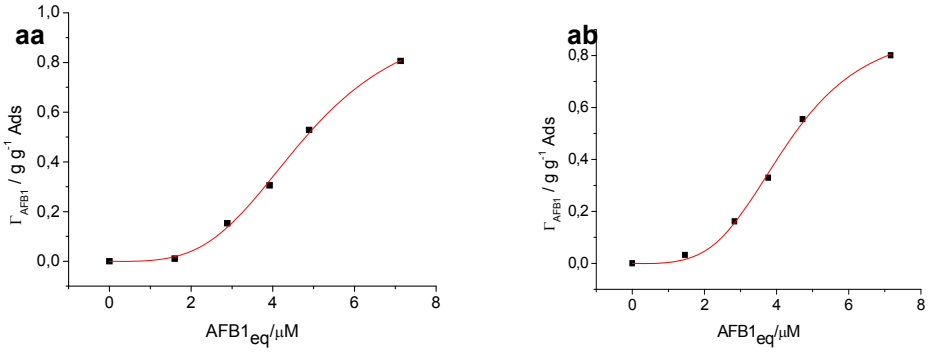

Figure 1. Adsorption isotherm of AFB_1 by Safmannan®, (a) pH 2, (b) pH 6.
Among the most important parameters we consider a saturation value of 0.918 ± 0.056 mg of adsorbed AFB_1/g PL at pH 2 and 1.002 ± 0.089 mg of adsorbed AFB_1/g PL at pH 6. The association constant per site was 0.233 ± 0.044 and 0.208 ± 0.063 ng/mL-1 (Table 1).

Table 1. Setting parameters obtained by Hill model to evaluate the adsorption isotherms from AFB_1 and Safmannan® at different pH.

Adsorbent	ppH	k_d (μM)	□□□□□□□□□	□$_{max}$ (g/g)	*n*	N	RR^2
Safmannan®	D2,0	4,291±0,189	0,233±0,044	0,918±0,056	3,829±0,453	5	00,996
	S6,0	4,806±0,303	0,208±0,063	1,002±0,089	3,619±0,459	5	00,996

k_d = dissociation constant, β = association constant, □$_{max}$ = masimum capacity of adsorption, *n* = number of sites for coorperativism, N = number of curve points. Each point is the mean of triplicates.

Conclusion. Aflatoxin B_1 was efficiently bound by PL through a cooperative attraction mechanism. This result shows the potential of this PL to prevent the toxic effects caused by the intake of ZEA
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09:30-10:00 AQUEOUS OZONE, A FRIENDLY METHOD FOR AFLATOXINS DEGRADATION

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Background: Aflatoxins (AF) are highly toxic and hepatocarcinogenic metabolic compounds produced by *Aspergillus* species such as *A. flavus*, *A. parasiticus* and *A. nomius* during infection and colonization of food raw materials as cereals, pulses and tree nuts and their by products. Several chemical methods have been shown to be effective on removing aflatoxins; however, they need to fulfill many characteristics to be approved by regulators agencies or, in some cases, they are still too expensive to be feasible on an industrial scale. One of the agents with great potential to reduce mycotoxins is ozone. It is effective against many mycotoxins and leaves no toxic residues after treatment.

Aim: to evaluate the use of aqueous ozone on degradation of AFB_1, AFB_2, AFG_1 and AFG_2.

Materials and Methods: Water saturated with ozone was prepared by bubbling gas, generated by passing ***extra-dry oxygen through*** an air-cooled corona discharge generator (Model CD-COM-HF-4) for 10 minutes (with the power generator at 100%; gas flow of 25 L / hours) in a bottle with 1000 mL of Milli-Q water, at 3 ºC. A concentrated ozone solution with *c.a.* 20 mg/L was obtained and was diluted with ozone demand free water. An aflatoxins stock solution (AFB_1 and AFG_1 – 2 mg/L each and AFB_2 and AFG_2 - 0.5 mg/L each) was used and it was diluted with ozone demand free water to the final working concentration. The degradation of mycotoxins by aqueous ozone was tested at 5 defined aqueous ozone levels: 0 (Control), 0.1, 1.0, 10 and 20 mg/L. A volume of 20 μL of each aflatoxin solution was applied into 2 mL vials, containing 200 μl of each aqueous ozone solution or ozone demand free water (Control) and left reacting for 30 minutes. To stop the reaction, 1200 μL of the mobile phase (see below) was added. Five replicates at each condition were made. Samples were analyzed using a HPLC equipped with a Jasco FP-920 fluorescence detector at 365 and 435 nm (excitation and emission, respectively), using photochemical post-column derivatization (PHRED unit). Chromatographic separation was performed on a reverse phase C_{18} column (Waters Spherisorb ODS2, 4.6 x 250 mm, 5 μm), fitted with a pre-column with the same stationary phase. The mobile phase used was pumped at 1.0 mL/min and consisted of an isocratic program as follows: water:acetonitrile:methanol (3:1:1, v/v). The injection volume was 100 μL.

Results and Discussion: AFB_1 and AFG_1 were more sensitive to ozone treatment than AFB_2 and AFG_2, since ozone acts preferentially against unsaturated compounds, by what may be classified by an electrophilic attack. The higher sensitivity of AFB_1 and AFG_1 is due to the 8,9 double bond forming the vinyl ether at the terminal furan ring (Mackenzie et al, 1998; Proctor et al, 2004), which is not present in AFB_2 and AFG_2. These results reinforce the relevance of this degradation strategy, since the more toxic aflatoxins (AFB_1 and AFG_1) (IARC, 2002; Proctor et al, 2004) are also the most sensitive ones to ozone. The highest degradation level for AFB_1 was 100%. The effective degradation of AFB_2, AFG_2 and AFG_1 was 8.06, 15.96 and 98.49%, respectively. As expected, the maximum degradation occurred at the highest ozone level (20 μg/mL).
Conclusion: Aqueous ozone can be used as a treatment for AF decontamination. Ozone foaming ability allows it to be trapped in a better way in water, since water is an industrial vehicle for washing and cleaning processes. However, gaseous ozone was found to be more effective then aqueous ozone, in the degradation of AFB_1 from dried figs (Zorlugenç et al., 2008).

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10:00-10:30 ADSORPTION OF AFLATOXIN B_1, ZEARALENONE AND OCHRATOXIN A BY MYCOTOXIN BINDERS FROM MÉXICO

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Background: Mycotoxin co-contamination of animal feeds and human food is a common and worldwide issue. Aflatoxin B_1 (AFB_1), a Group 1 carcinogen produced by *Aspergillus flavus* and *A. parasiticus.* This toxin is hepatotoxic, immunotoxic, antinutritional and has been linked to hepatocarcinoma (IARC, 2002). Zearalenone (ZEN) is an estrogenic mycotoxin produced by *Fusarium graminearum*, which can occur in corn, moldy hay, and pelleted feeds (CAST, 2003). Swine are most commonly affected, but cattle and poultry can also be affected by ZEN (Diekman & Green, 1992). Ochratoxin A (OTA) is another mycotoxin found in contaminated grains, wines and coffee. It is classified as possible human carcinogen (Group 2B) and is produced by *Aspergillus ochraceus* and *Penicillium verrucosum.* Human exposure occurs mainly through consumption of contaminated grain and pork products. Studies have correlated OTA exposure to the Balkan endemic nephropathy. OTA causes renal toxicity, nephropathy and immunosuppression in several animal species (IARC, 1993). In pioneering studies in Texas, inclusion of calcium montmorillonite clay (NovaSil) in animal feeds has been shown to notably reduce the adverse effects associated with exposure to the aflatoxins, by decreasing their bioavailability from the gastrointestinal tract (Phillips et al., 2006). However, comparable strategies for ZEN and OTA adsorption remain elusive. The extensive use of clay by the farm animal industry has led to the introduction of a variety of diverse products (labeled as mycotoxin binders) for use in feed. Thus, it is important to determine the effectiveness and safety of these products.

Aim: To characterize and compare the efficacy and relative safety of 12 different products distributed in México as mycotoxin binders utilizing: 1) equilibrium isothermal analysis for sorbent/toxin surface interactions for AFB_1, ZEA and OTA, 2) various mineralogical probes to determine potential correlations of mineral contents, and 3) a *Hydra vulgaris* bioassay.

Materials and Methods: The binders included Volclay (VOL), Zeotek (ZEO), Duotek (DUO), Mexsil (MEX), Mycosil (MYC), Mycosorb (MSB), Klinsil (KLS), Toxinor (TOX), Fixat (FXT), Myco-Ad (MCA), Mycofix (MIX) and Milbond (MLB) and were compared to NovaSil Plus (NSP). Isotherms for AFB_1 (Sigma Chemical) adsorption were conducted at pH 2 and pH 6.5. Isotherms were run in triplicate (5 mL per tube) and consisted of 11 different concentrations of AFB_1 with 0.1 mg of each binder. Controls consisted of water, water plus binder and AFB_1 (8 ppm). Analyses for ZEN and OTA sorption were performed at pH 2 and 6.5 with the binders that claimed to bind both mycotoxins. Binders were compared to Activated Carbon (AC), Cetylpyridinium-Low pH Montmorillonite (CP-LPHM) as positive controls for ZEN sorption. Isotherms for ZEN were performed using 0.05 mg of binder, ZEN at 4 ppm and 11 dilutions that ranged from 0.2 ppm to 4 ppm, while for OTA, single point isotherms were run using a concentration of 8 ppm. AC was used as control for OTA isotherms. Mineralogical analyses included determination of swelling volume (SV) and, X-ray diffraction (XRD) to confirm smectite presence, and fractionation procedures to calculate the percentages of sand, silt, and clay in each binder. The *Hydra vulgaris* bioassay was used as preliminary safety evaluation. The Hydra bioassay was performed by exposing the hydra to three different concentrations of each additive (0.1%, 0.3% and 0.5%), selected according to the inclusion rates recommended for animal feed.

Three healthy adult hydra were placed in a dish with 4.0 ml of medium containing 1mM $CaCl_2$ dihydrate and 0.458mM TES [N-tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid sodium salt] buffer (adjusted to pH 7) and were maintained at 18°C. Hydras were examined for signs of toxicity at 0, 4, 20, 28, 44, 68 and 92 h. The toxic endpoint was determined by the 'tulip' or 'disintegration' stage of the hydra. In each test, experimental treatments were compared with untreated and solvent controls.

Results and Discussion: Computer-generated isotherm data were analyzed, and the semi-empirical parameters of Q_{max} and K_d were calculated for each binder and the respective mycotoxin. L-shaped curve characteristics were observed for all the binders in the isothermal sorption of AFB_1 at pH 6.5 with varied r^2 values. The most effective binders for AFB_1 at both pH conditions were NSP (Q_{max} = 0.40), MLB, MCA and VOL while the least effective was MSB (Q_{max} = 0.01). Volclay had the highest SV value (4.0), suggesting high amounts of smectites while the other products had values between 0 and 2.92. XRD analysis confirmed the presence of smectite (montmorillonite) in most of the products except MEX, MSB, DUO and MIX. For ZEN adsorption, C-shaped curve characteristics were observed for most of the binders at pH 2 and 6.5 with varied r^2 values. AC and CP-LPHM showed different patterns of sorption (L and S respectively), suggestive of more effective binding properties. For OTA, the percentages of OTA bound in the clays were calculated, and none of the binders resulted effective as adsorbent when compared to the control, AC, which showed an effective adsorption pattern. For the mineral characterization, most of the binders showed evidence of smectite except for MEX, MIX, DUO and MSB. Most of the binders appeared to be safe, based on the hydra bioassay, except for MSB, DUO and ZEO.

Conclusions: In general, 1) NSP demonstrated the highest sorption capacity for AFB_1 at pH 2 and 6.5 followed by MCA, MLB and VOL; 2) most of the binders showed evidence of smectite, except MSB, DUO, MEX and MIX; 3) none of the binders showed an effective pattern of sorption for ZEN, except for controls, AC and CP-LPHM; 4) Preliminary data for OTA sorbents showed low binding percentages when compared to AC; 5) Most binders appeared safe for hydra except ZEO, DUO and MSB at 0.3% and 0.5% inclusion.

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11:00-11:30 BIOCONTROL OF *ASPERGILLUS FLAVUS* BY USING CERTAIN MICROBES – A STRATEGY TO REDUCE AFLATOXIN CONTAMINATION IN SORGHUM GRAINS

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Background: Sorghum grains suffer from severe infection and colonization by several toxigenic fungi and subsequent production of mycotoxins, posing a threat to human and animal health. Among all the mycotoxins, aflatoxins represent one of the most important toxicants which are considered as an important constraint for grain quality and production in sorghum. Various physical and chemical methods of reduction of mycotoxins have been recommended but only few have been accepted for practical use. Biodegradation of aflatoxins deploying microbes is an attractive strategy for the mycotoxin management.

Aim: This study aimed to explore the potential use of certain biocontrol agents for the inhibtion of toxigenic *Aspergillus flavus* growth and subsequent reduction of aflatoxin B1 (AFB1) in sorghum.

Materials and Methods: In this study, 4 various biocontrol agents deployed i.e. *Rhodococcus erythropolis* (MTCC 1526), *Pseudomonas fluorescens* (OU-593), *Bacillus substilis* (MTCC 121) and *Trichoderma viride* (MTCC 800) and culture filtrates were prepared using standard microbiological methods in the literature. The efficacy of these biocontrol agents on the growth of toxigenic *Aspergillus flavus* was assessed by preparing A. flavus spore suspension and testing of these biocontrol agents on aflatoxin B1 produced by this fungus on sorghum grains. Further, these *A. flavus* infected seeds were subjected to ELISA for the detection of varying levels of aflatoxin B1 in sorghum samples.

Results and Discussion: In this present study, we tested three bacterial species (*B. subtilis*, *P. fluorescens* and *R. erythropolis*) and a fungus (*T. viride*) for AFB1 degradation. *B. subtilis* inhibited the growth of *A. flavus* (72%) and reduction of AFB1 (54%) at a concentration of 200 ml/kg of sorghum grains. *P. fluorescens* culture filtrate showed an 74% inhibition of *A. flavus* growth and 62.6% AFB1 reduction. *R. erythropolis* showed a cent percent inhibtion of *A. flavus* growth and AFB1 reduction. Similarly, *T. viride* showed an inhibition of 65% *A. flavus* growth and 39% AFB1 reduction at 200 ml/kg of sorghum grain. This was further furnished in the literature that *Bacillus spp.*, *Pseudomonas spp., Rhodococcus spp. a*nd *Trichoderma spp.* Able to inhibit mycotoxigenic fungi and degrade mycotoxins including AFB1.

Conclusions: The inhibitory acitivity was likely due to the extracellular nature of the metabolites produced by these microbes in the growth medium. One of the striking features is that, these metabolites were stable over a wide range of pH and temperature (121o5 C for 30 6 minutes). Therefore, the potential use of these microbial metabolites to control mycotoxigenic fungi should be thoroughly investigated since many chemical fungicides are being out from the commercial market.

11:30-12:00 EFFECT OF PLANT EXTRACTS ON CITRININ PRODUCTION BY *PENICILLIUM CITRINUM*

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Background:

The excessive and indiscriminate use of organic pesticides has resulted in ill effects on soil health, health hazards to humans, toxicity to useful non-targeted animals and environmental pollution (Roy & Dureja, 1998). Therefore, alternatives to synthetic pesticides are needed. Effective phytocompounds are expected to be far more advantageous than synthetic pesticides, as they are easily decomposable, not environmental pollutants and possess no residual or phytotoxic properties (Rao 1990). The effects of plant extracts and essential oils on the growth of food spoilage and plant pathogenic fungi have been studied; however, there is hardly any data on the effect of these compounds on mycotoxin production by toxigenic fungi.

Aim:

The aim of this investigation was to study the *in vitro* effect of different plant extracts on citrinin production by *Penicillium citrinum*.

Materials and Methods:

The plants used were eucalyptus (*Eucalyptus globulus*) and marigold (*Calendula officinalis*). Previous studies have demonstrated that ethanolic and chloroformic extracts of these plants were inhibitory for the growth of several toxigenic food spoilage fungi. Plant extracts were prepared by mixing 50g of each plant with 250 ml of the solvent. The mixture was mechanically homogenised at 300 rpm for 1h. The extracts were filtered and evaporated to dryness at 45ºC. Dry extracts were dissolved in ethanol and sterilized by filtration. The *P. citrinum* strain was maintained on Potato Dextrose Agar. Spore suspensions were prepared and diluted in a sterile Tween 80 0.05% solution to a concentration of 10^6 spores/ml. Petri plates of Malt Extract Agar (MEA) and Czapek Yeast Extract Agar (CYA) containing the corresponding plant extract were inoculated with 2 μl of the spore suspension. The concentrations of extracts evaluated were 500, 250, 100, 50 and 10 μg/ml media. Controls were prepared by adding the same concentration of ethanol. The plates were incubated 7 days at 25ºC. Citrinin production was determined by TLC applying the “plug” method described by Filtenborg & Frisvad (1980).

Results and Discussion:

Only the eucalyptus ethanolic extract produced a considerable reduction on citrinin biosynthesis at the concentrations evaluated. On MEA, no significant differences were observed between all the treatments and the control. On CYA, the eucalyptus ethanolic extract produced a decreased in the amount of citrinin production at low concentrations (50, 100 and 250 mg/ml), but an increment to 500 mg/ml resulted in an accumulation of citrinin similar to that observed in the control. The maximum inhibition (42%) was registered with the ethanolic fraction of eucalyptus at 100 mg/ml on CYA. The marigold extracts were not effective to inhibit citrinin production by *P. citrinum* on any of the culture media. Moreover, the ethanolic extract of Marigold increased to 150% the amount of citrinin accumulated when suboptimal doses (50, 100 and 250 mg/ml) were used on CYA.

Conclusions:

Several essential oils and plant extracts have been reported to inhibit postharvest and food spoilage fungi in vitro such as *Penicillium* spp. (Smid et al., 1995; Arras and Usai, 2001). However, little attention has been given to the effect of these compounds on mycotoxin production by toxigenic species. When suboptimal doses of the natural antifungal are applied, growth might be partially inhibited and food spoilage is delayed, but a non-desirable effect on toxin production could be produced. The results of the present study draw attention on that effect and suggest the necessary evaluation of the influence of plant extract and essential oils on mycotoxin production. The use of natural antimicrobial compounds is important not only in the preservation of food but also in the control of human and plant diseases of microbial origin.

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12:00-12:30 EFFICACY OF STICKERS IN THE APPLICATION OF THE BIOCONTROL AGENTS *BACILLUS AMYLOLIQUEFACIENS* AND *MICROBACTERIUM OLEOVORANS* ON THE MAIZE SEED.

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Background: Maize is the host of *Fusarium verticillioides* fumonisins producers. Biological control represents an environmentally friendly alternative to the use of chemical pesticides.Treatment of seed with *Bacillus amyloliquefaciens* and *Microbacterium oleovorans* reduced *Fusarium verticilliodes* count from maize grains, and also significantly decreased the amounts of fumonisinas B_1 and B_2 detected in sampled grains from plants grown from treated seeds (Pereira, P. *et al*. 2007, 2009). In previous studies of production of biomass of *B. amyloliquefaciens* and *M. oleovorans*, the medium select were molasses (20 g l^{1}) + soy powder (10 g l^{1}) due to the great growth rate and high survival after freeze-drying process. Seed coating is a general term for the application of finely ground solids or liquids containing dissolved or suspended solids to form at covering the seed coat (Scott, 1989). The process of seed coating usually involves the use of adhesives, also know as stickers, to bind material to the surface of the seed.

Aim: To investigate the efficacy of four stickers in seed coating of freeze-drying and fresh cells inoculum, and to determine survival of *B. amyloliquefaciens* and *M. oleovorans* after storage time *in vitro* assays.

Materials and Methods: the production medium molasses (20 g l^{1}) + soy powder (10 g l^{1}), modified with NaCl at 0.99; 0.98; 0.97 and 0.96 a_w, were inoculated with 1% inoculum in exponential phase of *B. amyloliquefaciens* and *M. oleovorans*, and incubated at 30ºC and 140 rpm, for 24 h. Fresh cells were obtained in stationary phase of growth. The cells were protected by the addition of 10% sucrose solution before freeze-dried process. The cells were freezing directly at -20ºC for 4 h, immediately afterwards at -80ºC for 24 h and after were connected to a freeze-drier operating a chamber pressure <0,05mbar and -45ºC, for 72 h. Four stickers were prepared as water-based solutions: paraffinic oil 2%; carboxi methyl cellulose 1%; melaza 10% and gum arabic 5%. Seeds (DK747 MG RR2) were soaked for 15 min in the sticker solution (1ml/100 seed) and powder freeze-dried or fresh cells of the biocontrol agents were mixer in a plastic bag, after 24 h, the CFU/seed was determined from 5 coated seeds. The seed were homogenized with 5 ml of sterilized water in a vortex mixer and serial decimal dilutions were spread-plated onto the surface of Petri plates containing nutritive agar (Bardin and Huang, 2003). CFU counts on maize seeds were determined after the

coated seed were dried and after four weeks of storage in plastic bag at room temperature.

Results and Discussion: The maize seed inoculated with both formulations, powder freeze-dried and fresh cells, treated separately with the four stickers and stored at room temperature, showed different counts and viability of *B. amyloliquefaciens* and *M. oleovorans.*
The a_W have influence in the viability with high survival at 0.99 and decrease at 0.98; 0.97 and 0.96 a_W .The freeze-dried formulation showed high survival of *B. amyloliquefaciens* and *M. oleovorans* during four weeks, and also effect of the different stickers compared with control treatment were demonstrated. The high count of *B. amyloliquefaciens* and *M. oleovorans* was obtained with paraffinic oil 2% sticker at 0.97 and 0.96 a_W using the freeze-dried formulation.
The advantages of freeze-drying are protection from contamination or infestation during storage, long viability and ease of strain distribution (Smith and Onions, 1983).

Conclusion: This study has shown that freeze-drying formulation of both biocontrol agents at 0.97 water activity and paraffinic oil 2% as sticker could be applied to the maize seeds to improve the seed treatment and protection against *Fusarium verticillioides* colonization.

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12:30-13:00 PRODUCTION OF *FUSARIUM VERTICILLIOIDES* BIOCONTROL AGENTS *BACILLUS AMYLOLIQUEFACIENS* AND *MICROBACTERIUM OLEOVORANS* USING DIFFERENT GROWTH MEDIA AND EVALUATION OF BIOMASS FREEZE-DRYING.

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Background: Previous studies showed that *Bacillus amyloliquefaciens* and *Microbacterium oleovorans*, significantly reduced *Fusarium verticilliodes* count and fumonisin B_1 and B_2 content from maize grains (Pereira *et al*, 2007, 2009). *B. amyloliquefaciens* and *M. oleovorans* are more tolerant to ionic and nonionic potential stress that matric potential stress, and showed tolerance to heat-shock at 45ºC in the physiological adaptation assays (Sartori *et al.,* 2009). Formulation is necessary in order to optimize the efficacy, stability, safety and ease of application of the product (Rhodes, 1993).

Aim: To compare the viability and biomass production of *B. amyloliquefaciens* and *M. oleovorans* in different growth media, and the efficiency of freeze drying method, as possible formulation process.

Materials and Methods: *B. amyloliquefaciens* and *M. oleovorans* were grown in 100 ml of four different media: 1) nutrient yeast dextrose broth (NYDB). 2) molasses (20 g l^{-1}) + soy powder (10 g l^{-1}) 3) sucrose (g l^{-1}) + yeast extract (5 g l^{-1}) (Costa *et al*., 2001) 4) lactose (12,7 g l^{-1}) + tapioca (16,7 g l^{-1}) + peptone (8 g l^{-1}) + ammonium sulfate (1,8 g l^{-1}) (Rao *et al*., 2007). Media water activity were modified with NaCl to 0.99; 0.98; 0.97 and 0.96, incubated at 30ºC and 140 rpm. The growth were determined spectrometrically to 620 nm, and growth rate were calculated. Results of this study allowed to select two media: 1) nutrient yeast dextrose broth (NYDB) and 2) molasses (20 g l^{-1}) + soy powder (10 g l^{-1}).To test freeze-drying method operating chamber pressure <0,05mbar and -45ºC, for 72 h was used. Viability of *B. amyloliquefaciens* and *M. oleovorans* were determined before and after freeze-drying process.

Results and Discussion: *B. amyloliquefaciens* and *M. oleovorans*, showed highest growth rate in molasses (20 g l^{-1}) + soy powder (10 g l^{-1}) medium. Growth profiles demostrated that both bacteria have high growth rates at the highest a_w level, with growth reduced in the range a_w: 0,97-0,96. The biomass obtained in sucrose + yeast extract medium and lactose + tapioca + peptone + ammonium sulfate medium was not enough to freeze-dry.

B. amyloliquefaciens showed the highest survival after freeze-drying of biomass obtained in NYDB medium to 0,99 a_w, whereas at 0.98, 0.97 and 0.96 a_w the highest survival was obtained in molasses + soy powder medium.

M. oleovorans showed greater survival in molasses + soy powder medium at 0.99, 0.98 and 0.97 a_w than NYDB medium. Not survival of biomass obtained from NYDB medium at 0.96 a_w was observed.

Conclusion: molasses (20 g l ^{1}) + soy powder (10 g l ^{1}) was select for biomass production due to the great growth rate and the high survival after freeze-drying process. These results could have important implications to optimize the formulation process of the biocontrol agents.

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Celestún
Monday June 28

16:30-20:00 Round Table: Definition of the mycotoxin problems in the pecuary industry.
Moderator: René Márquez

Celestún
Tuesday June 29, 2010.

Session 13: Mycotoxins in swine and livestock.

08:30-09:00 INHIBITION OF *Aspergillus* SECTION *Flavi* BY LACTIC ACID BACTERIA (LAB) ISOLATED FROM BREWER´S GRAINS FOR SWINE FEED

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Background: Aflatoxins (AFs) are secondary metabolites produced mainly by *Aspergillus flavus* and *A. parasiticus*. There are four types of AFs: aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2). The presence of AFs in feeds causes significant economic losses and serious health problems. In fact, AFB_1 is one of the most potent natural liver carcinogens known. The use of biological control agents is a possible alternative to synthetic fungicides and pesticides. Lactic acid bacteria (LAB) have been widely studied to be applied in biopreservation. The inhibition action of LAB is based in the both competition against other microorganisms and production of various antimicrobial compounds such as lactic acid, diacetyl, acetaldehyde, acetic acid, hydrogen peroxide and bacteriocins.

Aims: To study fungal and lactic flora and to evaluate the effect of LAB on *A. flavus* and *A. parasiticus* AFs producing isolated from brewer´s grain for swine feed.

Materials and Methods: Samples of brewer's grain were collected from a craft brewery located in Villa General Belgrano (31º 59' S 64º 34' O), Córdoba, Argentina, from May 2008 to February 2009. Samples were collected at different storage times, at cero (extracted from the fermentation tanks after that the process was completed) and at seven days (taken from storage bag before feeding pigs). The pH, temperature and water activity (a_W) data were taken. Quantitative enumeration was done using the surface-spread method. Serial dilutions (10^{-1} to 10^{-3}) were made and 0.1 ml aliquots were inoculated by duplicate on the culture media DRBC and DG18. Plates were incubated at 25ºC for 7-10 days in darkness. The results were expressed as CFU per gram of sample (CFU g^{-1}) and fungal species were identified. Pour plate technique in

Man Rogosa and Sharpe Agar (MRS agar) was performed to count LAB. Plates were incubated in microaerophilic conditions at 37°C for 48 h. The results were expressed as CFU per gram of sample (CFU g^{-1}). All counts were performed by triplicate and the lactic flora was identified. A total of 137 *A.* section *Flavi* strains isolated from brewer´s grain were assayed for AFs production according to Geisen *et al*. (1996). Homologous interaction tests were performed using the technique of cross streaks and LAB isolated was assayed for inhibition on aflatoxin-producing fungi (Magnusson and Schnurer (2001).

Results and Discussion: The pH values ranged from 4.22 to 7.43, whereas a_W values were over 0.989 in the samples tested. Fungal counts showed a range of 1 x 10^2 to 3.9 x 10^6 and 6.9 x 10^3 - 6.6 x 10^6 in DRBC and 3 x 10^2 - 8 x 10^6 and 1 x 10^3 - 6.6 X 10^6 for DG18, at 0 and 7 days of storage, respectively. These fungal counts were similar with those reported by Cavaglieri *et al*. (2009) who worked with barley rootlets. Over 50% of the samples exceeded the maximum allowed, 1 x 10^4 CFU g^{-1} (GMP, 2008). These results suggest a high fungal activity determining a low quality and inadequate storage of the substrate. Yeasts had an isolation frequency above 75% in both kinds of samples. The toxicogenic genus with highest incidence was *Aspergillus* spp. (79 y 74 %) followed by *Penicillium* spp. (65 % y 52 %) at 0 and 7 days of storage, respectively. The most frequent species was *A. flavus* (75 and 92% at 0 and 7 days). These results agree with Simas *et al*. (2007). Counts of LAB ranged from 1.9 x 10^5 to 4.4 x 10^9 CFU g^{-1} at 0 and 7 day of storage. The most frequent genus isolated was *Leuconostoc* spp., followed by *Lactobacillus* spp. These results agree with Olstorpe *et al*. (2008). From 136 tested *Aspergillus* section *Flavi* strains, 89.3% and 76.3% at 0 and 7 days of storage, respectively, were AFB_1 producers. The AFB_1 production showed a range of 0.57 to 26.47 and of 0.21 to 32.14 ppm at 0 and 7 days of storage. Homologous inhibition tests between BAL showed that 83.3% of the strains had inhibitory activity with inhibition halos means from 22 ± 7.3 mm. Preliminary studies show the ability of some of these BAL to reduce the development, sporulation and sclerotia´s production of aflatoxin-producing fungi.

Conclusion: These brewer's grain present toxigenic moulds species which makes this alternative feed is not sufficiently suitable for animal consumption. The LAB could be applied as biocontrollers to minimize mainly the growth of aflatoxigenic fungal strains reducing economic losses in the swine production.

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09:00- 09:30 EVALUATION OF AN ALUMINOSILICATE TO DECREASE THE TOXIC EFFECTS OF AFLATOXIN B1 IN SWINE.

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Background: Aflatoxin B1 (AFB1) is a mycotoxin with carcinogenic, teratogenic, mutagenic and immunosuppressant properties; it is produced by several species of the *Aspergillus* genus (*flavus, parasiticus, nomius*y *pseudotamarii*). Its target organ is the liver (hepatotoxic). The effects of aflatoxins (AFs) in swine depend on the age, concentration in feed, and exposure periods. Problems related to contamination with these toxins have been reported in all stages of swine lifespan. Its most common effects are: weight loss, anorexia, ataxia, roughened hair, tremors, comma and death (Coppeck et al., 1989), other reported effects include decreases in feed conversion, hepatitis, nephrosis and systemic hemorrhages (Hoerr et al., 1983, Miller et al., 1982). Several aluminosilicates have been reported to reduce the effects of aflatoxins in swine diets (Ramos and Hernandez, 1997). Thus, the study proposal was to evaluate the reduction of the negative effects produced by AFB1 in contaminated feed by incorporating a phyllosilicate in swine diets.

Aim: to evaluate the efficiency of a mycotoxin binder AFUMTEK, to decrease the toxic effects of a diet contaminated with 680 µg/kg (ppb) of AFB1 in swine balanced feeds.

Materials and Methods: 18 swine were used, weaned at 21 days of age divided into 3 groups with 6 repetitions. They were housed in individual corrals with swine use floor. A week of contiditioning was considered. Commercial feeds were used, they were proven to be free of contamination with other mycotoxins (AFs, fumonisin B1, Ochratoxin A, DON and T.2). The treatments were: 1. Negative control diet, 2. Positive control diet with 680 ppb of AFB1, 3. Challenge diet with Afumtek adsorbent: 5 kg/t + 680 ppb of AFB1. The experiment took 21 days. Swine were weighed at the beginning of the experiment and individual weights were recorded weekly. Feed conversion was calculated weekly. At the end of the experiment all swine were sacrificed; during the necropsy, samples of tonsils, lungs, livers, gallbladders, kidneys, spleens and mesenteric lymph nodes. All organs were previously weighed. Samples were submitted to histopathology studies. Fat, iron, vitamin A and AFM1 were quantified in livers. Biochemical parameters were analyzed in sera (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyltransferase (GGT), glucose, local proteins, albumen, creatinine, triglycerides, cholesterol, urea, uric acid) and vitamin A. The information obtained was analyzed with the Tukey test using the SYSTAT statistical software; the significance value was based on a probability of 0.05.

Results and Discussion: significative statistical differences were observed in animal weight gain, total weight, feed consumption, alkaline phosphatase, GGT and total

proteins. In animals in which damages produced by aflatoxins were observed, a recovery was obtained from the challenge diet that included the adsorbent. The decrease in weight gain of the positive control group was of 31.2% compared to the negative control group. 52.3% of the weight gain difference was recovered in the challenge group. The rest of the evaluated parameters only showed numeric differences. The histopathological analysis showed the effects of consuming aflatoxins in livers and kidneys. Adding mycotoxin adsorbents in the diets of animals exposed to contamination with aflatoxins diminished economic losses attributable to these toxins, especially in large productive facilities.

Conclusion: AFB1 at the concentrations used in the experiment affected animals. The increase of the concentration of alkaline phosphatase and GGT enzymes indicate the effects of AFB1 in swine. The decrease of these enzymes in the group given a diet with adsorbent is another proof of the protection afforded by the adsorbent. An increase in profits is also expected in the group of animals that consumed the AFs and the binder due to weight recoveries.

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09:30-10:00 EFFICACY OF CURCUMINOIDS FROM TURMERIC (*Curcuma longa* L.) POWDER IN REDUCING THE TOXIC EFFECTS OF AFLATOXIN B_1 IN WEANLING PIGS

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Background: Aflatoxins (AF) are contaminants of feed ingredients used in livestock rations and in human food and are produced by the fungi *Aspergillus parasiticus* and A. *flavus*. Aflatoxin B_1 (AFB_1), the most potent among the AFs, has been shown to cause feed refusal, decreased feed efficiency, impaired reproduction, immune suppression, jaundice, and hepatitis/cirrhosis of the liver in pigs (Newberne, 1973; Robens and Richard, 1992). AFB_1 also increases free radical production, leading to oxidative damage and lipid peroxidation which might ultimately lead to cell damage and death (Surai, 2002). The yellowish pigments of turmeric (*Curcuma longa*) powder (TMP), referred to as total curcuminoids (TCMN) have been shown to have antioxidant properties (Soni *et al*., 1997) and to inhibit biotransformation of the AFs to their epoxide derivatives (Lee *et al*., 2001). The antioxidant properties of TCMN suggest that they may be able to prevent or reduce the toxic effects of aflatoxins (Galvano *et al*., 2001).

Aim: To determine if curcuminoids from turmeric would prevent or reduce the toxic effects of AFB_1 in weanling pigs fed dietary treatments for 21 days.

Materials and Methods: On day 14 post weaning, 30 crossbred weanling pigs were weighed, ear-tagged, and placed in individual pens with *ad libitum* access to feed and water. A 3 x 2 factorial design [3 concentrations of AFB_1 and two levels of curcuminoids (TCMN)] was used with 5 pigs assigned to each of 6 dietary treatments from day 14 to day 35 post weaning. Dietary treatments included: A) basal diet (BD) containing no AFB_1 or TCMN; B) BD supplemented with 0.5 mg AFB_1/kg of diet; C) BD supplemented with 1.0 mg AFB_1/kg diet; D) BD supplemented with 220 mg TCMN/kg diet; E) BD supplemented with 220 mg TCMN/kg diet and 0.5 mg AFB_1/kg diet; and F) BD supplemented with 220 mg TCMN/kg diet and 1.0 mg AFB_1/kg diet. Curcuminoids were supplied by turmeric powder containing 3.29% TCMN. AFB_1 was supplied by *Aspergillus parasiticus* (NRRL 2999) culture material containing 750 mg AFB_1/kg culture material. Feed consumption and weight gain were measured over the course of the study, and blood samples were collected on day 34 for hematology and serum biochemistries. All pigs were sacrificed on day 35, and livers and kidneys were weighed, with sections of liver from each animal fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin (H & E) for histologic evaluation. Response variables included growth performance, relative liver and kidney weight, serum chemistry, hematology, and selected histologic changes. Data were analyzed as a 3 x 2 factorial using the GLM procedures of SAS (SAS Institute, 2003).

Results and Discussion: Feed intake (FI) and body weight gain (BWG) were reduced ($P < 0.05$) by AFB_1 but were not affected ($P > 0.05$) by TCMN. No AFB_1 by TCMN interactions ($P > 0.05$) were observed for FI or BWG. There was, however, a significant AFB_1 by TCMN interaction ($P < 0.05$) observed for feed efficiency (G:F). TCMN decreased G:F in pigs fed no AFB_1, had no effect in pigs fed 0.5 mg AFB_1/kg diet, but increased G:F in pigs fed 1 mg AFB_1/kg diet. Relative kidney weights were not affected ($P > 0.05$) by dietary treatments. In contrast, relative liver weights increased ($P < 0.05$) in pigs fed AFB_1 and in pigs fed TCMN. No AFB_1 by TCMN interactions ($P > 0.05$) were observed for relative kidney or relative liver weight. Serum concentrations of urinary nitrogen, creatinine, Na, K, Ca, P, Mg, albumen, globulin, total protein, GGT and CPK were not affected ($P > 0.05$) by dietary treatments. Serum AST was increased in pigs fed AF ($P < 0.05$), as compared with controls, but was not affected ($P > 0.05$) by TCMN. There appeared to be a subtle increase in hepatocyte vacuolation associated with AF exposure. Results of this study are in contrast to previous study with broilers (Gowda et al., 2009) in which TCMN reduced the negative effects of AFB_1 on broiler performance, serum chemistries, and liver weight. Possible explanations for the contrasting results may include species differences and the concentration of TCMN used in the current study (220 mg/kg diet), which was selected based on its efficacy in broilers.

Conclusions: Dietary concentrations of AF as low as 0.5 mg/kg diet depressed growth performance of weanling pigs. TCMN from turmeric at 220 mg/kg diet was only marginally effective in preventing or reducing the toxic effects of AFB_1.

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10:00-10:30 INFLUENCE OF FUMONISINS ON SPHINGOLIPID METABOLISM IN PIGLETS

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Background: Fumonisins are cancerogenic mycotoxins capable of disrupting sphingolipid metabolism which has been reported to result in increased sphinganine (Sa) to sphingosine (So) ratios and in increased concentrations of 1-deoxy-sphinganine in plasma and tissues of animals (Riley and Voss, 2006; Zitomer et al., 2009).

Aim: The aim of this work was to investigate the influence of different concentrations of fumonisins in feed on the sphingolipid metabolism in piglets. To this end, an analysis method for sphingoid bases in plasma and tissues should be established and critically validated.

Materials and Methods: Two feeding experiments were carried out with piglets: In the first, a group of 6 piglets was fed 6.4 mg/kg fumonisins containing diet ad libitum over 6 weeks. In the second experiment, a group of 6 piglets was administered 2 mg of fumonisin B1 per kg body weight in addition to basal diet. In both experiments, a control group of 6 piglets received basal diet. Plasma samples were collected once a week. At the end of the second experiment, piglets were euthanized and tissue samples (liver, lung, kidney) were taken and immediately frozen with liquid nitrogen and stored at -80°C until analysis.
Plasma samples were analyzed as described previously with minor modifications (Schwartz et al., 2009). Aliquots of tissue samples were homogenized on ice in cold phosphate buffer and aliquots of the homogenate were analyzed by the same procedure as the plasma samples. The analytical method was comprehensively validated and the homogeneity of the tissue samples was tested.

Results and Discussion: In the first feeding experiment mean concentrations of sphinganine (Sa) and sphinganine to sphingosine (Sa to So) ratios in plasma were significantly greater in the fumonisin treatment group than in the control group from the third week of treatment on. At the end of treatment (6 weeks), mean Sa concentrations (N = 6) had increased from 6.0 ± 4.5 ng/ml (first week) to 93 ± 47 ng/ml and mean Sa to So ratios had increased from 0.24 ± 0.06 to 1.04 ± 0.22. Mean Sa concentrations and Sa to So ratios in the control group were similar to values in the fumonisin group in the first week and remained stable over the course of treatment.
In the second feeding experiment mean initial Sa concentrations and Sa to So ratios in plasma of piglets were similar in the fumonisin and in the control group and not statistically different to the values obtained in the first feeding experiment. However, after consumption of 2 mg of fumonisin B1 per kg body weight for only 7 days piglets of the fumonisin group had significantly greater Sa concentrations and Sa to So ratios in plasma than piglets of the control group. Mean values after 14 days of fumonisin B1 treatment are given in Table 1.

Table 1: Average concentrations of Sa, 1-deoxy Sa and average Sa to So ratios (± natural standard deviations) in plasma and tissue samples of piglets after consumption of control diet or control diet plus 2 mg fumonisin B1 per kg body weight for 14 days.

	Sa (ng/ml or ng/g)		Sa/So ratio		1-Deoxy Sa (ng/g)
	Control group	Fumonisin group	Control group	Fumonisin group	Fumonisin group
Plasma	4.8 ± 2.4	300 ± 207	0.24 ± 0.04	2.49 ± 0.48	traces
Kidney	109 ± 20	24600 ± 4300	0.15 ± 0.03	5.35 ± 0.32	553 ± 60
Lung	1270 ± 400	40100 ± 21000	0.15 ± 0.02	1.44 ± 0.60	1880 ± 1270
Liver	74 ± 11	13260 ± 7100	0.11 ± 0.02	3.06 ± 1.08	643 ± 319

There were big differences in concentrations of Sa between pigs of one feeding group. Differences in the Sa to So ratio were less pronounced. Nevertheless, each single pig of the fumonisin group had significantly higher Sa concentrations and Sa to So ratios in plasma and tissues than any of the pigs of the control group. In addition, 1-deoxy sphinganine, a novel fumonisin biomarker (Zitomer et al. 2009), was above the limit of quantitation only in tissue samples of piglets of the fumonisin group and in lung samples of the control group (182 ± 33 ng/g).

Conclusion: Consumption of fumonisin containing diet dose-dependently increases the concentration of Sa, 1-deoxy Sa and the Sa to So ratio. Different organs contain highly different concentrations of sphingoid bases and different Sa to So ratios. Tissue samples of piglets of one feeding group may contain greatly different concentrations of sphingoid bases. Differences in the Sa to So ratio are less pronounced so that the Sa to So ratio is the more reliable biomarker. The analytical method has been fully validated and seems promising for investigating the efficiency of feed additives for detoxification of fumonisins.

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11:00-11:30 EFFECT OF MYCOFIX® ADDITION TO THE SWINE FEED ON PIG PERFORMANCE AS A CORRECTIVE ACTION OF THE HACCP PLAN IN A COMMERCIAL FARM

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INTRODUCTION

Mycotoxins are substances of great concern in pig production, as even at low contamination levels they are having a negative impact on productive and reproductive parameters, carcass quality but also food safety, due to the possibility of accumulation of toxic metabolites or residues in tissues.

Strategies for mycotoxin prevention and control in order to mitigate their negative impact on swine performance include to identify critical points in the production chain such as the control of incoming and storage of the raw materials for swine feeds, mixing and storage of diets (feeds), especially for diets used during lactation, nursing (for sows and their herds) and growing phase.

There is some literature available documenting the use of mycotoxin deactivators in pig production under experimental conditions but not a lot of data of commercial farms is available.

The aim of this work is to verify the effectiveness of mycotoxin management on a selected Argentinean farm. As a corrective action in the HACCP plan and to minimize the negative impact of aflatoxins, ochratoxin, deoxynialenol and zearalenone, Mycofix® was added to swine feeds. On behalf of that, this work is considered a pioneer in that field.

MATERIALS AND METHODS

Two piglet groups were formed: CONTROL GROUP (CG, regular diet) n=110 and TREATED GROUP (TG, 0.5 kg Mycofix® Plus/ton) n=120. Zootechnical Productive Parameters were observed from birth until slaughtering.

Deoxynivalenol, aflatoxins, zearalenone and ochratoxins were analyzed in all feed samples.

Average Daily Gain (ADG), Feed Efficiency (FE) and Cumulative Weight Gain (CWG) were evaluated to measure the animal performance.

RESULTS AND DISCUSSION

The only mycotoxins detected were aflatoxins (levels < 15 $\mu g.kg^{-1}$) and zearalenone (maximum level < 350 $\mu g.kg^{-1}$).

Statistically nonparametric tests were used, comparing the median values from the two groups studied.

All the parameters compared, ADG, FE and CWG, had significant differences, with $P < 0.05$. The median values obtained were: CG-ADG: 0.523 kg and TG-ADG: 0.547 kg; CG-FE: 2.535 kg and TG-FE: 2.646 kg; CG-CWG: 90.4 kg and TG-CWG: 95.6 kg.

CONCLUSIONS

The inclusion of Mycofix® in diets of fattening pigs led to a positive effect in overall performance parameters, even with low concentrations of mycotoxins.

The results demonstrate that use of Mycofix®, when the mycotoxins levels are low, along the growing stage – until 70 days old– is highly beneficial for the Commercial Farm. Pigs were heavier at slaughter time and significantly more homogeneous animal's lots.

Focusing on food safety, a complete HACCP plan for prevention of mycotoxins in swine production ensures to obtain carcass without measurable mycotoxin residues.

The measurement of mycotoxin levels in raw materials for swine feeds must be considered as a part of the HACCP plan, since contamination is an indicator of low quality grain.

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11:30-12:00 HEALTH PROBLEMS IN PIGS RELATED TO MYCOTOXINS

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INTRODUCTION

Mycotoxins are produced and eliminated by fungus that growth in grains such as Corn, wheat, sorghum and others. This growth can occur at or before the harvesting, during storage or even worst in the animal feeder. The fungi metabolites are highly toxic when eaten by domestic animals. Some of the better known mycotoxins are: Aflatoxins, Fumonisin, Ocratoxin, Tricothecens and Ergot. In the domestic pig's diet, most of the used grains are, corn, sorghum and wheat, for this reason pigs are susceptible to become intoxicated. When the mycotoxin level is elevated, the feed has a peculiar flavour, image and taste, pigs are very cautious if something is wrong with the feed they will no eat it, they refuse the feed. That is why it is not common to see acute intoxications[1]. In pigs we usually observe gradual intoxications; the animals eat sublethal toxins doses for a long period of time. These kinds of intoxications observed in pigs affect their health status and their productive performance; there is also growth retardation. It is estimated in the United States that the economic cost due to mycotoxin is above 1.4 billion dollars a year.

The objective of this work is to share some clinical and pathological lesions observed in pigs intoxicated with mycotoxins that will help to the diagnoses in pig farms.

MYCOTOXINS CLASIFICATION

Aflatoxins are produced by two different types of fungi of Aspergillus group; *Aspergillus flavus* and *Aspergillus fumigatus*. Aflatoxicosis occurs in many parts of the world and affects birds, growing piglets, pregnant sows, calves and dogs. In experimental studies all species of animals have shown some degree of susceptibility. Its toxic effects include mutagenesis because of its effect on deoxyribonucleic acid (DNA), nuclear, also produce carcinogenic, teratogenic and inmunosupresion[2]. The organ with the greatest alterations in cases of poisoning with this mycotoxin is liver[1]

Ochratoxin and Citrinin are two mycotoxins that have the same effect on the animals affected; Ochratoxin is produced by *Aspergillus ochraceus* and Citrinin by *Penicillium citrinum*. Poultry and pigs are the species known to be more susceptible to Ochratoxin. The grains commonly affected are: Barley, corn, sorghum and wheat[3, 4]

Fumonisin is produced by *Fusarium moniliforme*; susceptible animal species includes horses and pigs. In horses and other equine, disease causes neurological symptoms that are described as leukoencephalomalacia. In pigs the lesions include pulmonary edema and hidrotorax[5, 6]

The group of **Trichothecenes** includes several structurally related compounds, produced by several families of fungi such as *Fusarium, Trichothecium, Myrothecium* and others. Susceptible animals include pigs, poultry, cattle and horses. Trichothecenes most frequently found in animal feed are: Deoxynivalenol or Vomitoxin, T2 toxin and diacetoxyscirpenol or DAS. Trichothecenes toxicity is based on the direct cytotoxicity; damage is seen primarily at epithelial tissues in the digestive system[7]. **Zearalenone** is produced by *F. graminearum* and *F. roseum* causing vulvovaginitis and other estrogenic effects in pigs. It is found in grains such as corn, wheat, barley and oats. *F. roseum* has the ability to produce either Zearalenone or Deoxynivalenol.

Alkaloids of *Claviceps*, are produced by Claviceps purpurea sclerotia, it is found in rye, barley, wheat and oats. The most produced alkaloids are ergotamine and ergonovine[8]. The species affected are cattle, pigs, sheep and poultry. Cause vasoconstriction by direct action on the muscles of the arterioles, repeated doses damage the vascular epithelium.

LESIONS OBSERVED IN ANIMALS AFFECTED BY THESE MYCOTOXINS

In swine clinic is common to find cases of ingestion of feed that are contaminated with mycotoxins that is something that occurs both in our country as elsewhere in the world. Most of the Clinical illnesses reported com from experimental intoxication with mycotoxins. The mycotoxin used as well the quantities and times administered are known.

In field cases by contrast, we assume that the animal may have ingested over a long period of time some mycotoxin quantities that have reached the level to cause problems. It is also common to see clinical infectious diseases in addition to the damage caused by mycotoxins. This is logical because as described from experimental cases the effects of some mycotoxins such as aflatoxins are immunosuppressive and this facilitates the manifestation of diseases that may have been in a subclinical form in the population or are newly introduced but otherwise not have been that easy to affect the animals.

AFLATOXIN INTOXICATION

Aflatoxins have a devastating effect on the productive performance of pigs. In animals affected most notable clinical signs are a low growth rate and a yellowish (Icteric) color in both the skin and hair. When making a postmortem examination the most striking lesions are yellowish (icteric) tissues such as skin, subcutaneous tissue and muscle (Fig. 1) and a color change in liver, gastric ulcers are occasionally observed (Fig. 2) and spleens reduced in size (Fig. 3). Histopathologic examination revealed a proliferation of bile ducts (Fig. 4) which has been described as one of the most characteristic lesions in this type of intoxication; hepatocytes show severe degenerative and necrotic changes. Liver damage caused by this intoxication is sufficient to explain the jaundice that is manifested in the abundance of yellow pigment in animal tissues. One of the complications most often seen in conjunction with this intoxication is Vitamin E deficiency. There is ample evidence that Vit. E is essential for vital body functions and that the organ where it is stored and from which is distributed to other tissues is the Liver[9]. Then when the liver is damaged by the Aflatoxin their functions are not properly carry out, vitamin E deficiency as well as Cirrhosis is observed. Vitamin E deficiency in pigs is related to; Blueberry Hear Disease, Yellow fat Disease, Reproductive problems and inmunosupression[9, 10].

The sows (200-300 kg) during gestation have a restricted feeding (2 to 3 kg per day) even if they are eating food contaminated with mycotoxins, the proportion of mycotoxins ingested per kilo of body weight is maintained below the dose that can cause damage, therefore no clinical signs are observed in sows. During lactation, sows eat greater amounts of feed, they can consume up to 8 or 9 kilos of feed per day. Aflatoxins are eliminated by the sow through her milk. So even if a sow do not show clinical signs of Aflatoxin intoxication, her litter might be ingested Aflatoxin by drinking the sow's milk. The quantities ingested are enough to intoxicate an animal less than 2 kilos[2]. In the region piglets have been observed within days of age with severe liver damage that surely were poisoned through breast milk. Some sows voluntary refuse feed, perhaps it may be a case of maternal instinct in the sow trying to protect her litter.

Fig. 1 Yellowish organs and tissues

Fig. 2 Gastric ulcer

Fig. 3 Spleen reduced in size

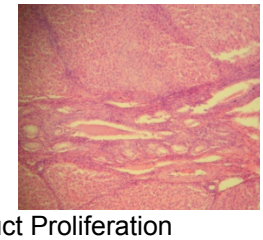

Fig. 4 Bile duct Proliferation

OCHRATOXIN INTOXICATION

Ochratoxin and citrine are nephrotoxic. In experimental studies the authors conclude that the OTA has a more severe effect on renal function compared with the effect of citrinina[3]. Like Aflatoxins, these mycotoxins have carcinogenic, teratogenic and immunotoxic effects. The pig is susceptible to the action of these micotoxinas[11]. Some stability-oriented studies of the toxin and the duration of the activity in affected tissues have shown that this toxin is highly stable. The damages they can cause in tissues are the result of an accumulation phenomenon[4].

Taking into account that from the pathological point of view the effects of Ochratoxin and Citrinin are similar it is possible that lesions observed in field cases are due to the action of both. However, in the mycotoxin analysis, in suspicious feed, Ochratoxin is the one regularly detected. Clinical signs reported as indicative of intoxication with Ochratoxin are polydipsia and polyuria but in pigs that consumed water ad libitum and are raised in floor cage these clinical signs are not easily registered. In postmortem examinations on animals that were presumed to be affected by OTA the renal lesions observed macroscopically were: white patches on the surface of the kidney which varied in size from a diffuse stippled to irregularly shaped spots easily distinguishable (Fig. 5). Another notable injury was cysts in the kidney, presented individually or multiple cysts in the same kidney (Fig. 6). In histopathological examination severe degenerative changes were observed in both proximal and distal tubules and a mild diffuse infiltrate of round cells and some plasma cells. Gastric ulcers have been associated with this type of mycotoxins.

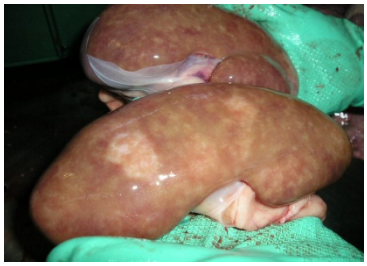

Fig. 5 Whitish spots

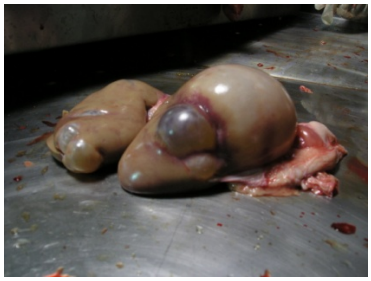

Fig. 6 Kidney Cysts

FUMONISIN INTOXICATION

In pigs the most affected organs are lungs and livers, kidney and esophagus might also be affected[12]. Among the first reports of the effect of this toxin for pigs is the description of respiratory failure in pigs fed on corn that was considered unsuitable for human consumption or poultry. The most consistent lesion found in pigs intoxicated with this type of contaminated grain was pulmonary edema[1]. In a recent study it was demonstrated that the fumonisin dose at which the animal is exposed is the factor that determines whether the lung or the liver is affected[6]. The pigs that received an amount equal to or greater than 16 mg fumonisin / kg. / day (via stomach probe) developed interlobular edema and pulmonary edema. Pigs that received less than the 16 mg / kg / day of fumonisin (via probe), as well as pigs receiving feed contaminated with the toxin at a rate of 200 mg fumonisin per kg of feed, developed jaundice and hepatocellular necrosis

In field studies in Mexico the most prominent lesions were pulmonary edema and jaundice. In some animals these lesions were observed meaning it can be concluded that in field cases liver or lung lesions are not mutually exclusive. There was a particular case where the participation of OTA was established based on both macroscopic lesions of pulmonary edema (Fig. 7), jaundice and microscopic lesions. Microscopically in lungs edema was observed at all levels; below the pleura and interlobular, the cells involved were abundant eosinophils. In liver hepatocellular necrosis as well as eosinophils were observed. This farm had several other diseases. One of the infections was Salmonellosis in fattening pigs. Salmonella cholerasuis a well known pathogen in pigs was isolated, the morbidity and mortality that occurred on that farm at the outbreak were higher than 45%. This outbreak occurred in animals of different ages and weight in the farm, their weight was between 40 to 110 Kg and affected animals of all ages and all weights.

It is interesting that in recent studies it has been demonstrated that fumonisin poisoning among other effects can have a severe decline in the population of intravascular macrophages in the lung. These cells are closely related to pathogen removal features or elements that can be harmful to the body. In pigs

that had received fumonisin at 20 mg per kg for 7 days, the pulmonary clearance mechanisms were depressed in a way that was statistically significativa[13]. Such findings provide a logical explanation for the phenomenon observed several times at the field level when there is Fumonisin intoxication other diseases have a greater strength and severity[13].

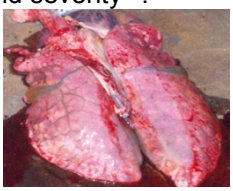


Fig. 7 Interlobular and Mediastinal edema, without pneumonic lesions

TRICHOTHECENS INTOXICATION

This group includes several mycotoxins that are linked from the structural point of view but may differ in both the fungus that produces as in the spectrum of animal species that affects every one in particular.

The toxins most commonly reported in cases of pigs are, deoxynivalenol or DON also Vomitoxin, nivalenol and 15-acetildeoxynivalenol. Both DON and Zearalenone produce neurological damage that decreases appetite. DON has a pro-inflammatory effect in the digestive tract increasing susceptibility to infections. In general, these toxins are very aggressive to the digestive tract epithelium, among the most common signs of presence of these toxins in food are vomiting or feed refusal (Fig. 8 and Fig 9) however this does not happen in all cases. The Zearalenone has estrogenic effects in sows that decrease productivity[14]. In sows 7-10 days pregnant may cause: degeneration of blastocysts, reduced number of piglets per litter, abortions, low conception rate, increased stillbirths, inactive corpora lutea (Fig. 10), increased splayleg and trembling piglets, sows with vaginal discharge (Fig. 11), rectal (Fig. 12), and vaginal prolapse. Zearalenone is found in milk and can be ingested by the piglets causing swelling and redness of the vulva (Fig. 13). In boars Zearalenone affects the semen quality; the males have increased size of nipples and more prominent sexual organs.

In Mexico, populations have been observed in which suddenly increases the number of cases of animals affected with gastric ulcer or in which it detects a high incidence of infectious diseases of diverse nature. In the case of gastric ulcers it was interesting to notice that once the mycotoxin problem was controlled the cases of gastric ulcers diminished.

The high susceptibility to infectious disease seen in field cases also may be related to these mycotoxins. In experimental studies the immune response was diminished gradually in conjunction with the dosage of toxin to which the pigs had been exposed[7].


Fig. 8 Vomitoxin: animals refuse to eat

Fig. 9 Vomit

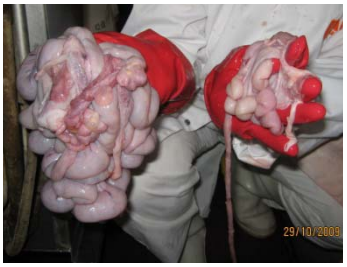

Fig. 10 Corpora lutea, hydrometra

Fig. 11 Catarrhal discharge

Fig. 12 Rectal prolapse

Fig. 13 Redness and swelling of Vulva

Claviceps ALKALOIDS INTOXICATION

The alkaloids are potent initiators of smooth muscle contraction from uterus and middle layer of the arteries. These alkaloids also simulate the action of dopamine in the central nervous system and inhibit prolactin release, preventing the growth of the mammary gland and inhibiting lactation. The alkaloids, in high concentrations, cause constriction of the arteries, therefore ischemia and dry gangrene can be seen in the limbs and appendages. This is exacerbated in cold climates[2,7]. This mycotoxin also causes liver damage, polydipsia and polyuria.

In one field case in Mexico, there was high mortality of piglets in the absence of obvious clinical signs of infection and with no response to antibiotic treatments in both sows and piglets. The only consistent clinical sign in all affected litters was hypogalactia or agalactia in sows. The piglets examined post-mortem showed empty stomachs, friable livers and mucoid degeneration in heart. In sows the lack of mammary gland development was evident and other significant lesions were white patches of irregular size and distribution in the liver, cirrhosis. Microscopically: Portal cirrhosis was observed; hepatocytes had severe degenerative changes mainly albuminous degeneration and fatty change. This case was particularly illustrative of the lack of knowledge that exists in cases of intoxication in more than one occasion the diagnosis given was infection with PRRS virus. The production of sorghum in the months before the outbreak had been somewhat depleted by having been infected with “cornezuelo”, which is the common name for Claviceps. By integrating the clinical history with the lesions found in different organs, it was determined that further measures should be taken to reduce the impact of intoxication. The productive parameters gradually returned to the normal level

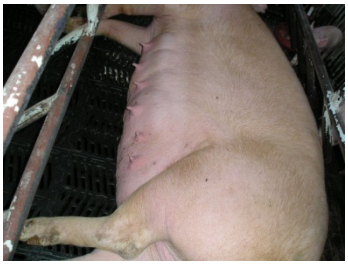

Fig.14 Agalactia post farrowing

Fig.15 Tail Necrosis

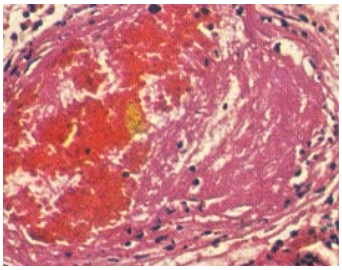

Fig.16 Endothelial thrombosis

In conclusion mycotoxins are products of metabolism of fungi that may be contaminating the grains and this contamination may not be apparent to the farmer or livestock producer. The rapid identification of the clinical signs and lesions suggesting mycotoxin intoxication can make a big difference in the final effect that this type of intoxication poisoning can have on the profitability of a production cycle. A recognized problem of contaminated servings expeditiously can be more easily controlled before it causes irreversible damage to the population. The support of diagnostic techniques should be used whenever available.

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12:00-12:30 ABATTOIR MONITORING AS A DIAGNOSTIC TOOL FOR MYCOTOXINS IN PIGS

Margarita Trujano[1]*, Raúl García[2], René Márquez[3], Julieta Sierra[4], Serafín Solorio[4]

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Introduction: The most important mycotoxins in pigs are: Aflatoxin, Fumonisin, Ochratoxin, Trichothecenes and Ergot[1]. Pigs ingest sublethal amounts of toxin but for a long period of time. These intoxications significantly affect the health of animals and this is manifested in a decrease in growth rate and productive efficiency. An important element in the control of intoxications is an efficient diagnosis that allows knowing not only the existence of these mycotoxins but also the severity of lesions. A macroscopical examination of lesions is an activity practiced in animals sent to the abattoir. This could be very useful but some details should be considered for maximizing the potential of the technique[2]

Objective: To demonstrate an innovated trial rating system in the abattoir for mycotoxins in pigs, the lesions suggestive of different mycotoxins, and a format for reporting these.

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Method: To properly assess the organs affected by mycotoxins: The targeted organs were selected. These organs were: lungs, livers, kidneys, reproductive tracts, spleens and digestive tracts. It is important the extent or severity of injuries to give a proper rating. The consistency of the organ, the color and size should also need to be examined. The following lesions were related to mycotoxins; Lungs, edema and yellowish color. Livers: color, texture, presence of fibrous tissue, white and reddish spots. Kidneys: color, size, cysts, white patches and red patches. In reproductive tract: presence of corpora lutea, hydrometra and cysts. In Spleen: Color, size, fibrous tissue. Digestive tract: ulcers, lymphoid reaction, presence of undigested food. The necrosis or friable lesions in liver and kidney, are detected by the fragility of the tissue between the fingers when handled

In the control sheet to track lesions, the values were included. In the assessment of lung edema the value given was 1 for moderate lesions and 2 for severe lesions (Edema between lung lobes or in the mediastinum). A value of 1 was given for Yellowish color.

The four lobes of the pig´s liver are evaluated individually. The value depends on the number of affected lobes: 0 = normal, 1 = One liver lobe is affected, 2 = if two lobes are affected 3 = If three lobes are affected and 4 = when all lobes are affected. The evaluated lesions are: fibrosis, friability, white spots and red spots. Yellowish color is given a value of 1.

In kidneys the lesions evaluated were: Irregular white or red, color, cysts, and size. Normal = 0, irregular white or red spots =1 if 1-10 spots were observed. =2 if there are

>10 spots. Yellowish color = 1. If the kidney is enlarged = 1. When cysts are observed depending on the size and number = 1 if there is one small. =2 if there are larger and more than one cyst.

In addition to the organs described other organs are examined qualitatively: Spleen (size, whitish, and infarction), digestive tract (lymphoid reaction, irritation, undigested food, ulcers) and reproductive system (Cysts, hydrometra, corpora lutea, corpus hemorrhagic).

The evaluation also undertakes a histopathological examination. Macroscopically the lesions could be mistaken but under histopathological examination the participation of mycotoxins can be confirmed or ruled out. Figure 1 shows an example of the graphs included in the report.

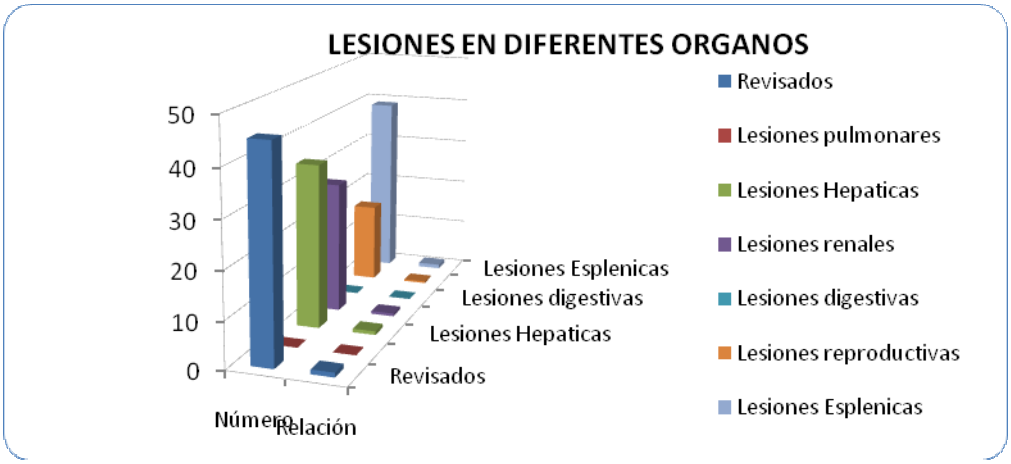

Fig. 1 Lesions observed in different pig organs at the abattoir.

Conclusions: The examination of organs at the abattoir is not very common but represents a useful diagnostic tool for monitoring mycotoxin Intoxication in pigs. This Monitoring together with histopathology analyses will let us know the type of mycotoxin and the severity of the lesion involved.

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12:30-13:00 GENOMICS: A NEW TOOL FOR STUDYING THE EFFECTS OF MYCOTOXINS IN LIVESTOCK

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Background: Mycotoxins are naturally occurring toxic secondary metabolites of fungi that may be present in feed ingredients and have been associated with various animal and poultry diseases. To date, most research conducted on the effects of mycotoxins in livestock has been at the level of the animal. The successful sequencing of animal genomes allows for the use of genomic (microarrays and real-time PCR) that will allow researchers to identify genes that are differentially expressed in response to mycotoxins. Identification of these genes may allow researchers to identify opportunities for using nutritional/pharmacological intervention to reduce or prevent the toxic effects of mycotoxins.

Aim: To demonstrate how genomic tools (microarrays and real-time PCR) can be used to identify genes that are affected by mycotoxins, and to identify opportunities for using nutritional/pharmacological intervention to reduce or prevent the toxic effects of mycotoxins.

Materials and Methods: Aflatoxin (AF) was selected because its physiological and toxicological effects are well known. In experiment 1, microarray analysis was used to identify shifts in genetic expression associated with affected physiological processes in chicks fed 0 and 2 mg/kg AFB_1 in order to identify potential targets for nutritional/pharmacological intervention. High quality ribonucleic acid (RNA) was extracted from liver tissue, purified, reverse transcribed, and hybridized to an oligonucleotide microarray chip. Microarray data were analyzed using a 2-step ANOVA model and validated by quantitative real-time PCR of selected genes. In experiment 2, the efficacy of curcumin, a natural antioxidant found in turmeric (*Curcuma longa*) powder (TMP), to ameliorate changes in gene expression in the livers of broiler chicks fed AFB_1 (1 mg/kg) was evaluated using real-time PCR. Based on results of experiment 1, genes associated with antioxidant function [catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione S-transferase (GST)], biotransformation [cytochrome P450, epoxide hydrolase (EH)], and immune systems (interleukins 6 and 2) were specifically targeted.

Results and Discussion: In experiment 1, AF reduced feed intake, body weight gain, serum total proteins, serum Ca and P, but increased liver weights. Microarray analysis identified 177 genes (false discovery rate less ≤13% and fold change ≥1.4) as being differentially expressed between controls and chicks fed 2 mg AFB_1/kg diet. Of these, 97 genes were up regulated and 80 genes were down regulated. Compared with controls (0 mg AFB_1/kg), various genes associated with energy production, fatty acid metabolism, growth and development, antioxidant protection, detoxification, coagulation, and immune protection were down-regulated, whereas genes associated with cell proliferation were

up-regulated in birds fed 2 mg AFB_1/kg (Yarru et al., 2009a). Based on the results of experiment 1, a natural antioxidant (curcumin) from TMP was selected and used in experiment 2 to determine if it would be effective in reducing or preventing the toxic effects of AF. In experiment 2, AF (1 mg/kg) reduced feed intake and body weight gain, and increased relative liver weight. Curcumin (74 mg/kg) ameliorated the negative effects of AF on growth performance and provided partial protection against changes in expression of antioxidant (SOD and GST), biotransformation (EH and cytochrome P450), and immune system (interleukin 6) genes in livers of chicks fed 1 mg/kg AFB_1 (Yarru et al., 2009b). Results of these two experiments indicate that the use of genomic tools is a feasible approach for addressing the issue of mycotoxins and mycotoxicoses in livestock.

Conclusions: Genomic tools (microarrays and real-time PCR) can be used to identify genes that are affected by mycotoxins, and to identify potential targets for nutritional/pharmacological intervention.

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Session 14: Mycotoxins in poultry.

16:30- 17:00 THE EFFECTS OF 1.2 PPM T -2 TOXIN ON PERFORMANCE, LESIONS, AND GENERAL HEALTH OF MALE BROILERS AND THE EFFICIENCY OF AN ORGANOALUMINOSILICATE (MYCOTOXIN BINDER).

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Background: general signs of trichothecene toxicity in broilers include weight loss, decreased feed conversion, feed refusal, vomiting, severe dermatitis, hemorrhage and death. Because T-2 toxin was one the first trichothecenes discovered, its effects on animals were early characterized. T-2 is a mycotoxin affecting broilers health and performance (CAST, 2003). The aluminosilicates do not adsorb T-2 Toxin (Brito et al., 2008).

Aim: a trial was performed to evaluate the toxic effect of a contamination (1.2 ppm) of T-2 and the efficiency of a commercial organoaluminosilicate (mycotoxin binder).

Material and Methods: Ninety Ross 308 male broilers were randomly allocated in five groups: group 1 (negative control group), group 2 positive control (1.2 ppm T-2 toxin), group 3 (1.2 ppm T-2 toxin + organoaluminosilicate binder low dose), group 4 (1.2 ppm T-2 toxin + organoaluminosilicate binder high dose), and group 5 (organoaluminosilicate binder).
The birds were fed their respective diets from 10 days of age, up to 39 days, date in which they were sacrificed. We take the individual weight of the birds at the beginning and at the end of the experiment. At day 39, consumption. weight gain, feed conversion and mortality were calculated. Lesions of the oral cavity, feces and feathers were scored. In the animals that were given the mycotoxin binder, parameters were obtained same that those of the group control, for what it is considered that the organoaluminosilicate is innocuous. This experiment was realized in the Instituto Internacional de Investigación Animal, located in the Municipio del Marquez, Qro. At 1800 msnm, being the semi-desert climate.

Results and discussion: the results shown, at the concentration of 1.2 ppm of T-2 Toxin, affects significantly: feed intake, weight gain, feed conversion and mortality. The presence of T-2 toxin in the diet of the animals caused lesions in the oral cavity. The effects of the T-2 toxin in the broilers were practically eliminated by the incorporation of any of both doses of the organoaluminosilicate in the experimental diet.

Conclusion: The T-2 toxin at 1.2 ppm in the diet of the birds affects the health and performance of the broilers. We observed that T-2 toxin is of dermal toxicity and that the oral lesions reduce feed intake. The organoaluminosilicate in the diet of the animals that were given T-2 toxin, reduced the negative effects caused by the aforementioned mycotoxin.

References:
Mycotoxins: Risk in Plant, Animal, an Human Systems. Task Force Report. No. 139. January 2003. Council for Agricultural Science and Technology.

Víctor Brito y Miguel Forat. Evaluación del efecto de diferentes secuestrantes de micotoxinas en dietas contaminadas con toxina T-2. IIIA 5 – 08Y – PI. 2008.

17:00-17:30 DIETARY AFLATOXINS AND 4,15-DIACETOXYSCIRPENOL: INDIVIDUAL AND COMBINED EFFECTS IN BROILER CHICKENS

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Background: Both aflatoxins (AF) and diacetoxyscirpenol (DAS) (4,15-diacetoxy-3-hydroxy-12,13-epoxy-trichothec-9-ene) are important mycotoxins for the poultry industry not only because they are feedstuff natural contaminants (Council for Agricultural Science and Technology, 1989) but also for their highly toxic effect (Ueno, 1977). The combination of AF and DAS evidenced a synergistic interaction effect for a further depression of body weight gain (Kubena *et al*., 1993) in agreement with the synergistic interaction found by Huff *et al.* (1988) between AF (2.5 mg/ Kg) and T-2 toxin (4 mg/ Kg). Contrasting to these results, Kubena *et al*. (1990) did not evidence a synergistic response at higher levels of these mycotoxins.

Aims: the present study was designed to assess the effect of both toxins single and in combination on some primary responses of productivity and health in broilers.

Materials and Methods: AF was produced by growing *Aspergillus parasiticus* NRRL 2999 on moist polished rice according to the method of Shotwell *et al*. (1966) with the modifications by West *et al*. (1973). DAS was produced and purified according to the method of Richardson and Hamilton (1987) with modifications "(M. Jaramillo and R. Wyatt, 2000, unpublished data)". Two hundred and sixteen day-old broiler chicks Ross were weighed and identified by individual wing band, and housed in electrically heated batteries under continuous lighting with feed and water available *ad libitum* during 21 days. A completely randomized 3 x 3 factorial experimental design was used in this experiment and individual birds were considered as the experimental unit. ***Parameters evaluated***: body weight (BW), feed consumption, body temperature (BT), mortality, biliary-liver function (Jaramillo, 2005), oral lesion score, liver weight, and the base of tongue and liver were removed and fixed in neutral buffered 10% formalin (pH 7.2) and processed for histology (Luna, 1968). ***Statistical analysis***: Body weight, feed consumption, body temperature, bile volume, and liver weight were subjected to an analysis of variance using the General Linear Models procedure (SAS Institute, Inc.). Liver function was subjected to log transformation prior to analysis. Oral lesion scores (OLS) were subjected to the weighted least square method to evaluate the effect of AF, DAS and their combinations on this variable. The histological changes were analyzed on a descriptive way. The percentage of mortality was calculated for the accumulated period (0-21 days). Significant difference were compared using the Tukey's Studentized Test. Statements of significance are based on $P< 0.05$ unless otherwise indicated.

Results and Discussion: In this study, the depression of BW caused by the administration of single AF and DAS was a response indicative of AF and DAS toxicosis through different mechanism of actions. Thus, whereas the depression of BW caused by AF has been associated with inhibition of protein synthesis, the one caused by DAS is associated with a decrease on feed consumption. AF and DAS in combination at any of their concentration evaluated here caused a significant reduction on BW that could be described as an additive interaction. Birds fed with single DAS 6.0 µg/g and in combination with AF 2.5 mg/kg exhibited a significant decrease of BT. The significant response on increase of bile volume in birds consuming AF indicated a biliary dysfunction. Feeding AF alone caused significant increases in the relative

weight of liver and might be interpreted as a hepatomegaly to compensate the damage on liver function exhibited by birds exposed to AF and the deterioration of liver function can be explained through the histological changes observed on these birds. Both mycotoxins showed liver´s histological changes that were characteristic of those seen in acute to subacute mycotoxicosis. Dietary AF caused a negative effect on mortality (17%) emphasizing the importance of this mycotoxin on poultry industry.

Conclusions: The present study demonstrates that single AF and single DAS and their combinations interferes the broiler chick health. Their toxic effect is exhibited through different responses implying some additive effects. The results of this research emphasize that DAS effect can be detected by broilers as early as 7 days of age through a reduction of feed consumption.

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17:30-18:00 AFOLABI AND SONS MYCOTOXIN STUDY NETWORK (ASMSN)

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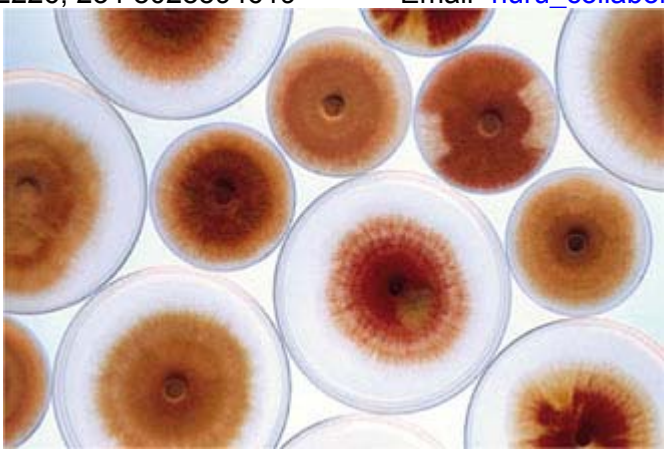

INTRODUCTION: The Afolabi and Sons Mycotoxin Study Network (ASMSN) is a body of scientists in the academia, industry, government regulatory agencies, farmers and other stakeholders in the food and feed sectors united by the need to create awareness on mycotoxins. A Non-Governmental, Non-Political and Non-Profit-making scientific organization founded in 2006 by a group of Scientists, ASMSN seeks to improve the living conditions of humanity, through increased support and promotion of studies on mycotoxins.

The network currently operates from National Agency for Foods Drug Administration and Control (NAFDAC) office in Lagos, Nigeria with members across the country in tertiary and research institutions, pharmaceutical companies, regulatory agencies, manufacturing and other related industries.

The network has organized and held four conferences since inauguration with support from regulatory agencies like SON and NAFDAC, food/feed industries and service providers like Biomin, Katchey, Neogen and Vicam. Other international bodies like the International Society for Mycotoxicology and MycoRed are expected to collaborate in future initiatives directed at reducing mycotoxin contamination in agricultural products. The previous conferences have provided massive data on occurrence and significance of food-borne mycotoxins in Nigeria.

CONCLUSIONS:

NSPRI 2010 will therefore focus on strategies for mycotoxin management in agriculture and agricultural products. Papers reporting experiences and experiments in the area of control interventions will be given priority.

Details of the 3rd ASMSN workshop which will take place during NSPRI 2010 will be provided soon.

REFERENCES:

Afolabi Rasaq Aremu

18:00-18:30 EFECTO DE LA INTERACCIÓN DE AFLATOXINAS Y LAS FUMONISINAS EN POLLO DE ENGORDA COMO MODELO DE ESTUDIO.

Del Río García JC[1], Ávila GE[2], López CC[2], Moreno RC[1], Méndez AA, Morales AAE[1], Moreno ME[1].

FES Cuautitlán – UNAM. Unidad de Investigación Multidisciplinaria "Alimentos, Micotoxinas y Micotoxicosis"[1], FMVZ –UNAM Depto. de Aves[2].

RESUMEN

El estudio del efecto de la combinación de las micotoxinas a tomado un gran significando para la salud humana y animal, ya que es la forma en que se presentan estos compuestos en la naturaleza, por lo tanto, el riesgo está relacionado a la presencia de dos o mas metabolitos tóxicos biológicamente activos y su combinación en el alimento, generando un efecto de interacción enmarcado dentro de los principios de toxicología de sinergismo o de aditividad. Se realizaron una serie de trabajos experimentales con la finalidad de ajustar las concentraciones de micotoxinas a utilizar en el trabajo final, del mismo modo estos sirvieron para preparar la metodología de recopilación de datos, muestreo sanguíneo y de órganos, así como de ajustar las técnicas de laboratorio para la evaluación del hematocrito, proteínas, transaminasas y bilirrubinas séricas. En la investigación final se observó que la presencia de aflatoxinas y fumonisinas (AFBs+FBs) disminuyen el peso de las aves ($p<0.05$). El peso relativo de hígado, riñón y bazo se ven afectados en presencia de algún tipo de micotoxina o su combinación respecto al testigo ($p<0.05$). Del mismo modo el hematocrito, proteínas, transaminasas y bilirrubinas se ven afectadas significativamente en las aves que consumieron AFBs+FBs ($p<0.05$). Este trabajo es el primer estudio en México que pone de manifiesto que concentraciones menores a 200 o 300 mg de FB /kg de alimento en combinación con 1 mg de AFB /kg de alimento inciden negativamente sobre el desempeño y la salud del pollo de engorda bajo las condiciones experimentales empleadas.

Palabras claves: Aflatoxina B_1 , Fumonisina B_1, Pollo de engorda, Interacción.

Celestún

Wednesday June 30, 2010.

Session 15: Mycotoxin in feed and its control.

08:30-09:00 EFFECTS OF FEEDING DIETS NATURALLY CONTAMINATED WITH AFLATOXINS ON ANIMAL PROTEIN

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Abstract

Background: The poultry sector provides 70% of animal protein to the Mexican people as meat and eggs. The birds are the most susceptible species to aflatoxins, chemicals that interfere with protein metabolism in animals.

Aim: A study was conducted to assess the effects of feeding naturally contaminated with aflatoxins to broilers on the capacity for protein synthesis.

Materials and methods: Seventy five Ross chickens were maintained in a production farm starting from 35 days until 58 days of age and assigned to 3 treatment groups with 25 birds for each treatment. Diets included two types of food concentrate; depending of the grow stage. It contains between 18% and 19.5 %of protein and 5 % to 9.5% of lipid. Both are with 12.5% of moisture. Two levels (5% and 10%) of rests of fish stores were supplemented in diets. The variable measured included feed intake, weight gain (GP) and feed conversion (CA) in the birds. On the 3 types of food it was determined the aflatoxins using enzyme- linked-immunosorbent assey (ELISA) with a detection limit 1.7 ppb (Ridascreen fast aflatoxin).

Results and Discussion: All the food samples contained aflatoxins in different levels. The concentrated food in a range of 1.67 to 2.12 ppb and the rests of fish stores at 2.28 ppb. All they into the official limit values in US (4 ppb). In the first week the animals of the control group won 0.740kg, in the second week, 1.5kg and in the third week 1.2kg. In the treated group with 5% of fish rests stores, in the first week 0.7kg; in the second week 2.7kg and in the third one 0.9kg. In the treated group with 10%of fish rests stores, in the first week 1.9kg; in the second 2.6kg and in the third one 0.9kg. The group 3 was the minor weight gain due to increase in feeding aflatoxins by foods. In the control group the effect of aflatoxins in the most lowed level was effective in the third week.

Conclusions: There were statistically reductions between treatments in the capacity for protein synthesis caused by aflatoxins in broilers.

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Métodos rápidos de inmunodiagnóstico para detección de aflatoxinas. 2006. Ridascreen fast aflatoxin. Biopharm Ind.

09:00-09:30 EVALUATION OF NUTRIENT CONTENTS AND SOME MYCOTOXIN LEVELS USING MAIZE IN ANIMAL NUTRITION IN BANDIRMA PROVINCE OF TURKEY

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Background: The flora of the fungies in the feed materials are inspected. In two groups as “field fungies” and “storage fungies”. Field fungies are present in the soil and they contaminate the plants in the period before the harvest. These fungies are Alternaria, Clodusporium, Fusarium, Rhizopus, Chaetomium, Cephalosporium and Helminthosporium. The feeds are contaminated by soil, water and infected plants. The spores of the fungies in these groups have the ability of living in the high levels of moisture like 22 % -25 %. These kind of fungies stop growing at 18 % moisture levels. The storage fungies are Aspergillus and Penicillium species. They present at the 18 % moisture levels. The moisture level decreases to 14-15 % as they develop.

Aim: To determine nutrient contents and some mycotoxin levels in using maize in animal nutrition in Bandırma province of Turkey

Material and Methods: In this study, a total of 108 maize samples were collected randomly during the years 2007 and 2008 from different feed companies in Bandırma province of Turkey. Feed samples were stored in plastic bags at 4^0C until the analysis. Samples were analyzed for contamination with aflatoxins using enzyme-linked immunoabsorbent assay (ELISA) method. The RIDASCREEN ® (Art.No: R5202) test kits (R-Biopharm AG; Germany) were used for the analyses. Also, chemical analyses and metabolic energy levels were made in 108 feeding maize samples which were taken at various times according to the AOAC methods.

Results and Discussion: In almost all of the maize samples that were analyzed Aflatoxin, Ochratoxin A, Zearalenon and T-2 toxin levels were belove the level that was stated by FDA. Chemical ingredients in maize samples were examined and it was determined that there weren’t major differences when compared with the 108 maize samples nourish matter percentage. The dry matter levels in maize was determined as 85.22 – 90.70 % in the analyses made and in this study nutrient contents and some mycotoxin levels were examined.

Conclusion: In conclusion, in the study of animal nutrition and especially poultry nutrition maize forms the major part in the diet. The mycotoxin levels of maize should be examined in standart periods. So that we can determine the harmful effect amount that may be caused by “carry over” from animal products to human beings.

09:30-10:00 ISOLATION AND IDENTIFICATION OF MICOTOXIGENIC FUNGI IN FISH MEALS USED FOR PREPARATION OF ANIMAL FEEDS

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ABSTRACT

The use of fish meal for the formulation of animal feeds has been questioned by sanitary authorities of many countries, linking it as causal agent of diverse animal pathologies. Considering that fungi have the capacity to invade different substrates and produce micotoxins, in this study we detect, isolate and identify micotoxigenic fungi in fish meals with the purpose to evaluate their quality and inocuousness. Two hundred and forty samples of fish meal from three fish processing plants (A, B and C) located at the northeastern region of Venezuela were evaluated. Percent humidity was determined by the method adopted by AOAC, while for the detection, recount and identification of fungi the methodology followed that recommended by Samson *et al*., using as culture media MEA agar at 2% with addition of 100 ppm of chloranphenycol and 50 ppm of streptomycin. Fungi identification was made from pure cultures, using agars Czapeck, CREA and G25N. Results showed considerable variation in the humidity content of the fish meals. The total recount of fungi varied between 3, 5 $x10^6$ C.F.U/g to 4,9$x10^6$ C.F.U/g in samples from processing plants B and A, for which most fungi species were *Aspergillus niger*, *A. terreus*, *A. flavus* y *A. versicolor*. In samples from processing plant B, the isolated fungi were identified as *A. penicilloides, Penicillium aurantiogriseum*, *P. citrinum* and *Alternata alternata*. It is concluded that by the type of isolated micoflora and its toxigenic capacity, fish meal represents an important risk factor for the development of animal pathologies.

10:00-10:30 DETERMINATION OF MYCOTOXINS IN FISH FEED FROM TILAPIA

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Background: Mycotoxins in food and feed can appear during different production stages, ranging from the culturing of raw ingredients until processing and storage (Sweeney and Dobson, 1998). Humidity and temperature are enhancing factors for the production of mycotoxins such as fumonisin and aflatoxin, which are produced by *Fusarium* and *Aspergillus* spp. respectively. Aflatoxins represent, among all known mycotoxins, the best characterized and most investigated foodborne contaminant to date, hence its importance and need to early determination and research (Eaton and Groopman, 1994). Culturing of tilapia (*Oreochromis* spp.), Mexico's most important fresh water fish, is realised primarily in subtropical environments, such as Nayarit state, localized on Mexican Pacific coast; due to the conditions of this place could favor feed contamination during aquaculture activity (Coker, 1997).

Aim: This study is designed to determinate the average concentration of total aflatoxin and fumonisin in feed for tilapia culture across the state of Nayarit.

Material and Methods: Samples were collected (during the summer of 2009) among the 10 most representative tilapia farms, by production, in Nayarit, México. Sampling technique was based in the European Union Regulation (No. 401/2006). Activity of water (A_w), temperature *in situ* and total aflatoxin and fumonisin, using competitive ELISA with Ridascreen® laboratory kit, were determined.

Results and Discussion: Average A_w value from all samples was 0.55. *In situ* temperature ranged from 26-35^{o}C. Presence of total aflatoxin was found only in one of the analyzed samples in a concentration of 1.97 ppb. As for total fumonisin, concentrations ranging from 0.52 to 0.78 ppm were found in six out of 10 samples. The temperature found drops easily on the viability range for fungi but not in the case of the A_w registered at that period of time, suggesting that the low mycotoxin values were probably produced either by xerophilic strains of these fungal genres or regular strains as well (Lacey, 1989) since secondary metabolites such as these toxins are formed from a few primary metabolism intermediaries, under sub optimal and stressing conditions like poor water availability (Swanson, 1987).

Conclusion: In México there is no current regulation on fish feed containing mycotoxin, however, based on the European Union Regulation (No. 401/2006 and No. 1881/2007), the concentrations found on this analysis did not trespass the maximum permissible limits, established as 2 ppb and 4 ppm for total aflatoxin and total fumonisin respectively.

References:

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11:00-11:30 OCCURRENCE OF MYCOTOXINS IN ROUND BALE SILAGE

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Background: Silage is an important feed for ruminant during winter period. It consists of the lactic fermentation of forage, either grasses or legumes, under anaerobic conditions. Round bale silage is generally made from unchopped forage of higher dry matter level (40-50%). Under those conditions, the capacity of lactic acid bacteria to ferment the material is lower but the osmotic conditions reduce the growth potential of most microorganisms (McDonald, 1991). Localized areas of higher humidity and/or air ingress through puncture of the plastic could promote the development of fungi and accumulation of mycotoxins (O'Brian *et.al.* 2008). This environment could also favor the degradation of some mycotoxins and the accumulation of metabolites (Richard et.al., 2007).

Aim: Study fungi diversity and the mycotoxins of fresh forage and four weeks old silage of forage from farms in northern temperate region of Canada.

Materials and methods: Forage and silage samples were collected from six farms of the northwestern region of the province of Québec (Canada) at two periods (June and August) of the same growing season. Fresh forage was sampled directly from newly bale forage (< 1 hour) before being wrapped with plastic. For the two sampling period, silage samples were collected four weeks later from the same round bales. Chemical parameters (pH, DM, %total-N, buffering capacity, nitrate, reducing sugars, ADF, NDF) and microbial (molds, yeasts, lactic acid bacteria, clostridia and enterobacteria) were determined for each sample. Mycotoxins were extracted by the method of Garon *et.al.* (2006) and analyzed by an Agilent 1100 HPLC-MS/MS at the Mass Sprectroscopy Facility (Guelph University).

Results and Discussion: All silages samples were well conserved with only one butyric silage (Farm H). Silage pH ranged from 4.5 to 5.7 (mean 5.0) and dry matter varied from 28 to 70% (mean of 48.3 %) (Table 1). Small number of samples from each farm limited statistical comparisons. Fermentation was mainly homo-lactic, with low ethanol and acetic acid, except for the butyric silage.

Microorganism's dynamics during silage fermentation was fairly normal on most farms (Table 1). Molds counts were under detection in most samples, with the exception of three silages prepared from first cut silage, having a maximum of 3.3 $\log_{10}$ g^{-1} fresh silage of farm D. Climatic conditions for the region in June 2008 were rainy, which restrict capacity to produced optimal quality silage. High moulds counts were not in relation with the pH since silage with the highest value had a pH of 4.6.

Table 1. Molds counts of fresh and ensiled forages (log_{10} g^{-1} fresh material)

Farm	Fresh forage				Silage	
	First cut		Second cut		First cut	Second cut
	log_{10}	DM (%)	log_{10}	DM (%)	log_{10}	log_{10}
A	5.43 [z]	47.2	2.88	54.5	< 2	< 2 [y]
B	5.98	45.2	4.99	56.1	2.0	< 2
C	5.81	57.9	n.d.	n.d.	< 2	n.d.
D	5.95	44.8	5.38	53.6	6.51	< 2
E	5.31	39.6	< 2	54.1	< 2	< 2
F	5.41	52.6	4.98	63.5	1.52	< 2
G	5.90	36.6	5.75	36.3	< 2	< 2
H	4.54	27.2	5.48	53.3	< 2	< 2

[z] Mean of log_{10} transformation of serial dilution plate counts data from both cut.
[y] Under detection limit of the plate counts method.

Most mycotoxins were present at low number in fresh forage (Table 2), with the exception of aflatoxins (AB1, AG1 and AG2) where concentration was higher. After fermentation, small decrease in fumonisin and citrinin had been observed, while the four aflatoxins and zearalenone increased in concentration (underlined data in Table 2). *Penicillium* spp. were the principal moulds isolated, followed by *Monascus* spp. Those results are similar to observations of O'Brian *et.al.* (2007) from Ireland were *Penicillium* spp. constitute the dominant species.

Table 2. Mycotoxins level between fresh and ensiled forage samples

	Detection classes [z]	Mycotoxins (% samples in classes)									
		DON	PAT	AB1	AB2	AG1	AG2	FUM	OTA	ZEA	CIT
Fresh forage	Below det. lev	0	33	0	0	0	0	73	47	0	7
	Low	60	67	33	80	33	13	***20***	53	100	93
	High	40	0	67	20	67	87	7	0	0	0
Silage	Below det. lev	8	31	0	0	0	0	92	38	0	15
	Low	54	69	23	62	31	8	0	62	85	85
	High	38	0	***77***	***38***	***69***	***92***	8	0	***15***	0

[z] Matrix did not allow quantification at time of experiment.

Conclusion: This preliminary investigation on the diversity of mycotoxins in round bale grass silage from northeastern Canada indicates that important mycotoxicoses causing fungi and some mycotoxins were present in fresh forage. Mycotoxins were present before ensiling and were not degraded during the first month of fermentation.

References:

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11:30-12:00 MYCOTOXIN CONTAMINATION 2009: WORLDWIDE OCCURRENCE IN COMPONENTS DESTINED FOR ANIMAL FEED

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From January 2009 until December 2009, a total of 9 030 analyses were carried out for the most important mycotoxins in terms of agriculture and animal production – aflatoxins (Afla), zearalenone (ZON), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA). In total, 2 660 different samples were analysed. Samples tested were diverse, ranging from cereals such as corn, wheat and rice to processing by-products, namely soybean meal, corn gluten meal, dried distillers grains with solubles (DDGS) and other fodder such as straw, silage and finished feed. Samples derived from different countries/regions all over the world, namely the Asian-Pacific region, Europe, Middle-East and Africa and the Americas (North and South America). The majority of the analyses were performed at ROMER Labs Diagnostic GmbH (Austria), ROMER Labs Singapore Pte Ltd (Singapore), ROMER Labs Inc (USA) and SAMITEC (Brazil). 75% of the samples were analyzed by HPLC, 25% by ELISA. Samples were mostly taken as part of routine analysis. Overall results showed 33%, 30%, 49%, 54% and 27% of samples contaminated (over detection limit) with Afla, ZON, DON, FUM and OTA. Data were additionally grouped according to occurrence in different geographical regions, on the occurrence in different raw materials and on the co-occurrence of different mycotoxins.

Highest contaminations (in % of total analysed) of aflatoxins were found in Africa (85% positive, 91 ppb average contamination), highest zearalenone contaminations were found in North Asia (59%, 326 ppb average) and South America (mainly Brazil 51% pos., 185 ppb average) and in North America (40% pos., 324 ppb average). In most analysed regions more than 50% of samples were affected with deoxynivalenol, highest levels in South Africa (88% pos., 1403 ppb). Fumonisin was most prevalent in North America (82% pos., 1800 ppb average), Brazil (87% pos, 4965 ppb average), South Europe (81%, 3411 ppb), and Africa (80%, 1488 ppb). South Asia showed highest ochratoxin A contamination (63% pos., 34ppb average).

Comparing different materials analysed it can be stated that maximum contaminations (for each mycotoxin) for aflatoxins were found in Vietnamese corn (6105 ppb), Japanese corn for zearalenone (7422 ppb), corn gluten meal from Malaysia (11836 ppb deoxynivalenol and 32510 ppb fumonisin). Highest contamination for ochratoxin was found in a finished feed sample from Pakistan (1582 ppb).

74% of all analysed samples were contaminated with one mycotoxin and 40% were contaminated with more than one mycotoxin.

12:00-12:30 LEVEL DETERMINATION OF DEOXINIVALENOL (DON) IN CANINE COMMERCIAL BALANCED FEEDING AT THE BOLIVARIAN OF REPUBLIC OF VENEZUELA.

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Background: El Deoxinivalenol (DON) a mycotoxin, it is main toxin in **tricothecenes** group, which are second metabolite toxins derived from fungus belonging to *Fusarium* genus. DON it is mainly produced by *F. graminearum* and *F. culmorum* which can be naturally developing in several cereals that are used to formulated commercial balanced feeding of animals directed human feeding an company. DON produced gastrotoxicity, vomit and rejection at some animals species, it could also produce inmunosuppression and last of muscle coordination (Vaamonde, 1996). The Food and Drug Administration (FDA), establish that feeding for puppets must have a permissive maximum level of 2mg/kg for DON. Venezuela at 2005 occur an outbreak of mycotoxicosis, this problem cause the last of 500 animals (Dog and Cats) and millionaires waste for commercial food manufacturers and animals owners (Sogbe, et al., 2006). DON information is too limited, so it is prevalence on commercial feeding and biological effects are so few.

Aim: Determine DON level in balance feeding of different brands for dog at the Bolivarian Republic of Venezuela.

Material and Methods: 37 samples were collected of food for dogs at the Bolivarian Republic of Venezuela (central region), at July of 2006. 28 of those were for adult dogs and 9 for puppies. Samples were analyzed for ELISA, using protocol specified on kits RIDASREEN®, then compared with levels permitted (DON) by the FDA.

Result and Discussion: 37 results were compared for DON in concentrated feeding for dogs with the permitted level for DON (2 mg/kg), finding levels between 0.44mg/kg – 4.38mg/kg. The 13.5% of samples obtained higher levels of DON compared to FDA permitted levels. Also finding that puppies feeding had a higher quantity of this mycotoxin. DON average detected in this essay for adult dogs was 1.03mg/kg (± 0.20) and for puppies was 1.11mg/kg (± 0.24). Dogs are known as one of the domestic animals more susceptible to toxicity for DON, which can be characterize by diminish of intake, rejection to eat, vomit and inmunosuppressive effect. Leading to a subit appearing of parasitic and infectious diseases, puppies could not respond to vaccinations. Toxins from the *Fusarium* genus, DON it is has the higher prevalence and does not look to be affected by conventional processing of balance feeding for puppies (Hughes, et al. 1999).

Conclusion: DON average results found at this essay indicated a poor supervision for this mycotoxin at commercial balanced feeding for dogs. DON quantity was higher at feeding for puppies that in adults been a higher toxic risk for the group of puppies.

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12:30-13:00 NATURAL OCCURRENCE OF MYCOTOXINS USED IN ANIMAL PRODUCTION IN MEXICO DURING YEARS 2007-2009

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Background: The mycotoxins are secondary metabolites that are produced by some genera of fungi. Although between 300 and 400 mycotoxins are known, those mycotoxins of most concern, based on their toxicity and occurrence, are aflatoxin (AF), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEA) and T-2 toxin (T2). Ingestion of mycotoxins leads to important loss of productivity and diminution of sanitary quality of by-products from animal consuming contaminated feeds (Rustom, I. 1997). Natural occurrence studies have shown extensive mycotoxin contamination in both developing and developed countries, it was estimated that 25% of the world's crops might be contaminated with mycotoxins. In particular, in Mexico have been reported the natural occurrence of several mycotoxins in feeds for domestic animals during the years 1977-1980 (Rosiles and Pérez, 1981). Concerning AF, its occurrence in maize has been reported in the 90% of the samples in a range from 2.5 to 30 µg/Kg (Ellis, et al, 1991), and in average of 66 µg/Kg in 42 samples analyzed (Carvajal and Arroyo, 1997). Other study reported the presence of mycotoxins in domestic pet foods, finding high concentrations in the 17.1% of the samples analyzed (Carvajal et al. 2001). In other hand, concerning fusariotoxins, in four fields in northeast Mexico, have been insolated strains of *Fusarium moniliforme*, which shows a potential production of fusariotoxins (Desajardins, A. et al, 1994), and Fumonisin B1, was detected in products derivated from maize, at 790 µg/Kg (Dombrink-Kurtzman, M. A, et al, 1999). In addition, analysis of 24 samples from Tlaxcala, México, indicates that approximately 70% of the monitored samples were contaminated with zearalenone, with levels ranging from 3 to 83 µg/kg of corn kernels. (Briones-Reyes, et al, 2007). Finally, in a study natural occurrence of mycotoxins indicate that a total of 57% of the samples analyzed contained detectable quantities of at least one mycotoxin, 11.2 % of these had mycotoxin concentrations higher than the maximum concentrations established in current regulations (Flores, et al 2006).

Aim: To recognize at the present time the natural occurrence of mycotoxins in grains, raw materials and feedstuffs used in animal production in Mexico.

Material and Methods: Grains, raw materials and feedstuffs samples were obtained from feedstuffs, poultry, swine and cattle production companies, within the framework of mycotoxins analysis service of the FES-Iztacala, UNAM, México. In all cases the feeds and farms industries carried out the primary sampling, these primary samples were homogenized and quartered to obtain a 1 kg laboratory sample. The mycotoxins analyzed in the samples depended on the specific production of the company from which they were obtained, analyzing from 1 to 4 mycotoxins, ascomycotoxins (AF and OTA) and fusariotoxins (T2 and ZEA). The methods used for analysis were High Pefrformance Liquid Chromatography and ELISA. The contents of mycotoxins in the samples were compared with respect of two references of regulations, the proposal of regulation for maximum levels for mycotoxins in grains and feedstuffs used in the poultry in México (SAGARPA, 2001) and the levels of mycotoxins established by Mercosur (FAO, 1995). The values used as reference of regulations considered as maximum permissible were: 20 µg kg^{-1} for AF, 10 µg kg^{-1} for OTA, 100 µg kg^{-1} for T2, and 150 µg kg^{-1} for ZEA.

Results and discussion: During years 2007-2009, 575 samples were analyzed for the content of 1 to 4 mycotoxins, AF, OTA, T2, and ZEA. In total, 1553 analysis were performed, 513 for AF, 444 for OTA, 415 for T2 and 181 for ZEA. The results show that 79.26% of the samples content detectable quantities of some mycotoxin, from which 15.38% have levels of contamination higher than regulations. The higher contaminated year for the period was 2009, where appeared 16.49% of the samples with levels by above of the regulations, in addition, appeared 14.05% and 11.56% on 2007 and 2008, respectively. In particular, the mycotoxin that shows the higher incidence was T2, with contamination in a range from 1 to 300.14 μg kg^{-1}, occurrence in 341 of 411 analyzed samples and 9.73% with levels higher than regulations. In general, the type samples which show the higher contamination were DDG´S and feeds. In summary, no one of the mycotoxins analysis in the tree years (1553 analysis) had levels that can cause mortality problems in the animal populations, Instead, the natural occurrence of mycotoxins can be noticeable in a pernicious reduction of the productivity parameters of the farms productions.

Conclusion: The 15.39% of samples analyzed during 2007-2009 was contaminated with levels above regulations.
The mycotoxin which shows the higher incidence was T2 and the samples more contaminated were DDG´S and feeds.

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13:00-13:30 STUDY OF FUNGI AND THEIR TOXIGENIC POTENTIAL ISOLATED FROM PAKISTANI POULTRY FEED AND FEED INGREDIENTS.

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Background: Agricultural products including, cereals, cereal by products and oilseed meals contribute as a major components of poultry feed. Mould contamination is wide spread in tropical countries where poultry production and processing are expanding rapidly. (Okoli *et al.,* 2006). Among different mycotoxins aflatoxins (AF) and ochratoxin A (OTA) are the most important contaminants of poultry feeds. Therefore regular monitoring of toxigenic mycobiota of agricultural based foods and feeds is essential to develop strategies for reduction and the prevention of mycotoxins contamination. Study of prevalence of toxigenic mycobiota of poultry/animal feeds is regularly and frequently reported from different countries including Brazil (Oliveira et al., 2006), Argentina (Dalcero et al., 1997), Nigeria (Osho *et al.,* 2007) and Spain (Accensi *et al*., 2004).In Pakistan limited studies are conducted in poultry feeds.

Aims: The aim of this study was to determine the mycobiota of poultry feed, feed ingredients and to evaluate aflatoxin and ochratoxin producing ability of isolated Aspergillus species.

Materials and methods: A total of 89 samples of commercial poultry feed, 30 samples of farm mixed poultry feed, 67 samples of wheat and 17 samples of wheat bran were collected from different grain markets, ingredient suppliers, poultry feed manufacturing units and home mix feed manufacturing poultry farms over a period of two years from august 2005 to august 2007. After through mixing representative sample was made and kept in polythene bags prior to inoculation onto culture media. These were inoculated on different culturing media and incubated at 27 ^{o}C in dark for 7-10 days. For mycotoxins analysis samples were processed through micro-scale extraction method and analyzed on HPLC with florescent detector.

Results and discussion: Out of total 89 samples of commercial poultry feed 62 (69.66 %) yielded fungi while remaining no fungi. Fungi belonging to four genera including *Aspergillus, Alternaria, Fusarium and Penicillium* were isolated from commercial poultry feed. Based upon total feed samples (n=89) frequency distribution of *Aspergillus, Alternaria, Fusarium and Penicillium* was 43.82, 1.12, 5.61 and 22.47 percent, respectively. Among different species isolated *A. niger* (19.10 %) was most frequently isolated species on basis of total samples and isolates followed by *P. verrucosum* (14.60 %), *A. flavus* (8.98 %), *A. ochraceous & P. chryosogenum* (7.86 %), *A. parasiticus and Fusarium spp*. (5.61 %). *A. carbonarius, A. fumigatus* and *Alternaria spp*. (1.12 & 1.54 %). High isolation frequency of Aspergillus from poultry feeds have also been reported from Spain (Accensi *et al*., 2004). Out of 39 isolates of *Aspergillus* from commercial

poultry feed, 31 (79.48 %) were found toxigenic. Aflatoxigenic isolates included *A. flavus* (100 %), *A. parasiticus* (100 %). Ochratoxigenic isolates included *A. carbonarius* (1/1, 100 %), *A. niger* aggregates (52.94 %) and *A. ocharceous* (100 %).In farm mixed poultry feed 25 (83.33 %) out of 30 samples yielded fungi while no fungus could be isolated from 5 (16.67 %) samples. Based upon total samples (n=30) frequency distribution of was 46.66, 10, 10 and 23.33 percent, respectively. *Penicillium verrucosum* was the most frequently isolated species followed by *A. flavus, A. niger* aggregates*, Alternaria spp., Fusarium spp., A. ochraceous, A. parasiticus, A. carbonarius and A. fumigatus.* Out of 14 isolates of *Aspergillus* from farm-mixed poultry feed, 9 (64.28 %) were found toxigenic. Aflatoxigenic isolates included *A. flavus* (2/3, 66.67 %), *A. parasiticus* (2/2, 100 %). Ochratoxigenic isolates included *A. niger* aggregates (2/3, 66.67 %) and *A. ocharceous* (3/3, 100 %). The total aflatoxins (AF) producing potential of toxigenic isolates on YES medium in the present study varied from 0.9 to 1987 ng/g and ochratoxin A (OTA) produced by these species on YES medium varied from 1.4 to 16720 ng/g. In wheat 45 (67. 16 %) out of 67 samples yielded fungi, while 22 (32.84 %) samples yielded no fungus. Based upon total samples, frequency distribution of *Aspergillus*, *Alternaria, Fusarium* and *Penicillium* was 34.88, 5.97, 7.46 and 26.74 percent, respectively. *P. verrucosum* was the most frequently isolated species in total samples followed by *A. niger* aggregates*, and other species.* Among *Aspergilli A. niger* aggregates (42.42%) was most frequently isolated species followed by *A. flavus* (18.18%), *A. ochraceous* (18.18%), *, A. parasiticus* (15.15%)*, A. carbonarius* (3.03%) and *A. fumigatus* (3.03%). Out of 30 *Aspergillus* isolates of wheat, 17 (56.67%) were found toxigenic. Aflatoxigenic isolates included *A. flavus* (3/6, 50%), *A. parasiticus* (4/5, 80%). Ochratoxigenic isolates included *A. niger* aggregates (6/14, 42.86%) and *A. ochraceous* (3/3, 100%). Their AFB1production potential ranged from 1.44 to 836.53 ng/g and maximum ochratoxin A (OTA) production was up to 15045 ng/g. similar to our studies Riba *et al*. (2008) from Algeria reported Aspergillus highes frequency followed by *Penicillium, Fusarium, Alternaria and Mucor*. In wheat-bran out of the total 17 samples, 10 (58.82 %) samples yielded fungi remaining no fungus. Based upon total samples, frequency distribution of *Aspergillus*, *Fusarium* and *Penicillium* was 35.28, 11.76 and 29.40 percent, respectively. Out of 6 *Aspergillus* isolates of Wheat-bran, 1 (16.67 %) was found toxigenic. No aflatoxigenic isolates was found toxigenic. Ochratoxigenic isolates included *A. ochraceous* (100 %).

Conclusion: The present study has provided information about contaminating toxigenic mycoflora of poultry feeds and feed ingredients. It is first report describing isolation of OTA producing Aspergilli in poultry feeds in Pakistan. This information will help to make strategies to avoid and prevent this hot issue.

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Session 16: Mycotoxins in dairy cattle and milk.

17:00-17:30 FACTORS AFFECTING CARRY-OVER OF AFLATOXIN M1 IN THE MILK OF DAIRY COWS GIVEN AFLATOXIN B1 IN THEIR FEED

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Aims:
In the course of a national residue control program to monitor farm milk for the presence of the mycotoxin aflatoxin M1 (AFM1), occasional samples were found to exceed the maximum residue level (MRL) of 50 ng/l. Efforts to incriminate a feedstuff contaminated with aflatoxin B1 (AFB1), the natural precursor of AFM1, failed to locate any feed constituent containing more than the regulatory limit of 20 µg/kg. In this work, cows were fed a measured quantity of AFB1 to examine what factors might impinge on the daily carry-over (CO) of AFM1 into their milk.

Materials and Methods:
Forty-four 500g aliquots of ground corn meal (CM) were weighed and placed on separate sheets of aluminium foil. A 5 mg standard of AFB1 (Sigma) was dissolved in organic solvents, and the equivalent of 80µg of dissolved AFB1 was evenly distributed onto each aliquot of CM; the solvent was allowed to dry for 3 h in a fume hood. Each aliquot of spiked CM was placed into a plastic bag and thoroughly mixed by shaking; all were stored at 4°C until the day of feeding.
Eight Israeli-Holstein cows in the Agricultural Research Organization research dairy were individually fed an AFB1-containing aliquot of CM containing 80µg AFB1, daily for 5 d, at 08.30 h. A total mixed ration (TMR) was given at 11.00 h each day. The CM and TMR were examined for AFB1 content. The cows were milked thrice daily. Feed intake and milk yield were recorded. Blood was taken before the trial and on Day 3 of feeding AFB1. Daily milk samples, representative of the daily yield (comprising 1% of each milking), were taken one d before, during the 5 d of AFB1 administration and 4 d thereafter, for AFM1 analysis and somatic cell count level (SCC) determination. Daily tank milk samples from cows receiving the same TMR but no AFB1 were analyzed for AFM1 as a reference. The milk of each cow was examined for agents causing mastitis.

Results and Discussion:
Very low levels of AFB1 were found in the CM and the TMR, whereas 2 spiked CM samples were shown to contain 72µg of AFB1. There were no effects of AFB1 administration on daily milk yield, nor on blood parameters indicating ill-health or liver damage. SCC was found to be elevated on some days of the trial in 6 of the cows, indicating sub-clinical mastitis. The mean milk yield and AFM1 CO into the milk for the 5 d of AFB1 administration are detailed in the Table below.

Cow	Lactation	Days in milk (d)	Milk yield (kg/d, ±SD)	Carry-over (% ±SD)
889*	1	203	37.2 ± 2.2	9.8 ± 2.3
902*	1	198	46.2 ± 2.5	12.1 ± 1.8
801*	2	302	33.7 ± 1.8	6.4 ± 2.0
910*	2	143	32.7 ± 1.4	10.3 ± 2.0
723*	4	123	37.6 ± 2.0	6.6 ± 1.6
809*	4	167	38.6 ± 6.6	6.8 ± 1.9
672	4	156	35.2 ± 2.4	6.9 ± 1.6
478	6	212	30.4 ± 3.3	3.4 ± 1.6

* Cow with elevated SCC on 2 or more d of the 5 d trial

The mean CO was 7.8%, which is higher than CO reported by other such trials where similar Holstein cows were milked twice daily (1,2). Stage of lactation and milk yield are the main factors known to influence CO. For example it was found that cows (milked twice daily) in early lactation (14-28 d in milk) and late lactation (238-252 d in milk) had mean CO of 6.2% and 1.8%, respectively (1). The cows in the present trial could be deemed "mid-late lactation", and so CO in early lactation might have been even higher than 7.8%. It was also shown that cows with high milk yield (40 kg/d) had a higher CO than cows with low milk yield (16 kg/d) (1). The mean milk yield in the current trial was 36 kg/d, which is similar to the national mean yield in Israel (35-40 kg/d). In a more recent trial with intensive husbandry, it was found that milk yield was the major factor affecting the total excretion of AFM1 (2). Cows that were around 140 d in milk and were milked twice daily with high milk yield (40 kg/d) and low milk yield (16 kg/d) had mean CO of 2.5% and 1.4%, respectively.
Considering possible causes for the high CO recorded in this trial, it is surmised that the thrice daily milking, compared with twice daily in previous trials, contributed to this effect. In addition, sub-clinical mastitis, (reflected in the elevated SCC), which was well evidenced in some of these cows, may have brought about an increase in blood-udder permeability (2), also leading to an elevated CO.

Conclusions:
AFB1 was transferred into cows' milk as AFM1 at a higher daily rate than previously found. This is likely to be due to the thrice daily milking in this trial, compared with twice daily in previous trials, and / or to the presence of sub-clinical mastitis.

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17:30-17:55 MICOTOXINAS EN RASTROJOS DE MAÍZ DESTINADOS AL CONSUMO DE BOVINOS PRODUCTORES DE LECHE EN TEPATITLÁN, JALISCO, MÉXICO.

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Antecedentes: La contaminación por micotoxinas en los forrajes (rastrojos o ensilados) puede presentarse durante el cultivo así como en el almacenamiento. Aún cuando han sido caracterizadas más de 400 micotoxinas, pocas se han reportado en los forrajes, destacando las aflatoxinas, deoxinivalenol, fumonisinas y zearalenona (Seglar et al., 1997). Los principales efectos de las micotoxicosis en rumiantes se caracterizan por la reducción de la producción y calidad de la leche, fallas en la respuesta inmunológica y terapéutica, además de algunos signos inespecíficos como reducción en el consumo de alimento, condición corporal deficiente, pelo áspero y problemas reproductivos (Whitlow & Hagler, 1998).

Objetivo: Evaluar la contaminación por aflatoxinas, deoxinivalenol, fumonisinas y zearalenona en el rastrojo de maíz cosechado en la localidad La Villa municipio de Tepatitlán, Jalisco.

Materiales y métodos: Se obtuvieron 68 muestras de rastrojo de maíz (planta completa), cultivados durante el ciclo primavera-verano 2007 bajo un diseño de bloques completamente al azar (4 observaciones/variedad o híbrido de maíz). Los genotipos estudiados correspondieron a 4 híbridos de maíz blanco, ciclo vegetativo tardío (H-318, H-319, H-321, H-358); 7 variedades de maíz amarillo de polinización libre, 6 de ciclo vegetativo precoz (1058, 1060, 1065, 1068R, 1069, 1463A) y 1 de ciclo intermedio precoz (1071); y 6 variedades experimentales mejoradas. Todas las muestras fueron analizadas por duplicado mediante la técnica de inmunoensayo enzimático de tipo competitivo para determinar aflatoxinas totales (AFT), deoxinivalenol (DON), fumonisinas (FBs) y zearalenona (ZEA). El presente estudio corresponde a la etapa preliminar del proyecto “Evaluación micotoxicológica de subproductos destinados a la alimentación animal”, el cual se realiza bajo el marco del proyecto Iberoamericano CYTED 109AC0371.

Resultados y discusión: El presente estudio permitió detectar la co-ocurrencia de micotoxinas en el rastrojo de maíz analizado. Se encontró AFT en el 76.5% de las muestras estudiadas (media: 7.43 μg Kg^{-1}; rango: ND–28.2 μg Kg^{-1}); DON en el 100% (media: 6.04 mg Kg^{-1}; rango: 1.74–22.44 mg Kg^{-1}); FBs en el 16.7% (media: 1.04 mg Kg^{-1}; 0.26–1.96 mg Kg^{-1}); ZEA en 91% (media: 507 μg Kg^{-1}; ND–9758 μg Kg^{-1}). Los niveles de AFT presentes en el rastrojo de maíz se encontraron por debajo del límite permitido por la Norma Oficial Mexicana NOM188-SSA-2002 (20 µg Kg^{-1}) a excepción

de una muestra. Todas las muestras analizadas presentaron niveles de FBs menores a los recomendados por la FDA (50 mg Kg^{-1}). No existen límites establecidos por Normas Oficiales para ZEA y DON en México, sin embargo, se recomienda que los niveles de DON no excedan de 5-10 mg Kg^{-1} en alimentos para ganado vacuno, y de 500 μg Kg^{-1} para ZEA (EC, 2006). Los resultados mostraron que el 53.8% y 53.4% de las muestras superaron los niveles recomendados para DON y ZEA, respectivamente. La co-ocurrencia de ZEA y DON ha sido reportada en otros países (Raymond et al., 2000). Si bien los efectos reproductivos de ZEA en vacas productoras de leche son menores que los observados en cerdas, posiblemente por la degradación de esta toxina por los microorganismos del rumen, puede observarse reducción en la fertilidad (Scudamore et al., 1997). Por su parte, DON puede afectar el consumo de alimento además de disminuir la respuesta inmune (Charmley et al., 1993).

Conclusiones: La co-ocurrencia de micotoxinas en el rastrojo de maíz de 17 genotipos cosechado en una localidad del estado de Jalisco, puede considerarse de riesgo a la salud por el efecto sinérgico potencial de dichas toxinas. Los niveles de micotoxinas obtenidos en este estudio serán confirmados por HPLC.

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17:55-18:20 ESTUDIO *IN VIVO* DE DOS MODELOS DE EXPOSICIÓN A AFLATOXINA B_1 EN RACIONES DE BOVINOS CON Y SIN ADSORBENTES Y SU CORRELACIÓN CON AFM_1 EN LECHE.

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Antecedentes: Las aflatoxinas son metabolitos secundarios producidos principalmente por *Aspergillus flavus* y *A. parasiticus* con efectos inmunosupresivos, mutagénicos, carcinogénicos y teratogénicos en humanos y animales (Task, 2003). En bovinos productores de leche el consumo de alimento contaminado con aflatoxina B_1 (AFB_1) determina la presencia de aflatoxina M_1 (AFM_1) en leche; la tasa de biotransformación varía de 0.32 a 6.2 % (Magan y Olsen, 2004). En México las Normas Oficiales NOM 188-SSA1-2002 y NOM 184-SSA1-2002 establecen el límite máximo permitido para AFB_1 y AFM_1 de 20 µg kg^{-1} y 0.5 µg L^{-1} respectivamente. Entre las estrategias para la reducción de las micotoxicosis se incluye el uso de adsorbentes de micotoxinas que se adicionan a las raciones durante el proceso de elaboración (Díaz y Smith, 2005). Estas sustancias se caracterizan por disminuir la biodisponibilidad de las toxinas en el tracto gastrointestinal del animal huésped al formar complejos que se eliminan con las heces.

Objetivo: Comparar dos modelos de exposición a aflatoxina B_1 en raciones de bovinos contaminadas artificialmente con y sin adsorbentes (FIXAT, QUITAFLAX ZEO y extractos de cultivos de *Saccharomyces cerevisiae* (ECSC) para determinar su correlación con la presencia de AFM_1 y parámetros de calidad de la leche.

Materiales y métodos: Se seleccionaron 12 animales de la raza Holstein clínicamente sanos, los cuales fueron expuestos a 40 µg kg^{-1} de aflatoxina B_1 en base materia seca (880 µg AFB_1/animal) utilizando dos modelos de exposición denominados Núcleo e Integral. Los adsorbentes minerales se incluyeron a razón de 40g/animal/día (0.4% en la ración) y el orgánico (ECSC) a 15g/animal/día (0.01%). El modelo Núcleo utilizó un diseño experimental de Cuadrado Latino con 4 grupos o tratamientos: T-1 (testigo AFB_1); T-2 (AFB_1 + montmorillonita); T-3 (AFB_1 + clinoptilolita) y T-4 (AFB_1 + ECSC*)* y 4 períodos de 11 días. Cada periodo se dividió en dos subperiodos (A: 6 días y B: 5 días), el primero permitió administrar la ración libre de AFB_1 y excepto en el testigo fueron incluidos los adsorbentes, lo cual permitió monitorear la presencia de AFM_1 previo a la exposición a AFB_1, en el segundo la AFB_1 se administró en corrales individuales en una sola dosis cada tratamiento (AFB_1 con/sin adsorbente en 280 g de concentrado). El modelo Integral se realizó bajo un diseño experimental por bloques, con un grupo conformado por 4 vacas que cursaron por 4 periodos de 11 días (periodos descritos previamente), el mismo grupo recibió los 4 tratamientos sucesivamente. La AFB_1 y los adsorbentes se integraron en toda la ración y se suministró en el comedero del corral experimental. La determinación de AFB_1 en la ración y de AFM_1 en la leche se realizó mediante las técnicas de HPLC descritas por AOAC 2003.02 y 994.08 para AFB_1 y

AOAC 2000.08 para AFM_1. Los resultados fueron analizados mediante ANOVA y las diferencias estadísticas se establecieron por la prueba de Tukey ($P < 0.05$) utilizando el paquete estadístico Sigma STAT v3.1 para Windows. .

Resultados y discusión: Los resultados reportados bajo el modelo Núcleo mostraron una tasa de biotransformación de AFB_1 a AFM_1 de 3.2 % y una eliminación promedio de AFM_1 en la leche del testigo de 1.184 µg L^{-1} con porcentajes de reducción en la eliminación de AFM_1 de 19 %, 17.6 % y 11.2 % respectivamente para T-2, T-3 y T-4, sin encontrarse diferencia estadística entre tratamientos. La exposición a AFB_1 bajo este modelo favoreció su absorción con un consecuente aumento en el porcentaje de biotransformación. Larrson *et al,* (1989) reportaron que además del hígado la mucosa nasal olfatoria posee gran capacidad para formar metabolitos derivados de AFB_1, condición que pudo favorecer la presencia de AFM_1 en la leche debido al uso de un núcleo conteniendo niveles elevados de AFB_1. En el modelo Integral la tasa de biotransformación fue de 1.8 %, con niveles de eliminación promedio de AFM_1 de 0.585 µg L^{-1}, los porcentajes de reducción de AFM_1 en la leche fueron de 33.2 %, 36.8 % y 12 % para T-2, T-3 y T-4 respecto al testigo, mostrando diferencia estadística entre tratamientos ($P< 0.05$). Debe destacarse que la tasa de biotransformación observada bajo este modelo fue similar al promedio reportado por Magan y Olsen, (2004) para estudios realizados con alimento naturalmente contaminado con AFB_1, además la aplicación de Modelos Matemáticos permitió observar la relación lineal entre ambos metabolitos.

Conclusiones: El modelo Integral demostró mayor confiabilidad para evaluar la eficiencia de los adsorbentes de micotoxinas *in vivo*. Los adsorbentes minerales montmorillonita (FIXAT) y clinoptilolita (QUITAFLAX ZEO) redujeron significativamente ($p < 0.05$) los niveles de AFM_1 en leche a niveles por debajo de lo reglamentado en México, mientras que el adsorbente ECSC no demostró eficiencia.

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18:20-18:50 CONTAMINACIÓN CON AFM_1 EN QUESOS FRESCO, ADOBERA Y ASADERO ELABORADOS ARTESANALMENTE EN LA REGIÓN DE LOS ALTOS, JALISCO

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Antecedentes: La aflatoxina M_1 (AFM_1) es un metabolito 4-hidrolizado de la AFB_1 y puede encontrarse en la leche y consecuentemente en otros productos lácteos. La afinidad de la AFM_1 a la caseína de la leche permite incrementar su concentración en quesos (Oruc et al., 2006). La presencia de AFB_1 y AFM_1 es considerada de riesgo a la salud, ambas toxinas fueron clasificadas como agentes carcinógenos para humanos del grupo 1 por la Agencia Internacional de Investigaciones sobre el Cáncer (IARC, 2002). En México las Normas Oficiales NOM 188-SSA1-2002 y NOM 184-SSA1-2002 establecen el límite máximo permitido para AFB_1 y AFM_1 de 20 μg kg^{-1} y 0.5 μg L^{-1} respectivamente.

Objetivo: Determinar los niveles de contaminación por AFM_1 en quesos elaborados artesanalmente en la región de los Altos, Jalisco.

Materiales y métodos: Durante los meses de enero a septiembre de 2009 se obtuvieron 150 muestras (500g) de quesos tipo fresco, adobera y asadero elaborados en fábricas artesanales de las localidades de San Miguel el Alto, Santa Maria del Valle, Capilla de Guadalupe, San Ignacio Cerro Gordo y Tepatitlán, Jalisco. Todas las muestras fueron procesadas mediante la técnica de Inmunoensayo de tipo competitivo (Inmunolab.GmbH). Los resultados fueron analizados mediante ANOVA utilizando el programa Sigma STAT v3.1 para Windows.

Resultados y Discusión: Se encontró contaminación con AFM_1 en el 87% de los quesos analizados. Los niveles promedio en el queso fresco, adobera y asadero fueron de 0.195 μg kg^{-1}, 0.257 μg kg^{-1} y 0.169 □μg kg^{-1} respectivamente, similares estadísticamente ($P>0.05$). El nivel máximo detectado de AFM_1 se observó en una muestra de queso adobera 1.512 □μg kg^{-1}. El 10.7% (16/150) de las muestras de queso estudiadas presentaron niveles que superaron los niveles permitidos por la regulación en México. Los resultados observados en el presente estudio son comparables con los encontrados en Turquía por varios investigadores (Aycicek et al., 2005; Yapar et al., 2008; Ardic et al., 2009), reportándose en algunos estudios alto porcentaje de muestras que superan el límite máximo permitido por el Codex Alimentario de Turquía (250□ ng kg^{-1}), mientras que en Italia, Pietri et al. (1997) encontraron 91% de muestras de queso con niveles de 5 a 100 ng kg^{-1}, sólo una muestra superó el límite permitido. En diversos países no ha sido posible establecer los niveles permisibles para los productos lácteos como los quesos debido a la dificultad de identificar el factor de conversión estandarizado para productos derivados de la leche. Existen pocos datos disponibles en

la literatura nacional e internacional relacionados a factores de la concentración en los diferentes productos lácteos y esos datos son pobremente aplicables a los procesos de manufactura nacional de quesos.

Conclusiones: Se observó contaminación con AFM_1 en quesos artesanales elaborados en la región de los Altos, Jalisco, lo cual implica riesgo a la salud pública. Deben realizarse nuevos estudios durante otras épocas del año debido a las imprevisibles condiciones ambientales y climáticas, así como por las deficiencias de algunos sistemas de producción agrícola y pecuarios que permitan prevenir o disminuir el riesgo de exposición a micotoxinas en animales y humanos.

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PHYCOTOXIN SECTION

Valladolid

Monday 28

Phycotoxin Session 17: Cyanobacterial and Phytoplankton Toxins

10:30-11:30 THE ROLE OF CYANOBACTERIA IN HARMFUL ALGAE BLOOM (HAB) EVENTS

Keynote Conference

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Increasingly, harmful algal blooms (HAB's) are being reported worldwide due to several factors, primarily - eutrophication, climate change and more scientific investigation. HAB organisms include those causing: PSP (paralytic shellfish poisoning), DSP (diarrhetic shellfish poisoning), NSP (neurotoxic shellfish poisoning), ASP (amnesic shellfish poisoning) and CTP (cyanobacteria toxin poisoning). All but CTP organisms are mainly a marine occurrence. CTP's occur in freshwater lakes, ponds, rivers and reservoirs throughout the world. Organisms responsible include an estimated 40 genera but the main ones are *Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Microcystis, Nostoc and Oscillatoria (Planktothrix).* Cyanobacteria toxins (cyanotoxins) include cytotoxins and biotoxins with biotoxins being responsible for acute lethal, acute, chronic and sub-chronic poisonings of wild/domestic animals and humans. The biotoxins include the neurotoxins; anatoxin-a, anatoxin-a(s) and saxitoxins plus the hepatotoxins; microcystins, nodularins and cylindrospermopsin.

Concern for animal and human health impairments due to toxic cyanobacteria arises from several sources of information. The main one concerns reports of animal poisonings from ingestion of toxic waterblooms beginning with the later part of the 1800's. It was not until the 1950's, with pioneering work of scientists in Canada, The United States, Australia and South Africa, that we began to understand that cyanobacteria could indeed produce highly toxic compounds. The animal and human intoxication reports were first summarized in the 1960's. A major effort to compile all available information on toxic cyanobacteria including issues of human health, safe water practices, management, prevention and remediation has been published by the World Health Organization. This book is designed as a manual for water and health

authorities in addressing all issues of toxic cyanobacteria that they may be confronted with. Along with the WHO manual came a study on the risk assessment of cyanotoxins in drinking and bathing waters. Risk assessment of microcystins indicate that a level of 1 μg L^{-1} should be considered a guideline value for maximum allowable concentration (MAC) based upon an adult consumption of 2 L day^{-1}. Other MAC's for the neurotoxins and cylindrospermopsin will not be set until more basic toxicology and epidemiology are available. In addition the State of Oregon in the U.S.A. has set a regulatory level of 1 μg g^{-1} for microcystins in human food supplements made from the filamentous cyanobacterium *Aphanizomenon flos-aquae* and Brazil has set a regulatory level of 1 μg g^{-1} for microcystins in drinking water supplies. Health authorities from several countries are also evaluating the risks of cyanotoxins in algae and may also adopt guideline or regulatory levels for microcystins in drinking waters and algae food products.

Confirmation of human deaths from cyanotoxins is limited to exposure through renal dialysis. Microcystins were implicated as the major contributing factor in liver failure and death of at least 52 humans, in 1996, at a haemodialysis center in Caruaru, Brazil. This tragic but avoidable event point to the importance of understanding cyanotoxins as health hazards in drinking waters and then communicating this information, including the correct identification of cyanobacteria responsible for poisonings, to water authorities and public health officials. Since most of the worlds reservoir and lake based water supplies are subject to increasing nutrient levels, it is probable that episodes of cyanotoxin poisoning will continue unless measures are taken to improve our understanding of their role in water-based diseases. For these reasons it is important to consider the prevention, management and mitigation of CyanoHABs.

11:30-12:20 BIOACCUMULATION OF CYANOBACTERIAL TOXINS BY COMMERCIALLY IMPORTANT FISH AND INVERTEBRATES FROM TWO MEXICAN LAKES, LAKE CATEMACO AND LAKE PATZCUARO

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Background: Cyanobacterial toxins pose a well-recognized threat to human and environmental health, particularly via direct exposure to these toxins in drinking water or related routes. Less understood is the bioaccumulation of cyanobacterial toxins in freshwater food-webs, and associated potential for exposure to, and consequent toxicity of, these accumulated toxins. We identified two freshwater systems in Mexico, specifically Lake Patzcuaro (Michoacan) and Lake Catemaco (Veracruz), that are characterized by recurrent blooms and otherwise persistent dominance of known toxigenic cyanobacteria, including *Microcystis* and *Cylindrospermopsis* species, respectively. Associated with both of these systems are locally and commercially important species of fish and invertebrates (e.g. snails) that may represent novel vectors for bioaccumulated toxins, and thereby present a unique opportunity to understand health impacts of this mode of exposure.

Aim: Our studies focused on the identification and measurement of cyanobacterial toxins, including microcystins (MCYSTs), cylindrospermopsin (CYN) and saxitoxins (STx)/paralytic shellfish toxins (PSTs), in Lake Patzcuaro and Lake Catemaco, as well as bioaccumulation of these toxins in representative species of fish and invertebrates from these lakes, which are consumed by humans.

Methods and Materials: Integrated 5-m core samples, subsequently separated by filtration into particulate and dissolved fractions, along with bloom material, were collected from Lake Patzcuaro and Lake Catemaco. Samples of fish, including

“charales” (*Chirostoma* spp.), *Goodea* sp. and carp (*Cyprinus carpio*), were obtained from commercial catches, as well as local markets (i.e. charales) near Lake Patzcuaro. Samples of “tegogolo” snails (*Pomaceae patula catemacensis*) were obtained from local vendors in the vicinity of Lake Catemaco. Samples were quantitatively analyzed for MCYSTs, CYN and STx/PSTs by commercially available ELISA, as well as other methods, including LC-MS/MS (CYN and MCYSTs) and protein phosphatase inhibition (MCYSTs) as appropriate. Bioaccumulation factors (BAFs) were calculated for fish and tegogolos.

Results and Discussion: In Lake Patzcuaro, a daily recurring bloom of *Microcystis/ Aphanizomenon* was found to produce several variants of the MCYSTs that were measured at appreciably high levels in both particulate and dissolved fractions throughout the lake. In Lake Catemaco, which is characterized by a general dominance of *Cylindrospermopsis* sp., both CYN and STx/PSTs were detected in particulate material from the lake, although levels were quite low. Moreover, representative species of fish (i.e. *charales*, *Goodea* spp. and *C. carpio*, Lake Patzcuaro) or invertebrates (i.e. *tegogolo* snails, Lake Catemaco) from each lake were found to bioaccumulate these respective toxins at levels above those found in the water column. Results will be discussed, particularly with respect to the possible threat these accumulated toxins may pose to human and environmental health.

Conclusions: We identified presence, and apparent bioaccumulation, of three known cyanobacterial toxins, MCYSTs, CYN and STx/PSTs, in the two lakes, Lake Patzcuaro and Lake Catemaco, investigated. This is the first report of these cyanobacterial toxins in these two important freshwater systems. In particular, the bioaccumulation of these toxins by species that are consumed locally, as well as sold commercially, represents a potentially unique system for future investigation of the human health effects associated with cyanobacterial toxins in the food-web.

12:20-13:00 HEALTH RISK FOR ELITE CANOEING AND KAYAKING PADDLERS IN EUTROPHICATED WATER WITH CYANOBACTERIAL BLOOMS (*Microcystis* sp AND *Planktothrix* sp).

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Background: The Olympic rowing and canoeing venue "Virgilio Uribe" in Mexico city, receives effluents from wastewater treatment plant causing anthropocentric eutrophication in the water body that stimulates the growth of blooms and scums of cyanobacteria (*Microcystis* sp and *Planktothrix* sp). This species of cyanobacteria produces a secondary metabolite toxin known as: microcystin which has been known to cause serious health impairments as reported in numerous cases of irritation of the skin and/or mucous membranes, and from documented cases of illnesses after exposure through drinking water as well as accidental swallowing or aspiration of scum material (Chorus *et al*., 2000). We can therefore hypothesise if the elite flatwater athletes who spend time training in polluted water are at higher risk of presenting symptoms due to contact with microcystin.

Aim: The objective of the study was to calculate the concentration of microcystin in the above mentioned Mexico City venue and in the volume of water in the wet t-shirt of a canoeist after finishing a 1000m race, making a simple estimate of the levels of microcystin that could affect the athlete after one training day.

Material and methods: 23 male (18 kayak paddlers in K1s and 5 canoe paddlers in C1s) took part in this study. The weights of the paddlers as well as their cotton t-shirts were evaluated before and after a 1000m race using an electronic scale. In the water venue we evaluated the concentration of microcystin LR with (EnviroLogix Kit).

Results and Discussion:

Table 1. Calculation of the concentration of microcystin, body weight and volume of water in t-shirt (Mean ± SD)

Variable	Samples (n=3)	Kayakist (n=18)	Canoeist (n=5)
Microcystin in the water (µg/l)	3.53 ± 0.14		
Body weight (kg)		72.83 ± 2.29	67.4 ± 3.76
Dry weight t-shirt (gr)		0.13 ± 0.01	0.14 ± 0.02
Wet weight t-shirt (gr)		0.15 ± 0.01	0.16 ± 0.03
Quantity of water in the t-shirt in 1000m race (ml)		24.16 ± 4.95	16.2 ± 5.34
Calculation of the indirect concentration of microcystin in 1000m (µg/l)		0.084 ± 0.017	0.056 ± 0.018
Extrapolated microcystin in t-shirt after 12 km (µg/l)		1 ± 0.25	0.67 ± 0.22

The World Health Organization's (WHO's) recommended guidelines for drinking water limits the amount of microcystin to 1 µg/l (WHO, 1998). In this study the concentration of microcystin in the water is more than twice as the recommended guidelines. A recommended limit ranging from 10 to 400 µg/l of microcystin has been suggested for the safe practice in managing recreational waters (Chorus et al., 1999). The kayak paddlers wet theirs t-shirt more than their canoeing counterparts, so that the former are at higher risk of exposure to microcystin concentration.

Conclusion: The concentration for a 12 km training session was calculated as being 1 µg/l and 0.67 µg/l for kayaks and canoes respectively meaning that great care must be taken while training since this sport requires prolonged, intense training sessions with paddlers covering distances of 3000-4000 km per 10 months training season (García-Roves *et al*, 2000). Since kayak paddlers are exposed for a longer time, lakes or artificial venues have to have good recreational water quality in order to avoid intoxication and disease.

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Yucatan I

Monday 28

Poster Session X: Cyanobacterial and Phytoplankton Toxins

Moderator: **Cruz Lozano-Ramírez** (Mexico)

P-108 *IN VITRO* CITOTOXICITY OF CYANOBACTERIAL CRUDE EXTRACT FROM ANTIOQUIA (COLOMBIA)

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INTRODUCTION: The occurrence of cyanobacterial blooms are a potential dangerous related to water quality, since some species of cyanobacteria produces several toxins (Hudnell 2008). Human and animal illness and in some cases death have occurred from exposure to cyanotoxins, toxic secondary metabolites produced by cyanobacteria, through skin contact and consumption of potable and recreational waters (Oehrle 2010).

Toxicity assays are important to ensure good water quality but only few studies have determined the potential exposition to these toxins (Anjos 2006). The main research has focused on the predictions of influence of environmental factors in the formation of harmful cyanobacterial. (Sarnelle 2010). In recent years, in vitro-toxicity-tests involving the use of cultured cells have been developed to provide a substitute for the mouse bioassay (Masango 2008). In the current study, toxicity of algal samples collected during October of 2009 were measured using Vero and U-937cells assays. Also, liquid chromatography coupled with mass spectrometry (LC-MS) analyses evidenced some cyanotoxins.

MATERIALS AND METHODS

Collection of cyanobacterial samples

From a cyanobacterial bloom occurred at October 2009 at Antioquia /(Colombia), 500 g of material was collected and stored at -4°C until lyophilization.

Extracts preparation

Lyophilized cyanobacterial samples (160 g) were extracted with 1000 mL of 80% methanol (5x200), with ultrasonication for 15 min; after that, solvent was evaporated until dryness in a rotaevaporator. Then extract was separated using silica gel and sephadex colums and finally until obtain 20 fractions, according to tlc.

***In vitro* assay of citotoxicity**

The evaluations were performed in vitro cytotoxicity of 5 cell lines which were: U-937 (human promonocytic cells), Vero (kidney cells from African green monkey), using the enzymatic MTT micromethod. The tests were performed twice with 2 replicates for each concentration tested and the results are expressed as the lethal concentration 50 (LC_{50}) which was calculated by the Probit method. The amphotericin B ® was used as the control of cytotoxicity.

Mass spectrometry

The LC–MS experiments were carried out on an Agilent 1200 binary pump. Injection volumes were 10 μl. The mobile phase consisted of 0.2% formic acid (solvent A) and acetonitrile (solvent

B) with the following linear gradient programme: star with 10%B at 20 min 55% B. Flow rate of 0.8 ml/min.

RESULTS AND DISCUSSION: A high-biomass of cyanobacterial blooms was detected being *Microcystis* sp. was the main microalga; columns chromatography on silica gel and then sephadex brought a good separation of chlorophylls and fatty acids; high concentrations of carotenes were also separated, according to nmr spectroscopy. The last fractions contained several compounds were monitored through HPLC/MS, using mass scan mode to detect the peaks at *m/z* 1045, 1024, 953, 934 corresponding to several compounds reported before by others authors (Prakash 2009). Additionally Microcystins AR, WR, LR were detected in other peaks at m/z 953, 925, 728, 484, 440, 375, 213, 135; 1068, 1040, 934, 599, 626, 375, 213, 135; 728, 682, 599, 375, 213, 135, corresponding to specific fragmentations of each compound.

Although all compounds displayed high toxicity (Table 1), Vero cells were less sensitive than U-937 and main toxicity was detected in fraction 9, which contain nitrogen compounds, according to tlc using Dragendorf's reactive. These cells could be a more accurate and fast method to detect cyanotoxins instead of mice.

Table 1. Results in vitro assay of citotoxicity from cyanobacterial fractions.

	CL_{50} (µg/ml) X ± SD	
Name	U-937	Vero
A4	41.2 ± 6.2	39.7 ± 8.0
A5	> 200.0	> 200.0
A7	84.8 ± 2.8	51.3 ± 6.3
A8	29.7 ± 0.34	> 200.0
A9	16.8 ± 2.0	> 200.0
Amphotericine B	35.9 ± 5.5	61.5 ± 4.0

CONCLUSIONS: Analysis of the microcystins in algae has been successfully accomplished by using both HPLC- MS and bioassays, Both offer good potential for the analysis of microcystins in algae.

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P-109 SEASONAL VARIATION OF TOXICITY IN MARINE MACROALGAE OF VERACRUZ, GULF OF MEXICO

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Background. Algae are consumed by a lot of herbivorous, chemical defense is a powerful barrier against these herbivorous, these substances can be a risk for human consumers and they are an ecological element for the survivability of algae (Lozano, 1991). The chemical defense of algae is a collection of a wide variety of substances (Magallanes *et al.,* 2003). The toxicity of some algae has been an evolutionary factor that have been important in relation to distribution and abundance of these organisms (Lubchenco and Cubit,1980; Hay, 1996).

Aim. The setting up of biological tests using guppy *Poecillia reticulata* and plecostomus *Hypostomus plecostomus* in order to get a toxicity degree comparison related to the algae collecting season.

Materials and methods. Algae were collected in several localities in Veracruz State during February 2006; April 2007; September 2007. The total number of collected algae species was 17. Algae were frozen to avoid decomposition and were transported to laboratory. After, epibiontic organisms were eliminated from algae. The algae were extracted using distilled water, ethanol or acetone. The extracts were used for toxicity essays using guppys, *Poecillia reticulata* and plecostomus *Hypostomus plecostomus.*

Resuts and discussion. Macroalgae present a dissimilar toxicity activity depending on collecting season. *Acantophora* is a good example of this behavior. However *Cymopolia barbata* produces toxic metabolites during any collecting season. *Cymopolia* was toxic with any solvent used. It is possible that cimopol, a sesquiterpene isolated from *Cymopolia* be the responsible substance. Some Rhodophyta produce very toxic brominated phenols, however this kind of substance has not been isolated from *Gracilaria* genus. Two species of Gracilaria were toxic and could be an agar source.

Conclusion. Information about toxic macroalgae in Mexico is scarce. Knowledge of toxic macroalgae biology can give us useful information about how to use them as biological control against overpopulation of exotic introduced fish species in some water bodies, these algae could be a good alternative to more aggressive biocides.

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P-110 BIOASSAYS TO DETECT TOXICITY IN CULTURES OF MARINE MICROALGAE

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Background. As it happen in many places around the globe, dinoflagellates are economically important in Mexico. These organisms produce many molecules that present a diverse toxicity levels. The dinoflagellate growth population is known as Harmful Algae Bloom (HAB) and these events are ecologically important because toxic molecules can be concentrated along food chain producing severe health troubles in a variety of organisms, humans included (Cortés-Altamirano *et al*. 2006; Tiffany *et al*. 2001). Isolation and controlled conditions culture of dinoflagellates will let to know life cycle and metabolite production in a better way. Although it exists several research centers that make investigation on dinoflagellate culture is a very evident reality that is impossible that an investigation center could to get a complete collection of these organisms. Therefore is better to obtain organisms from the places where the HAB is produced in each country and cultivated them in order to make experiments for understanding physiological issues and, dynamic of HAB's and the imminent toxicity (Band-Schmidt *et al.,*2006; Rodriguez-Palacio *et al.,* 2009).

Aim. Test for toxicity in marine dinoflagellate cultures by bioassays using brine shrimp *Artemia salina* and guppy *Poecillia reticulata.*

Material and method. Dinoflagellate cultures were harvested during exponential phase. After, cultures were lyophilized in order to get cellular disruption; metabolites were obtained using a buthanol plus water solution. Extracts were dried up; at this point they were added with 9% salt-water solution. Each strain was tested three times with a control experiment. When brine shrimps were hatched they were nourished with *Chlorella vulgaris* for the three initial days; after three days are placed in a flask and algal extract is added. Organisms in control experiment were nourished with *Chorella*. Experiments with guppys were carried out in triplicate and just salt-water solution was added.

Results and discussion. Extracts from *Amphidinium* sp., *Chattonella marina, Heterocapsa pymaea, Gyrodinium uncatenum, Gyrodinium instriatum, Prorocentrum rathymum, Protoceratium reticulatum* were tested during exponential phase. Extracts were classified considering the behavior of experimental organisms when they were nourished with *Chlorella*. Key: (T) toxic; (MT) moderately toxic; (NT) not toxic.

Conclusion. Toxic molecules in nature represent a wide research topic in several science fields. Bioassays using *Artemia salina* and some ornamental fishes could be a powerful toxicity detecting test as good as mouse bioassays. An opportune specific detection test for harmful algae could help us to take ecological and fishery actions in order to avoid damage in human populations.

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Valladolid

Monday 28
Afternoon

Oral Phycotoxin Session 18: Dinoflagellate and harmful microalgae.

16:00-16:20 STRAINS OF TOXIC AND HARMFUL MICROALGAE, FROM WASTEWATER, MARINE, BRACKISH AND FRESH WATER

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Background. Microalgae are economically important in Mexico because some species could be potentially toxic. These explosive population growths are named harmful algal blooms (HAB´s) and are frequently recorded in Mexico. In relation to the research project called "Establecimiento de una colección de cultivos de microalgas. Primera etapa" developed in UAM-I, and the project "Fitoplancton tóxico y nocivo del Pacífico tropical mexicano: caracterización de las especies y comunidades, y variables ambientales asociadas a los eventos tóxicos y nocivos" developed in Instituto de Ciencias del Mar y Limnología, UNAM (Project PAPIIT DGAPA), we have set up potentially toxic microalgae cultures from Gulf of Mexico (Garrapatas tideland, Barberena river, Carpintero lagoon in Tamaulipas State; Chalchoapan and Catemaco lakes in Veracruz State), from mexican Pacific Ocean Guerrero, Colima and Michoacán States, and from interior water bodies as Miguel Hidalgo dam, Chapultepec lake and several wastewater treatment plants. Cultures of microalgae have greatly contributed to the better understanding of the morphology, phylogeny, life cycles, physiology and ecology of the species (Coats *et al.,*1984, 2002).

Aim. Set up potentially toxic microalgae cultures.

Material and methods. Living microalgae cultures were concentrated by inverse filtration techniques, microbiological handgrip on microbiological agar, seriated dilutions and micropipette isolation on f/2 medium (Guillard & Rytter, 1962; Guillard, 1975), L1 (Guillard & Hargraves, 1993) and/or L1SE (Rodríguez-Palacio, 2009). Culture media were prepared with pre-filtrated and sterilized water from the same localities. These cultures are non-axenic, semi-continuous, maintained with 12:12 light-darkness cycle and 18-20^{0}C ± 1^{0}C with a 90.5 µmol m^2 s^{-1} and 166.8 µmol m^2 s^{-1} light irradiation, respectively. Strains are inoculated depending of growth rate in each species.

Results and discussion. Between potentially toxic algae cultures we have set up are *Alexandrium tamarense, Amphidinium* sp. *Heterocapsa pigmea, Gyrodinium instriatum, Gymnodinuim catenatum, Karlodinium veneficum*., *Prorocentrum gracile, Prorocentrum micans, Prorocentrum triestimum, Prorocentrum mexicanum, Prorocentgrum rathymum,*

Protoceratium reticulatum, Pseudo-nitszchia delicatisima and *Scrippsiella trochoidea.* Fresh water and brackish species are *Anabaena* sp*., Bacilaria paxilifera*, *Chattonella marina, Cylindrotheca closterium, Nostoc* sp*., Pseudoanabaena.* All these species have been reported as potentially HAB producers. This type of research in UAM-I open an important investigation field and it is a powerful support for researchers who need work with alive organisms for experimental projects and teaching.

Conclusion. Microalgae cultures are a powerful tool for understanding the biological characteristics of these organisms because with traditional sampling methods is possible the organisms be damaged or morphological characters could be altered during fixing process. Moreover, these cultures can be used for toxicity tests and for the establishment of mitigation actions in order to protect human populations.

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16:20-16:40 FIRST REPORT OF *Pyrodinium bahamense* var, *bahamense* (GONYAULACALES: GONIODOMATACEAE), TOXIC DINOFLAGELLATE IN CAMPECHE BAY, MEXICO.

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Background: A potentially toxic dinoflagellate species is *Pyrodinium bahamense* Plate (1906) var. *compressum* (Böhm) Steidinger, Tester et Taylor (1980). Recently confirmed the toxicity of the var, *bahamense* (Landsberg *et al*., 2006). But in Mexico the cases of paralytic poisoning have been associated with the variety *compressum*.

Aim: The objective of this paper is to add to the inventory of phytoplankton species to *P. bahamense* to Campeche Bay, Mexico.

Materials and Methods: Hydrographic data and phytoplankton samples were obtained by collecting seawater samples from December 2007 to November 2008 in Campeche Bay in two station georeferenced, at three depths. Samples were fixed *in situ* with an iodine solution to 1% (Sournia, 1978). Additionally, circular tows were made with a conical net with mesh size of 35µm at each sampling point. The quantification of phytoplankton cells was carried out according to the method of Utermöhl (Hasle, 1978). The abundance values are expressed in cells per liter (Célls L^{-1}). For observation and identification of phytoplankton species were performed with fresh samples using a microscope Olympus CH30. The identification of species in fresh was carried out with the aid of specialized taxonomic keys.

Results and Discussion: Morphologic and cell size to *P. bahamense* of Campeche Bay, are closely related to var, *bahamense*, who presented a mean length (excluding apical horn and spinal) of 43µm and a transverse diameter of 41.5µm, close to those reported by Steidinger et al. (1980), for the coasts of Florida and Jamaica, who quantifies average length of 43µm (excluding apical horn and spinal) and 40µm transverse diameter, whereas Badylak et al. (2004) reports an average length (excluding apical horn and spinal) of 41µm and an transverse diameter of 43µm. For the coast of Belize, Faust et al. (2005), who mentions ranges 33-47µm medium in length (excluding apical horn and spinal) and 37-52 µm transverse diameter. Just as these authors have documented that the compression cell observed was lower compared with *P. bahamense* var. *compressum*, coincides with what is recorded by this study.

Conclusion: This study is the first reports the presence and abundance of *P. bahamense* var *bahamense*, for the Campeche Bay, thus completing the wide distribution in the southeastern Gulf of Mexico. This species recently reported as a producer of saxitoxin, representing a latent risk to public health.

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16:40-17:00 GROWTH AND TOXICITY OF *Pseudo-nitzschia australis* STRAINS ISOLATED FROM TODOS SANTOS BAY, BAJA CALIFORNIA, MÉXICO

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Background: Domoic acid (DA) is a potent marine biotoxin that causes the Amnesic Shellfish Poisoning. This toxin is naturally produced by several species of the genus *Pseudo-nitzschia*. As other types of toxic algae blooms, *Pseudo-nitzschia* outbreaks are becoming more frequent, posing an increasing threat to wildlife, human health and seafood safety. Environmental sampling has revealed the presence of DA in the northwestern Pacific coasts of México (Sierra-Beltrán *et. al.,*1997; Gárate-Lizárraga *et. al.,* 2007; García-Mendoza *et al.,* 2009) in which important aquaculture activities takes place.

Aim: To characterize temperature optimal growth conditions and production of domoic acid of *Pseudo-nitzschia australis* isolated from Todos Santos Bay, Mexico, for its grown and toxicity.

Materials and Methods: Two strains of *P. australis* (BTS1 and BTS2) were isolated by micropipetting single cells or chains of cells, from water samples collected from Todos Santos Bay, Baja California, México. The isolates were identified by scanning electron microscopy (SEM) based in ultrastructural description of Hasle and Fryxell (1970) and Hasle and Syvertsen (1996), and the species-especific LSU rRNA-targeted fluorescent probes based on the whole-cell hybridization protocol (Miller and Scholin, 1998). The isolates were cultured (triplicate) in L1 medium (Guillard y Hargraves, 1993) modified by silica enrichment to 250 μM and nitrate to 100 and 500 μM. The cultures were maintained in controlled environment chambers at 10, 12, 14, 15, 18 y 20 ºC with irradiance levels of 250 μmol quanta seg^{-1} m^{-2} provided in a 12:12 light:dark cycle. Domoic acid content in cells and in the medium, samples were in the stationary phase of growth of the cultures and processed according to the method of Quilliam (2003), using the fluorenylmethoxycarbonyl (FMOC) derivatization protocol and was quantified by HPLC with fluorescence detection.

Results and Discussion: *P. australis* presented optimum growth in a narrow interval of temperature (12-15 ºC) when cultivated in media with two silicate:nitrate ratios. *P. australis* presented low growth rate and biomass accumulation at 10ºC and null growth at temperature of 18 and 20ºC. The highest content of particulate domoic acid (pDA) was detected in cells cultivated at a 0.5 silicate to nitrate ratio. The lower concentrations were detected in cells cultivated at 10ºC and at temperatures of optimum growth for both strains (14ºC-BTS1, and 12ºC BTS2). Only for the strain BTS2, an increment in pDA production with temperature was observed. The dissolved domoic acid (dDA) showed

the highest difference between strains. In culture of the BTS1 strain dDA represented 24 to 68% of the total DA, this amount was detected in almost all culture conditions, whereas for the BTS2 strain was detected at 14 and 15ºC, dDA represented 0.6 to 14% of total DA in this strain.

Conclusions: *P. australis* has a narrow temperature range for optimal growth in culture conditions (12-14˚C). Differential responses were observed in the two strains evaluated; however, domoic acid content was related to growth characteristics of the cells. Higher cDA was detected in cells that presented lower growth rates. dDA could represent a high proportion of total DA produced by the cells.

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Valladolid

Tuesday June 29

Oral Phycotoxin Session 19: Shellfish Toxins and Diarrhetic Toxins (DSP).

08:30-09:00 DETECTION OF ASP AND PSP SHELLFISH TOXINS: COMPARISON OF PSP-ELISA VS MOUSE BIOASSAY AND ASP-ELISA VS HPLC IN DIFFERENT PRESENTATIONS OF SEAFOOD.

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Abstract

In this work we carried out a study to determine the viability of Enzyme-Linked Immunosorbent Assays (ELISAs) for the detection of Amnesic (ASP) and Paralytic (PSP) hydrophilic toxins in nine natural contaminated seafood species. We have compared these results with those obtained by HPLC and Mouse Bioassay (MBA). We have analyzed mussels, clams, barnacles, razor-shells, scallops, cockles and anchovies in fresh, frozen, boiled and canned presentations mainly from European origin.

PSP and ASP toxins were analyzed in one hundred and thirty-eight shellfish samples using competitive PSP-ELISA (Ridascreen) and ASP ELISA (Biosense) vs MBA and vs Liquid Chromatography with Ultraviolet Detection (LC-UV) respectively, both reference methods considered in the European Legislation.

Immunoassays were able to quantify toxins in all matrices and in all range of concentrations, showing excellent Pearson's correlations coefficients (r = 0.974 for PSP and r = 0.973 for ASP), and also to detect PSP and ASP with a lower limit of detection (LOD) than the MBA or the HPLC method.

The high correlation coefficient found between MBA and ELISA in all matrices evaluated suggests that the immunoassays could be helpful tools to determine whether a sample would be positive or negative in the MBA, thus reducing the number of animals employed in the analyses. In addition, ASP ELISA was able to detect DA in seven different species and in several presentations. The excellent correlation coefficients found for both toxins show that matrix effect does not affect the accuracy of the ELISA assays, therefore offering a good alternative as screening methods in routine monitoring programs for ASP and PSP toxins. Also, these results are very relevant if finally EFSA recommendations, concerning the legal limit reduction in marin biotoxins are legislated, since these methods could be a good alternative to detect these toxins, due to their limit of detection, if finally allowed limits are changed. Also, these results are very relevant if finally EFSA recommendations concerning the reduction of legal limits for marine biotoxins are legislated, since these methods, due to their low limit of detection, could be valuable alternatives to current official methods if finally allowed limits are changed.

09:00-09:30 HARMFUL ALGAE BLOOMS AND PARALYTIC SHELLFISH TOXINS IN BAHIA DE ACAPULCO, GUERRERO

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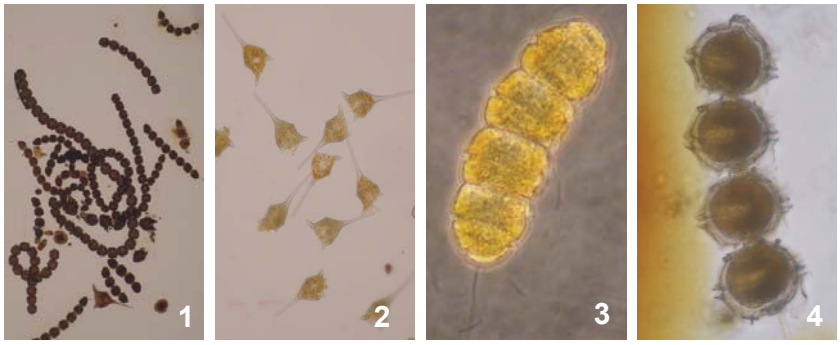
Harmful algae blooms (HAB) occur frequently in the Central Mexican Pacific coasts. During the 80´s and the 90´s decades, records of harmful algae blooms (HAB) in the Bahía de Acapulco were scarce. From 1991, staff o the State Laboratory of Public Health was trained in the identification of toxic microalgae and toxicity determination (mouse bioassay) by the FDA. Sixteen georeferenced samplings stations for monitoring harmful blooms have been established. As a result of the monitoring program, microalgae blooms were mainly caused by *Gymnodinium catenatum* (Fig. 1), *Ceratium balechii* (Fig. 2), *Cochlodinium polykrikoides* (Fig. 3), *Pyrodinium bahamense var. compressum* (Fig. 4) and *Akasiwo sanguinea*. Of these, two species produce paralytic toxins, *G. catenatum and P. bahamense* var. *compressum* (Mancilla-Cabrera *et al*., 2000; Orellana-Cepeda *et al*., 1997; Gárate-Lizárraga *et al*., 2008). According to Secretaria de Salud (Secretary of Health) about 138 human intoxication and six deaths occurred during 1995, which have impact the public health, economy and tourism in the state of Guerrero.



Figures 1-4. HAB forming microalgae in Bahía de Acapulco; 1) *Gymnodinium catenatum*, 2) *Ceratium balechii*, 3) *Cochlodinium polykrikoides*, (4) *Pyrodinium bahamense* var. *compressum*.

Objectives: Identification of harmful blooming species and the detection of paralytic shellfish toxins and their toxicity in bivalve mollusks.

Materials and methods: Seawater samples were monthly collected at the surface level. For cell counts, water samples were fixed using acid Lugol and analyzed with the standard technique of Sedgewick-Rafter chamber using a Phase contrast inverted

microscope. Shellfish toxicity was determined by Standard Mouse bioassay in: violet oyster (*Chama mexicana*), silvered oyster (*Crassostrea sp*.), queen clam (*Chione purpurissata*), Margarita scallop (*Spondylus princeps*), Lions-paw scallop (*Nodipecten subnodosus*), Chinese snail (*Muricanthus princeps*), and Mejillón tichinda (*Mytella arciformis*).

Results and discussion: Fourteen blooms of *G. catenatum* were observed from 1993 to 2006 and they reached densities of 2.5–3.3 x 10^6 cells L^{-1}, meanwhile less bloom events where recorded for *P. bahamense* var. *compressum.* Toxicity in the bivalve mollusks ranged from 20.65 and 7309 µgSTXeq 100 g^{-1}. The higher toxicity value was found in the violet oyster in November 2001 in Las Palmitas, Acapulco, during a *P. bahamense* var. *compressum* bloom. During 2005, 2006 and 2007, blooms of *G. catenatum* and *C. polykrikoides* were increasing inside the bay. The HAB caused by *G. catenatum* showed the highest abundances during December 2005 (1604 x 10^3 cells L^{-1}), January 2006 (10,000 x 10^3 cells L^{-1}) and December 2007 (1,942 x 10^3 cells L^{-1}). The high densities of *G. catenatum* results in toxicity values over 217 µgSTXeq 100 g^{-1} in December 2005, 112 µgSTXeq 100 g^{-1} in January 2006 and 1,152 µgSTXeq 100 g^{-1} in December 2007. A toxicity value of 1,163 µgSTXeq 100 g^{-1} was recorded in January 2008.

Conclusion: The increase of HAB in this bay has been noticeable during the last years and could be due to eutrophication processes caused by residual waters. The presence of paralytic toxins in bivalve mollusks was firstly linked to *P. bahamense* var. *compressum* and more recently to *G. catenatum*. The presence of *Cochlodinium polykrikoides* replaced *G*. *catenatum* during 2008.

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09:30-10:00 COMPARISON OF THREE METHODS OF ANALYSIS FOR THE DETERMINATION OF PARALYTIC SHELLFISH TOXINS IN LOBSTER (*Homarus americanus*) HEPATOPANCREAS.

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Background:

Historically, human intoxication with Paralytic Shellfish Toxins (PST) has occurred following consumption of bivalve molluscan shellfish. Due to this trend, bivalves have been the primary focus of PST method development and validation efforts. Currently, the AOAC mouse bioassay (MBA), Official Method 959.08, is considered to be the standard method for regulatory analysis of these toxins (AOAC 2009a) worldwide. Lawrence et al. (2004) developed an analytical method using pre-column oxidation prior to HPLC with fluorescence detection for the determination of PST in shellfish. This method was adopted for Official First Action in 2005 as Official Method 2005.06 (AOAC 2009b). The major limitation to widespread regulatory use of the Lawrence method is the time required to process samples containing significant amounts of the PST (Ben-Gigirey et al., 2007).

Rourke et al. (2008) published an adaptation of a method reported by Thomas et al. (2006) for analysis of PST using post-column oxidation (PCOX) The new PCOX method was found to be beneficial in a regulatory setting because of the higher throughput potential and faster turnaround times for positive samples. Results obtained using the PCOX method for bivalves were comparable with those obtained using the MBA and the Lawrence pre-column oxidation method. The PCOX method has been validated (van de Riet et al. 2009) and is currently the subject of a full AOAC collaborative study.

In the regulatory environment, routine analyses for PST are most often performed on bivalve molluscan shellfish and comparison studies have been carried out (Rourke et al 2008). To date, no comparisons of analytical results from PST analysis of lobster hepatopancreas (LHp) using any of the three methods have been published. The PCOX method has been modified (mPCOX) and validated using this matrix and in order to gain international acceptance of the mPCOX method, comparison to other more established methods is necessary.

Aim:

The objective of the study was to determine the comparability between the MBA, Lawrence and mPCOX for PST determination in LHp.

Materials and Methods:

The samples used in this study were collected as part of a larger survey to determine PST levels in LHp harvested from Eastern Canada between 2008 and 2009. Following initial analysis as part of the larger survey, samples were stored at -30˚C. Of the samples originally tested, 30 samples were selected for this comparison study to ensure that samples would provide a large range of PST concentrations and toxin profiles common in Eastern Canada. The samples were thawed, blended, and sub sampled into two aliquots of >5 g. One aliquot was shipped to Health Canada for analysis by the method developed by Lawrence et al. (2004). The other aliquot was retained in Dartmouth for analysis by mPCOX and MBA. Hydrochloric acid extraction as per van de Riet et al. (2009) was used for both the mPCOX and MBA. The MBA was performed according to the official method protocol with minor modifications to reduce the number of mice used. Due to matrix impacts, the PCOX method was successfully modified for PST measurement in LHp (mPCOX) by addition of SPE clean-up using Waters Oasis® HLB cartridges prior to analysis using HPLC with fluorescence detection.

Results and Discussion:

PST concentrations in LHp ranged from <20 µg/ 100 g tissue to >300 µg/ 100g tissue. In general, similar PST profiles were observed in LHp extracts regardless of whether the mPCOX or Lawrence methods were employed. Saxitoxin was the main contributor to total PST levels in LHp and most samples also contained Gonyautoxins 2 and 2 in most LHp samples, while neosaxitoxin was observed in a few of the 30 samples. LHp extracts were not as clean as observed for bivalves using both the mPCOX and Lawrence methods. Early eluting co-extractives were an issue in some chromatograms using the Lawrence method. This may be attributed to the fact that no additional clean up steps were added such as the SPE cleanup in the mPCOX method. This may explain the identification of gonyautoxin 1,4 (GTX 1,4) in some samples using the Lawrence method while similar results were not found using mPCOX. Recovery study data from the mPCOX method ranged from 92% to 129%. Recovery testing in LHp is ongoing for the Lawrence method.

Both analytical methods were found to have good correlations with the MBA results. (r^2>0.95) Correlation between the two HPLC based analytical methods was also found to be excellent (r^2 >0.96).

Conclusion:

PST in LHp can be determined using the MBA, mPCOX or the Lawrence method as all three methods report comparable results.

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10:00-10:30 EFFECT OF DIARRHETIC TOXINS (DSP) FROM *PROROCENTRUM LIMA* IN FISCHER 344 RATS: STUDY OF THE HEPATOCARCINOGENIC CAPABILITY OF DSP USING THE RESISTANT HEPATOCYTE MODEL

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Background

Harmful microalgae blooms are natural phenomena that can affect water bodies all around the world. In Mexico, one of the must important toxic dinoflagellate species is *Prorocentrum lima,* capable of produce diarrheic toxins (DSP). The ingestion of contaminated organisms with DSP toxins is considered the main vector for diarrheic syndrome development to the population, causing severe intoxications. DSP toxins, like the Okadaic Acid (OA) and the dinophysistoxins (DTX), have been widely studied because of their acute effects on environment and humans. On the other hand, they have been also related to the promotion of hepatic and gastrointestinal tumors under chronic exposures. It is important to use biological models just like the "resistant hepatocyte model" due to the liver versatility in procarcinogens substances activation. This characteristic is useful as an objective and valuable test for the carcinogenic capability of some chemicals (natural and artificial) in short time assays.

Objectives

1. Establish the hepatocarcinogenic capability of DSP toxins extract of Prorocentrum lima (PRL-1 strain) using the "resistant hepatocyte model" in Fischer 344 rats
2. Determine the toxin profile and quantify the toxicity of PRL-1 extracts throughout the mousse bioassay and HPLC/MS.
3. Describe and quantify the hepatic damages in the rats exposed to the DSP toxins extracts.

Materials and Methods

P. lima strain (PRL-1) was acquired from CODIMAR (CIBNOR). The cultures were kept under controlled conditions at CIBNOR. The extraction of DSP toxins from PRL-1 strain was carried out with methanol (MeOH) [100%] and dichloromethane [100%]. Extracts were dried and stored at 4°C until used. To determine the toxin profile from *P. lima* strain, the dry extract was reconstituted with MeOH [80%], filtered [0.45μm], injected in an HPLC/MS (ES+) system, and finally compared with a certified standard solution of OA (CRM-OA-c, NRC Canada). On the other hand, the mousse bioassay (MBA) was also used to determine/compare the extract toxicity. According to Solt and Farber (1976) modified model, two male rats batches (Fischer 344

strain) were used to analyze the liver tumor promotive capability of the DSP extract from PRL-1. In one batch, ten rats were exposed to the complete treatment (DEN as a primer and 2AAF as a promoter). The other batch contained 20 rats, which were treated with DEN as a primer and DSP toxins (11.42µg OA/day). The livers were stained with GGT+ and evaluated with the AnalySIS© software to quantify the affected areas. Both treatments were statistically analyzed using a t-*test* ($p<0.05$).

Results and Discussion

There were harvested up to 350mL of *P. lima* cultures, with an average cell density of $20x10^6$cell/mL in a 12mL of total DSP toxin extract. Three main toxins were detected using HPL/MS (ES+): OA (92.86 µg/mL; 2.041 pg/cell); DTX1 (60.41 µg/mL; 1.33 pg/cell), and DTX2 (40.00 µg/mL; 0.09 pg/cell). The toxicity of *P. lima* extract was determined with MBA, obtaining an average of 0.015 MU/mL (0.06 µg OA eq./mL; 2.94 pg OA/cell; 0.048 µg DTX1 eq./mL; 2.35 pg DTX1/cell). According to the toxicity values obtained in HPLC/MS compared with the MBA, it was evident the difference between these two methods. This corroborates that the MBA overestimates the results. In the "resistant hepatocyte model" the rats exposed to DSP extract showed severe damages, the most notorious were diarrhea, edema extending from the skull to the anterior extremities, nose and eye bleeding, dyspnea, lethargy, limited movement, lung congestion, otitis media and death. The individuals from the control group (DEN+2AAF) did not show any evidence of abnormalities or harms. The average number of tumor focus from the batch treated with DEN+2AAF was 14.25 focus/cm^2, and for the batch treated with DEN+DSP was 0.084 focus/cm^2. Comparing the percentage of GGT+ area and the average focus number there is a significant difference ($p<0.05$) between both batches (DEN+2AAF *vs*. DEN+DSP).

Conclusion

This work showed evidence of the low activity of the DPS extract from PRL-1 as a tumor promoter using the "resistant hepatocyte model" in rats (strain Fischer 344). These results were obtained in a bioassay carried out in 25 days with a toxin dose of 11.42µg OA/day. However, organisms showed negative effects when were exposed to DSP extract: diarrhea, dyspnea, lethargy, anterior extremities inflammation, bleeding and death. Other internal harms were recorded in the lungs, stomach, and intestines. The organisms from the control group did not show any of the symptoms described above.

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Oral Phycotoxin Session 20: Methods, ecology, exposure and new toxins.

10:30-11:00 COMPOUND DETECTION FROM STYPOPODIUM ZONALE BY MEANS OF A GEL CHROMATOGRAPHIC AUTOMATIC SYSTEM WITH A LASER POLARIMETRIC DETECTOR

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Background: The seaweeds are source of natural products, as secondary metabolites that are chemical defense or deterrents in front of the attack of other organism. Substances as steroids and carotenoids have been isolated from brown seaweed of *Dictyotaceae family*. *Stypopodium* genera that are widely distributed in tropical and subtropical seas and it has been characterized chemically for the diterpenes productions(1). This genus is highly represented in the Cuban seashores, and it has ecological interest because it contains ficotoxins to prevent the fish attacks and they are detected by “in vivo” assays.

Aim: To partially characterize the composition of the algae *Stypopodium zonale* using a gel chromatographic system with laser automatic polarimetric detector.

Materials and Methods:

Stypopodium zonale (Lamouroux) Paperfuss specie (Dictyotales, Ochrophyta) was collected at a depth of 1 to 6 meters at City of Havana seashore located at 23° 05’ 55’’ N and 82° 26’30’’ W, during March, 2008. Samples were kept at -20° C in the laboratory until the preparation of extracts.

Three thousand grams of wet alga, thoroughly washed and selected, were used to prepare two types of crude extracts, one of them in acetic acid (1 mol. L^{-1}), in relation 1:3 (W/V) and other in water: ethanol solution at 50%. Both extracts were homogenized with Ultraturrax T 25 at 5093.9 x g. Afterwards, the extracts were maintained at 10° C with vigorous shake at short intervals, it was centrifuged at 1292.7 x g and 24° C during 15 minutes. Pellets were discarded. Later on, supernatants were centrifuged at 3309.3 x g during 20 min. at 4° C. Finally, the extracts were concentrated using a vacuum rotatory evaporator and lyophilized at temperatures lower that - 50° C.

A new liquid chromatographic system with polarimetric detection and a computer program (2,3) allowing the output of chromatograms to a display on line and electronic data storing was employed. The chromatographic system includes the laser polarimetric detector, having a measuring interval of one second, the chromatographic columns, the continuous flux polarimeter tubes of 50 and 100 mm, the programs for data acquisition,

processing and storing, and the technical know-how for its most efficiently application. Thirty minutes is all the time needed to obtain a chromatogram by this method which is reasonably shorter than the time required for any other known comparable technique, and offering, besides, lower operation cost.

The combination of molecular exclusion liquid chromatography and laser polarimetric detection has turned into a carbohydrate separation and quantification system for the compounds of the referred algae.

Toxicological analyses: An alternative method with *Artemia saline* eggs counted and "*in vivo*" assay with intra-peritoneal way in mouse were employed to determiine the toxicity of the extracts.

Results and Discussion:

Low concentrations of high, medium and low molecular mass optical active compounds were detected in *Stypopodium zonale* which were present in the chromatograms of both acidic and alcoholic extracts.
The toxic compounds identified are shown in the following table:

Table 1: Toxicity detected in extracts according to both methods.

Extracts	**Acute Toxicity (ip) ($mg.kg^{-1}$)**	**Chronic Toxicity (ip, 30 days) ($mg.kg^{-1}$)**
Ethanol: water (IP)	500- 250 (Toxic)	600-150
Acidic extract	1000 – 500 (less toxic)	nd

A. salina nauplius (ED_{50} means)	295.49 $\mu g.ml^{-1}$ (mildly toxic)
Ethanol: water	319.97 (mildly toxic)
Acidic extract	27.36 (highly toxic)

Conclusion: It is possible to separate and detect fractions of high, medium and low molecular mass in the extracts of *Stypopodium zonale* with the liquid chromatographic gel system with polarimetric detection employed in the present paper.

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11:00-11:30 ANTIBACTERIAL AND ANTIFOULING ACTIVITY OF MARINE ALGAE IN THE GULF OF CALIFORNIA

Ruth Noemí Aguila-Ramírez[1*], Anabel Arenas-González[1], Bárbara González-Acosta[1], Claire Hellio[2], Claudia Judith Hernández- Guerrero[1*], Kim Mason[2]

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Background: Seaweeds are a promising source of bioactive compounds that can be used in the treatment of human diseases, cancer or control and prevent the colonization of fouling organisms. This study evaluated the antibacterial potential and antifouling ability of six species of seaweeds from the Gulf of California by testing organic extracts against strains of *Staphylococcus aureus* and *Escherichia coli* and against bacteria, fungi and microalgae. *Laurencia johnstonii, Ulva lactuca* and *Dictyota flabellata* showed a moderately active bacterial antibiosis, being the ethereal extracts more active against the bacterial strain *S. aureus.* The best antifouling results were obtained with *Ulva lactuca* and *Laurencia johnstonii*, which presented the greatest range of inhibition against all strains tested.

Aim: Identify promising species of particular interest to pharmaceutical and marine industries.

Material and Methods: *Dictyota flabellata, Padina concrescens*, *Laurencia johnstonii, Gymnogongrus mertinensis, Ulva lactuca and Codium fragile* were collected. The extraction was done of acetone/MeOH 1:1, obtaining a first crude extract. This extract was suspended in distilled water and repeatedly extracted with ethyl ether. The ether phase was dried over anhydrous sodium sulfate. The aqueous phase was extracted again with butanol. The antimicrobial activity tests against *Escherichia coli* and *Staphylococcus aureus* were done by agar diffusion technique. For antifouling bioassays, we tested 7 strains of marine and 4 terrestrial bacteria with the extracts at different concentrations determined by minimum inhibitory concentrations of broth dilution method in microtiter, the antimicroalgal bioassay were evaluated against different phases benthic marine microalgae, we compared the minimum inhibitory concentrations to control seawater by the method of Tsoukatou *et al.* (2002). For antifungal bioassay was tested in all extracts using the method described by Hellio *et al.* (2000), against five strains of marine fungi.

Results and discussion: Of the 6 species tested against bacteria pathogenic to humans, 50% had bacterial antibiosis moderately active, with one representative species from each group, the ether extracts being the most active against *S. aureus* strain*.* Butanol fraction *D. flabellata* was active against the same strain. Against *E. coli,* extracts showed no activity. The halo of positive control disc with commercial antibiotic presented a diameter of 20 mm, similar to that presented by the ether extract of *L. johnstonii.* In the antifouling activity *U. lactuca* and *L. johnstonii* showed the highest activity against all organisms tested. So against strains of marine bacteria inhibited the growth with MIC values of 0.1 to 1 μg ml^{-1}, while the rest of the species had low activity

with values greater than 50 μg ml^{-1}. Against terrestrial bacteria none of the extracts was active. In the tests against strains of microalgae, *U. lactuca* and *L. johnstonii* were moderately active against all strains. *C. fragile* and *P. concrescens* showed moderate activity against three strains of tropical microalgae. Against marine fungi, *U. lactuca* and *L. johnstonii* showed the highest activity and *P. concrescens* was moderately active against strains *Zalerion sp. and Monodictys pelagica*.

Conclusion: The results obtained here confirm that the seaweed *Laurencia johnstonii, Ulva lactuca and Dictyota flabellata* are rich in compounds with antibacterial or antifouling so will require studies to identify the compounds responsible for these activities.

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11:30-12:00 QUANTITATIVE EXPOSURE ASSESSMENT TO PHYCOTOXINS BY RECREATIVE HARVESTERS: FIRST RESULTS.

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Background: Phycotoxins are secondary metabolites produced by toxic phytoplankton. Filter-feeding them, bivalve shellfish accumulate phycotoxins until become unfit for human consumption. Phycotoxins expansion during the last decades leads to consider them as an important food safety issue. Data on phycotoxins concern mainly acute intoxications, but little information is available on chronic exposure. Thereby a consumer exposure assessment to phycotoxins “at normal levels” is necessary, taking firstly into account high exposed people i.e high shellfish consumers. Recreative shellfish harvesters are high shellfish consumers, but available data on their exposure to phycotoxins are lacking.

Aim: To develop a quantitative Monte Carlo exposure assessment model for phycotoxins in shellfish (Voes, 2001) and to assess the potential human exposure levels, specifically for recreative shellfish harvesters.

Materials and Methods: This assessment specifically focused on three phycotoxins of concern to humans: Okadaic acid (lipophilic toxin), Domoic acid (Amnesic Shellfish Toxin) and Saxitoxin (Paralytic Shellfish Toxin). Exposure assessment involved determining shellfish consumption by recreative harvesters and analyzing data on the occurrence of these phycotoxins in shellfish consumed. By combining the estimated individual phycotoxins concentrations in shellfish with available consumption data for the recreative harvesters, the daily intake of phycotoxins from shellfish by individuals was calculated. For calculations, data are incorporated in a probabilistic MCMC (Markov-Chain, Monte-Carlo) simulation method. This method repeatedly selects values randomly from contamination and consumption distributions to create exposure distributions. Consumption data was obtained by a previous study on shellfish harvesters’ consumption (Picot *et al.*, 2009) and contamination data were extracted from Ifremer database (Ifremer, 2008).

Results and Discussion: Contamination data are limited and irregular (analyses were made only when toxic phytoplankton crosses the threshold). Therefore two scenarii were envisaged: maximum of exposure (resulting of a simulation only with available contamination data i.e concentrations in the case of at risk situations) and minimum of exposure (available data were completed with “zero” when analyses were not performed). For the scenario “maximal of exposure”, using the simulation model, mean levels of exposure were estimated to: 0,032 µg Okadaic Acid equivalent.kg^{-1}.day^{-1}; 0,21 µg Domoic Acid equivalent.kg^{-1}.day^{-1}; 0,12 µg Saxitoxin equivalent.kg^{-1}.day^{-1}. For the scenario “minimum of exposure”, mean levels of exposure were estimated to: 0,012 µg

Okadaic Acid equivalent.kg^{-1}.day^{-1}; 0,066 µg Domoic Acid equivalent.kg^{-1}.day^{-1}; 0,042 µg Saxitoxin equivalent.kg^{-1}.day^{-1}.

Conclusion: Authorities had not been able to set Tolerable Daily Intakes of phycotoxins from shellfish because chronic toxicology data and consumption data were lacking. But results from the exposure assessment model suggested that the presence of phycotoxins in shellfish at normal contamination levels should be take into account and contamination data must be performed to improve simulation. This work is the first step on exposure assessment to phycotoxins by recreative harvesters.

References:

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12:00-13:00 EMERGING MARINE TOXINS: SPIROLIDES, GYMNODIMINES, PINNATOXINS, PTERIATOXINS, AND PROROCENTROLIDES

Michael A. Quilliam[1], Shawna L. MacKinnon[1], Patricia LeBlanc[1], William Hardstaff[1], Nancy Lewis[1] and Rex Munday[2]

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In 1991, routine monitoring of bivalve molluscs at aquaculture sites along the eastern shore of Nova Scotia revealed a novel and highly potent toxic response in mice following intra-peritoneal injections of lipophilic extracts. The mice exhibited rapid death (termed "fast-acting" toxicity) preceded by neurologic symptoms different from those of other known shellfish toxins. Extraction of shellfish digestive glands and bioassay-directed fractionation revealed several novel macrocycles, consisting of a spiro-linked tricyclic ether ring system and an unusual seven-membered spiro-linked cyclic imine moiety. The toxins were named spirolides. The cyclic imine feature has been identified as the active pharmacophore. This structural feature has since been found in other marine toxins such as gymnodimine, pinnatoxins, pteriatoxins and prorocentrolides. None of these toxins have been categorically linked to human intoxication, although there have been anecdotal consumer reports of vague symptoms (gastric distress, tachycardia) after consumption of spirolide contaminated shellfish. The biological origin of spirolides has been shown to be the marine dinoflagellate, *Alexandrium ostenfeldii*, and the toxins have now been detected in many countries. Many analogues and metabolites of the cyclic imine toxins have been detected in shellfish or dinoflagellates by LC-MS/MS, only a few of which have been isolated and structurally characterized. This presentation will review the discovery, structure determination, analysis, toxicity and pharmacology of spirolides and other cyclic imine toxins

Yucatan I

Tuesday June 29

Poster Session XI: Phycotoxins in the Atlantic area

P-111 KNOWLEDGE TRANSFER NETWORK FOR PREVENTION OF MENTAL DISEASES AND CANCER IN THE ATLANTIC AREA (PHARMATLANTIC)

María José Chapela, Luis M. Botana, Martiña Ferreira, Jorge Lago, Juan M. Vieites, **Ana G. Cabado*.**

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Climate change on the Atlantic Area is responsible for the settlement of favourable ecological conditions for marine compounds reproduction because marine organisms develop unique metabolic and physiological capabilities to be able to survive in such new habitats. These sometimes negative effects can be taken in a positive way because marine compounds can be studied by research centres of the Atlantic Area as new chemicals that should be used by enterprises to be benefited from such research in the future (chemistry, pharmacology, environmental, maritime, fishing, etc…).

PHARMATLANTIC is a 36 months EU project promoted by the Pharmacology Department of University of Santiago de Compostela (Veterinary Faculty). It was created on the necessity to provide involved industries situated in the Atlantic seaboard with research and innovation advances in prevention of mental diseases and cancer already developed by the most specialized centres in this field at EU level. A partnership of 10 EU institutions from Spain (University of Santiago de Compostela and ANFACO-CECOPESCA), Portugal (CIIMAR and University of Algarve), France (CNRS), United Kingdom (Queens's University Belfast and Agri-Food & Biosciences Institute) and Ireland (Cork Institute of Technology and Dublin City University) and the main research centres, universities and industries are represented in this project.

The main objective of PHARMATLANTIC is to build a stable network of knowledge transfer between researchers and industries by developing a joint method for the use and exploitation of marine resources through previous research in order to further transfer to the enterprise from involved sectors.

The specific objectives are:

- Data exchanging among research centres to create a joint valid methodology and industrial applications at transnational level.
- Establishment of a joint method aiming to transfer knowledge from research centres to companies.

- Exchanging and transfer of knowledge and innovation addressed to the industries of the sectors, particularly the pharmaceutical industry; raising awareness on the importance of innovation to companies of involved sectors.

Short term outputs:
- Establishment of a microalgae collection.
- Scaled specifications for microalgal culture in bioreactors.
- Comparative analysis of developed methods with current state of art.
- Development and introduction of new efficient (simplified, inexpensive, reliable) alternative biotoxin detection, refinement and control methods.
- Creation of an Internet Accessible Database.

Expected mid and long term outputs:
- Constitution of at least one transfer network in each one of involved Regions of participant partners.
- Study about state of knowledge transfer in the involved territories and at EU level.
- Achievement of a valid methodology of knowledge transfer from research centres to enterprises at EU level.
- Transnational Working Group of Knowledge Transfer and Valorisation.
- Creation of Innovation and Knowledge Transfer Network PHARMATLANTIC.

PHARMATLANTIC has an innovative character highlighted under all its aspects: thematic, implementation and results expected. Even its background is based on the innovative idea of using marine harmful organisms, which have resulted as a consequence of climate change and global temperature rising, in order to be used as a new source of chemicals for research in the fields of pharmacology.

The project theme is also based on a new and innovative approach which consists in establishing a reliable protocol in order to obtain bioactive compounds from marine harmful organisms with effect on neurodegenerative diseases and cancer.

P-112 ADVANCED TESTS ABOUT NEW TOXINS APPEARED IN THE EUROPEAN ATLANTIC AREA (ATLANTOX)

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Global warming is causing negative impacts in marine ecosystems all over the world. One of the effects derived from the rise of ocean temperature is the increasing frequency of harmful algal blooms, and the subsequent risks for seafood consumers and economical losses for coastal communities that depend on the exploitation of marine fish and shellfish resources and on tourism. This is an issue of transnational importance that requires joint actions in order to find adequate solutions. Besides the increasing number of toxic episodes, global ocean warming causes changes in the distribution of phytoplanktonic communities; thus, toxic microalgal species typical from warm or temperate waters may appear in higher latitudes or new locations. Toxic episodes referring to new toxins appeared for first time in the Gulf of Mexico, Gulf of California and Indo-Pacific ocean regions, but intoxications due to toxins so far unknown in European waters have been recently reported.

EU authorities consider therefore a priority the development of quick and reliable methods to detect new toxins, as well as the enhancement of knowledge about their effects on human health, since EU currently lacks legislation regulating maximum permitted limits for these compounds. The ATLANTOX project, launched in April 2009, is fully coincident with this aim, establishing tools and methods for the detection of newly appeared marine biotoxins which will contribute to the safe and sustainable management of coastal environments in the EU Atlantic Area.

ATLANTOX follows the priorities and objectives described in the EU Atlantic Area Operational Programme: protect, secure and enhance the marine and coastal environmental sustainability. The general objective of this project is to support and accelerate the development and introduction of a proper and efficient method of fast alternative controlling, based on antibodies and functional assays for biotoxins that causes problems of food safety with touristic and economic consequences.

Specific objectives are:

- Developing a reliable method of detection and control of marine toxins, which applies to the whole maritime zone of Atlantic Area, specially tetrodotoxin, palytoxin and cyclic imines such as spirolids.
- Establishment of a collaborative network for the collection and analysis of samples of marine toxins in the Atlantic Area.
- Promoting the exchange of knowledge on the topic addressed by promoting, among others, the mobility of researchers in the Atlantic Area.

- Enhancing the sustainable management of marine resources in the Atlantic coastal area.
- Encouraging the adoption of transnational tools to protect marine ecosystems.
- Avoiding negative impacts that the emergence of marine toxins exerts on the economies of Atlantic coastal areas linked to concepts of food safety and tourism promotion.

Expected short term outputs:
- Biotoxin and reference material production.
- Toxicological studies about new toxins appeared in the Atlantic Area coastline and identification of risks for humans.
- New and reliable detection methods.
- ITC outputs: webpage, Internet accessible database about toxins.
- Technical outputs: evaluation plan, evaluation report and communication and dissemination plan.

Expected long term outputs:
- Enhancing cooperation through stable research networks
- Contributing to the objectives of the EU research policy
- Replacement and/or reduction of in vivo toxin detection

ATLANTOX project is leaded by University of Santiago de Compostela (Spain). Other members of the partnership are: Institute of Agri-Food and Land Use of Queen's University Belfast (United Kingdom), Agri-Food & Biosciences Institute (United Kingdom), Cork Institute of Technology (Ireland), CIIMAR (Portugal), CNRS (France) and ANFACO-CECOPESCA (Spain). IFRÉMER (France) and IPIMAR (Portugal) participate as supporting partners.

More information: www.atlantox.com.

MYCOTOXIN SECTION
YUCATAN I
Poster Presentations
(Afternoons 13:30-17:00)

Poster Section I: Mycotoxins in field crops. Groups 1, 2 and 3.
Monday June 28
Group 1 (P-1 to P-6): Each presentation in 10 min.

P-1 AFLATOXIN PRODUCTION FOR MEXICAN STRAINS OF *Aspergillus flavus* IN IMMATURE CORN GRAINS.

Valdivia Flores, Arturo Gerardo*, María Carolina de Luna López, Raúl Ortiz Martínez, Teódulo Quezada Tristán, Armando Martínez de Anda, Ernesto Moreno Martínez.

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Background: Aflatoxins (AF) are a group of secondary metabolites produced when *Aspergillus spp.* fungi growth in stored seeds or plants in development (Diener and Davis, 1987). AF have a high impact in both human and animal health, causing significant economic losses in agriculture, animal industry, and food quality. The AF B_1, B_2, G_1, and G_2 (AFB_1, AFB_2, AFG_1, AFG_2) are found in Mexico as natural contaminants in foods or feeds (Flores *et al.,* 2006). AF metabolites could be founded in blood, urine, feces, muscles, eggs, also.

Aim: to evaluate the AF production of Mexican strains of *A. flavus* in immature kernels of endemic maize varieties in Aguascalientes, Mexico.

Materials and Methods: The fungus culture (Strains Cuautitlán and Tamaulipas; Unidad de Investigación en Granos y Semillas, FES- Cuautitlán, UNAM) was fulfilled in potato-dextrose agar medium along 18 days. The inoculum was prepared (1 X 10^7 spores / mL) in 5.0 mL of mineral oil solution (1.0 %), and added to 100 g of kernels. We used immature corn grain (3/4 of milk line) of eleven common varieties in the Aguascalientes state, Mexico: five were commercial hybrids (Cal-Oro, 3002, Gartz 8366, As910, C-526), three resistant to plant pathogens (30R39, 3028W, 30G40), a tolerant (C-922) and two without breeding (Criollo and Palomero); The grain moisture was adjusted to 15.0%, and placed in a loosely closed jar. The jar was kept in an incubation oven (29º C) during 14 days, in sterile conditions. The extracted solid phase eluate was derivatizated and the AFB_1, AFB_2, AFG_1, and AFG_2 concentrations were measured by high-performance liquid chromatography (HPLC) with fluorescence detection (Scott, 1995).

Results and Discussion: Cuautitlán and Tamaulipas strains of *Aspergillus flavus* were grown properly in immature corn grains and the AF occurred in a sufficient concentration to be measured. The means of AFB_1, AFB_2, AFG_1, and AFG_2 production (mg/kg) were: 88.1, 9.9, 1.5, and 0.5, respectively. Four maize varieties (Criollo, Cal-Oro, 3028W and 30R39) were more sensitive to growth of both fungus strains; moreover, its AF production (AFB_1 + AFB_2) was significantly higher than others maize varieties ($P < 0.01$; 13.4 *vs*. 40.0 mg/kg).

Conclusion: These results suggest that AF production is affected by genetic characteristics of fungus and corn and environmental conditions. This fact means that the AF food and feed contamination should be considered a multifactorial event.

References:

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P-2 DETECTION OF *FUSARIUM* SPP, DEOXYNIVALENOL AND ZEARALENONE CONTAMINATION IN WHEAT FROM MEXICO CITY

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Background: The filamentous fungus *Fusarium graminearum* is one of the most important pathogens of small grain cereals. It is best known as the pathogen responsible for *Fusarium* head blight (FHB) disease of wheat, also referred to as head scab. The economic damage caused by FHB includes reduced yields, discolored, shriveled kernels, mycotoxin contamination, and reduced seed quality. *Fusarium*-infected grains are often contaminated with mycotoxins, such as trichothecenes, fumonisins and zearalenone, which make them unsuitable for use as food and feed. The occurrence of mycotoxins in agricultural commodities has long been recognized as a potential hazard for human and animal health.

Aim: the aims of this work were to apply PCR analysis for the detection of *Fusarium* in wheat commercialized in Mexico City, to test for the presence of the tri5 and PKS4 genes in these isolates and to determine the incidence of DON and ZEA in wheat for human consumption in this city.

Materials and methods: A PCR method involving genes of the trichothecene (TRI5) and zearalenone (PKS4) biosynthesis pathways was utilized. A liquid extraction and clean-up procedure was utilized for the analysis of deoxynivalenol (DON) and zearalenone (ZEA) in food samples; for trace analysis, liquid chromatography coupled to UV detection was employed.

Results and discussion: The mean recovery of DON and ZEA in spiked samples ranged from 83.7-90.1 %. Linearity, limit of detection and quantification were assessed for the analytical performance of the methodology. A total of 35 wheat samples were subjected to PCR for the detection of toxigenic *Fusarium* spp. PCR indicated the presence of the tri5 and PKS4 genes in five and seven samples, respectively. DON was detected in 68.6 % of the samples, none of the samples exceeded the maximum permitted level of DON established by the European Union for these cereal; on the other hand, ZEA was detected in 45.7 % of wheat, 12.5 % of the positive samples surpassed the maximum permitted level. DON and ZEA contamination was found in 51.2 and 71.4 % of the samples where a positive amplification of the tri5 and PKS4 gene was obtained; this reinforces the idea that even though the mycotoxigenic fungi are present, it is not always an indicator of mycotoxin contamination.

Conclusions: There is a high incidence of deoxynivalenol and zearalenone on grain with quality suitable for milling, baking and pasta making in this City. These results back up the necessity to take a vigilant attitude in order to prevent human intake of mycotoxins; this information is necessary and of high priority in order to protect consumer health from the risk of exposure to these toxins.

References

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P-3 *FUSARIUM* MYCOTOXIN CONCENTRATIONS IN STRAW, CHAFF, AND GRAIN OF SOFT RED WINTER WHEAT EXPRESSING A RANGE OF RESISTANCE TO *FUSARIUM* HEAD BLIGHT

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Background: Fusarium head blight (FHB) or scab, which is caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.), is an increasingly important problem in the north-central region of the United States. During years of heavy FHB infection, veterinary diagnostic laboratories have, on occasion, unexpectedly found unusually high concentrations of both deoxynivalenol (DON) and zearalenone (ZEA) in wheat straw, as well as FHB-infected grain. Because swine are sensitive to concentrations of DON and ZEA as low as 1 ppm, these mycotoxins are particularly problematic when wheat straw is used for bedding in less than optimal production settings. Similarly, where straw is used as a source of roughage for cattle in total mixed rations (TMRs), concentrations of mycotoxins, to which ruminants are fairly resistant, might be found at clinically relevant, high concentrations in straw. Although there is a large body of literature on mycotoxin content in FHB infected grain, little is known of both the range and concentrations of mycotoxins in wheat straw[1-4].

Aim: To investigate mycotoxin concentrations in the straw, chaff, and grain of 60 soft red winter genotypes from the 2008 Northern Uniform Winter Wheat Scab Nursery.

Materials and Methods: The 2008 Northern Winter Wheat Scab Nursery (Fig. 1) was grown at the Bradford Research and Extension Center near Columbia, MO and spray-inoculated at 75% heading with a macroconidial suspension of *F. graminearum* concentrated to 50,000 macroconidia/mL. The nursery was maintained under overhead mist irrigation through heading and evaluated for incidence and severity 18 - 21 d after inoculation. At harvest, a 3-meter long sample of each genotype was cut at ground level, dried, and separated into the spike and straw. Straw samples were ground and the spikes were threshed using a wheat head thresher to separate wheat grain from the chaff. Grain and chaff were collected and ground. Ground samples were extracted with acetonitrile/water (86/14), passed over SPE clean-up columns, and the eluants taken to dryness. Samples were derivatized with TMSI/BSTFA/Pyridine/TMCS 1/1/1/1 and analyzed by GC/MMS in the SIM mode with an Agilent MSD 5975B MSD for 17 *Fusarium* mycotoxins.

Results and Discussion: The FHBI for the 60 genotypes evaluated ranged from a low of 9.9% to a high of 61.9% and averaged 35.7%. Significant concentrations of DON and ZEA were detected in the grain, chaff, and straw samples, while 15-ADON and zearalenol concentrations were negligible (<0.5 ppm) in the grain but measureable in the chaff and straw. 3-ADON and nivalenol were not detected in any of the samples. The DON and ZEA concentrations in the grain samples averaged 4.7 and 4.4 ppm,

respectively, across the 60 genotypes, and were significantly correlated with resistance level (r=0.56 and r=0.51 for DON and ZEA, respectively). In chaff samples, both mycotoxins were present at higher concentrations than in the grain, averaging 16.9 ppm (DON) and 42.9 ppm (ZEA), but the concentrations of these mycotoxins in chaff were poorly correlated with resistance (r=0.32 and r=0.37; DON and ZEA, respectively). In the straw samples, DON concentrations averaged 3.5 ppm, but, surprisingly, the ZEA concentrations were very high, averaging 55.5 ppm across all 60 entries. The correlation with resistance was much lower (r=0.21), for concentrations of these mycotoxins in the straw than in the grain, indicating that *Fusarium* mycotoxin concentrations in the chaff and straw cannot be reliably predicted by the scab resistance level of the cultivar.
Conclusions: The finding of significant levels of vomitoxin and zearalenone in the wheat straw is potentially clinically relevant to livestock producers.

References:

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P-4 INFLUENCE OF ENVIRONMENTAL FACTORS ON GROWTH AND MYCOTOXIN BIOSYNTHESIS OF FUSARIUM. PERSPECTIVES ON FUTURE CLIMATIC CONDITIONS IN SPAIN

Patricia Marín[1], Alejandra Cruz[1], Naresh Magan[2], Miguel Jurado[1], **Covadonga Vázquez[3],** María Teresa González-Jaén[1*]

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Background: *Fusarium verticillioides, F. proliferatum* and *F. graminearum* are the most important maize pathogens. They produce a number of mycotoxins, among which fumonisins and trichothecenes are the most relevant. *Fusarium* growth and mycotoxinn production result from the complex interaction of several factors (biotic and/or abiotic). Environmental factors, especially water stress and temperature, significantly influence fungal growth and toxin production. Although stress conditions may be only transient, in a situation of changing climatic conditions, they may become more permanent in certain regions and, therefore, information about the behavior of the different fungal species regarding growth patterns and toxin production in environmental stress conditions might become critical to improve prediction and control of mycotoxin risk.

Aims: The study aimed to know the effects of interacting conditions of temperature and water potential on growth rate of Spanish field isolates of the fumonisin-producing *Fusarium verticillioides, F. proliferatum* and the trichothecene-producing *F. graminearum:* (i) To compare the effects of temperature and water potential on growth and expression of *FUM1* and *TRI5* genes (key genes of the biosynthesis of fumonisins and trichothecenes, respectively) (ii) examine the temporal kinetics of the effect of ionic solute potential on growth and *FUM1* and *TRI5* gene expression in these three species.

Material and Methods: The strains analyzed were isolated from cereals from Spain and their ability to produce fumonisins or trichothenes had been previously determined. The experiments were carried out in and standardized *in vitro* systems described elsewhere (Jurado et al., 2008). The solute potentials used were: -0.7, -2.8, -7.0 and -9.8 MPa (a_w: 0.995, 0.982, 0.955 and 0.937) and the temperatures, 15, 20, 25, 30 and 35ºC. Plates were incubated for 10 or 12 days. Quantification of the expression of toxin biosynthetic genes was performed by real time RT-PCR using species specific assays previously described (López-Errasquín et al., 2007; Jurado et al., 2010 and Marín et al., submitted).

Results and Discussion: Detailed two dimensional growth maps were obtained for the three species which showed differences in optimal, permissive and marginal conditions in relation with temperature and water potential. *F. verticillioides* and *F. proliferatum* tolerated higher temperatures and water stress than *F. graminearum.* The three species showed marked differences in the regulation of toxin biosynthetic gene expression. In particular, *F. verticillioides FUM1* gene showed an increasing transcription induction at high water stress values. These results are in agreement with the occurrence of these species in surveys carried out in different agroclimatic regions in Spain.

Conclusions: Increasing temperatures and drought are conditions expected according the new scenarios caused by climatic change predicted for Spain. In this scenario, fumonisin risk might increase, especially due to *F. verticillioides.* The role of mycotoxins is also discussed in relation with host range and environmental conditions.

References:

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This work was supported by the Spanish Ministry of Science and Innovation (AGL 2007-66416-C05-02/ALI) and by the UCM-BSCH (GR58/08).

P-5 EVALUATION OF CORN HYBRIDS YELLOW TO *FUSARIUM VERTICILLIOIDES* IN EXPERIMENTAL FIELDS OF THREE LOCATIES IN THE PORTUGUESA STATES, VENEZUELA.

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Background

Are large species of mold that can colonize maize, but Fusarium verticillioides (F. moniliforme Syn) is a pathogenic species most commonly found in this crop, due to prevailing in the tropics and subtropics and are common inhabitants of soil (Bean and Echandi, 1989).

Objectives

To assess the incidence of Fusarium verticillioides field (FV) and total molds in corn produced under environmental conditions and different agronomic managements in the rain cycle of 2009.

Materials and methods

Evaluate eleven hybrids were yellow in the towns of Santa Cruz, El Playón and Acarigua in Portuguesa state. For the analysis of incidence of mold samples were collected from 2-3 kg of wet grains without mechanical or insect damage. The incidence of FV, including mold, was determined by direct seeding of hundred grains of each hybrid disinfected with sodium hypochlorite (3.27% NaClO) for ½ minute, washed 5 times with sterile distilled water, dried in petri dishes with sterile filter paper and planted in the media surface-malt-salt agar with pH 5.8 at a rate of 10-12 seeds / plate. After 7 days of incubation at 23 $\pm$ 2 ° C, grains were examined under stereoscopic microscope (Mazzani et al., 1999). The results were expressed as% of grains colonized by FV and total molds. For fungal incidence rate was used the scale proposed by Mazzani et al. (1999) that the incidence is rated as low (0-15%), intermediate (16-30%) and high ($\geq$ 30%). Confirmation of the identity of FV isolates was performed by the traditional method.

Results and discussion

FV was identified in all samples tested in the three localities. The hybrids showed incidences from 14% in D2A-713 (low) to 34% in DK-7088 in El Playón, ranking of medium to high for all except for D2A-713, Santa Cruz, the incidence ranged from 7% D2A - 694 to 32% in D2A-31 ranking of intermediate to high for all except for D2A-694 and Acarigua the incidence ranged from intermediate to high for all hybrids, ranging from 18% in D2A-720 up to 55% in DOW - 2B688, resulting in the latter town the largest presence of mold.

Conclusions.
The town of Acarigua FV was confirmed that one of the potentially toxigenic mold species important in commercial corn fields. Routine evaluation of the incidence of FV allows tracking of the new hybrids on the market.

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P-6 *FUSARIUM* SPECIES ISOLATED FROM CORN KERNELS

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Background: Some of the ear rots induced by *Fusarium* species are also important due to their relationship to human and animal diseases feed contaminated grains. *F. verticillioides* (Sacc.) Nirenberg (G) used to be known as *F*. *moniliforme* Sheldon, and *F*. *graminearum* Schwabe are species that, besides inducing corn ear rots are also able of producing mycotoxins which means sanitary risks Desjardins *et al.* (1994).

Aims: To know the *Fusarium* species profiles in corn grains that could come from or could induce corn ear rots.

Materials and methods: Sixteen white corn samples from the state of Puebla were analyzed to know the Fusaria present. The mold presence was determined plating 400 corn grains; previously surface disinfected with NaOCl 1%, in PDA plates, incubated at± 25°C for 7 days. The *Fusarium* isolates were purified and determined to the species level Nelson *et al*. (1983), Booth (1971).

Results and Discussion: The *Fusarium* isolates were 4753 which belonged to 9 species: *F*. *oxysporum* Schlecht (2434), *F. subglutinans* (Wollenw. & Reinking) used to be know as *F*. *moniliforme var subglutinans* Wollenw y Reink (966), *F. verticillioides* (Sacc.) Nirenberg (G) (586), *F*. *graminearum* Schwabe (549), *F. anthophilum* (A. Braun) Wollenw., used to be know as *F*. *moniliforme* var *anthophilum* (A. Braum) Wollenw (123), *F*. *poae* (Peck) Wollenw (44), *F*. *tricinctum* (Corda) Sacc. (38), *F*. *sporotrichioides* Sherb. (8) and, *F. proliferatum* (Matsushima) Nirenberg used to be know as *F*. *moniliforme* var *proliferatum* (Matsushima) Nirenberg (5).
The most abundant of the isolated species was *Fusarium oxysporum* in spite that there are no reports of it as a ear rot inducer, it was considered important because it is able to produce a great number of different important mycotoxins Savard & Blackwell (1994). *Fusarium verticillioides* and *F. graminearum*, besides inducing pink ear rot can also produce mycotoxins Desjardins *et al.* (1994).
Other of the isolated species that also induce corn ear rots but can or can not produce mycotoxins were *Fusarium subglutinans*, *F. anthophilum*. Some other of the isolated species in lower amounts were *F. poae*, *F*. *tricinctum*, *F*. *sporotrichioides*, and *F*. *proliferatum* species which match those cited by Savard & Blackwell (1994)

Conclusions: It is important to continue to do this kind of inspections in order to knowing the field problems as well as to guarantee the grain quality.

Poster Section I: Mycotoxins in field crops.

Monday June 28

Group 2 (P-7 to P-12): Each presentation in 10 min.

P-7 *FUSARIUM* HEAD BLIGHT AND DEOXYNIVALENOL ACCUMULATION IN WHEAT GERMPLASMS.

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Background: Fusarium head blight (FHB) is one the main diseases of wheat caused mainly by *Fusarium graminearum* Schwabe [telomorph *Gibberella zeae* (Schwein.) Petch]). This species produces trichothecenes type B, such us deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol and zearalenone.
The disease causes significant yield losses and reduced grain quality due to mycotoxins occurrence, mainly deoxynivalenol (DON). This toxin affects both human and animal health. Previous studies indicate that trichothecenes, particularly deoxynivalenol, are the most frequently identified mycotoxin in samples of *Fusarium*-contaminated grains worldwide and these grains may not be suitable for human or animal consumption.

Aim: This study was done to evaluate the behaviour of wheat germplasms in relation to FHB disease severity and DON accumulation.

Materials and Methods: The germplasms were planted at the experimental Station EEA INTA Pergamino including 15 lines (cross between MAYOOR//TKSN1081/ AE.SQ222) and 10 commercial varieties: Buck Halcón, ProINTA Granar, ACA 303, Klein Sagitario, Klein Escorpión, INIA Tijetera, Klein Chajá, BioINTA 1001, Klein Jabalí y Buck 75 Aniversario.The germplasms were planted under field conditions, during 2006/07 harvest seasons. The inoculation was done for aspersion, during the anthesis period with the selected *F. graminearum* (KBC7) strain. The inoculum concentration (spores) was of 2.5 x 10^5 spores ml^{-1}. The germplasms were evaluated at the maturity stage based on a scale of double digit for incidence/severity evaluation. The scale values ranged from 0 to 10. Those entrances that presented symptoms were also analyzed to determine the DON content. For DON detection and quantitation HPLC was used according to AOAC (1995).

Results and Discussion: In both harvest seasons (2006-2007) the behaviour of the different cultivars was variable in relation to the symptom levels and DON accumulation. The cultivars Klein Sagitario and Klein Escorpión showed the lowest incidence and DON content, while Buck Halcón cultivar showed the highest incidence. Significant correlation (Pearson) was observed between disease severity rate and DON accumulation during

2006 and 1007 harvest seasons, (r=0,43143; P<0,0004 and r=0,55604; P<0,0002 respectively).

Conclusion: These results suggest that the selection of germplasm with low visual FHB symptoms could be indirectly associated with low DON accumulation.

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P-8 ENDOPHITIC INFECTION BY TOXIGENIC STRAIN OF *FUSARIUM VERTICILLIOIDES* ON PLANT DEVELOPMENT: A COMPARATIVE STUDY BETWEEN RESISTANT AND SUSCEPTIBLE MAIZE HYBRIDS

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Background: In Córdoba, Argentina, corn is one of the most important cereal grains produced, being a significant income for regional economy. The fungus *Fusarium verticillioides* is a pathogen that is associated with disease at all stage of maize (*Zea mays L*) plant development, including seed rot, damping- off, roots rot, stalk rot and kernel or ear rot and produces fumonisins, food-borne carcinogenic mycotoxin. Consumption maize contaminated with fumonisins has been implicated in a number of animal and human illnesses, including esophageal cancer and neural tube defects. The substantial efforts consist of understand the toxicology of fumonisins as well as the maize-*Fusarium* pathosistem.

Aim: To investigate the fungal distribution and the time course for expression of disease symptoms in maize seedling infected by fumonisin-producing strain of *F. verticillioides*.

Materials and Methods: Two maize hybrids that had exhibited resistance (LT 622 MG) and susceptibility (HX 31P77) to *Fusarium* ear rot in previous experiments (Presello et al., 2009) were evaluated after seed infection with an inoculum of 10^{12} CFU/ml of *F. verticillioides* (2024 strain, UNRC). Three replicates of 10 seed each were planted in 24-cm diameter pots containing autoclaved sand under greenhouse conditions. The seedlings were harvested on days 7, 14 and 21. After harvest, the plants were washed and the roots were separated from the aerial parts and the seeds were removed. Plant height and principal root length were measured on fresh material. Then, the different plant parts were dried in an oven at 60 °C until constant weight for later determination of total biomass, aerial parts and root dry weigh. Data of growth of seedling were compared for significant difference, based on t test, between inoculated and non- inoculated kernel at each sampling date and treatment, as well as for eventual differences between hybrids. The fungal distribution was determined placing surface-disinfected segments from the root, mesocotyl, node, stem and oldest leaf on PDA. A total of 10 segments per plant from each 24 plants were analyzed. Segments from which the fungal grew on PDA were scored as positive and the percentage of positive responses was calculated for each part plant. In order to evaluate the ability to produce fumonisins, a conidial suspension of *F.* verticillioides 2024 strain was directly inoculated onto soaked, autoclaved, whole maize kernels. The samples were incubated at 25 °C in the dark for 4 weeks. Separation and purification of the toxin were performed in the fermented maize following the methodology of Voss et al. (1996). Samples (100 µl) from the watered extracts were diluted with acetonitrile (100 µl) and they were analyzed by HPLC as described Shepard et al. (1990).

Results: The strain of *F. verticillioides* showed to be a highly fumonisin producer on maize kernels. Evidence of seedling disease depending on the hybrid was observed. For HX 31P77 hybrid, when considering total biomass, *F. verticillioides* negatively affected *Zea mays L.* growth at 14 days and persisted for at least 21 days after planting of inoculated seeds. The reductions in both dry mass of root and arterial parts plant (leaves and stems) of infected seedling were significantly greater than noninfected seedling at 14 (P < 0, 04) and 21 (P < 0,001) days. At 21 days the mean height and principal root length of the seedlings were decreased (P < 0, 05). In contrast, the most resistant hybrid in the 21 day assay there was no significant difference on total biomass, dry mass of root and arterial parts plant between the inoculated and non inoculated treatments. However, at day 14, total biomass and dry weights of aerial parts of infected seedling exceeded that of non infected seedling (P < 0, 001). Height and principal root length by days 14 and 21 were significantly higher for infected than for noninfected kernels (P < 0,001 y P < 0, 04, respectively). *F. verticillioides* was isolated from both hybrids inoculated at each sampling date, but the highest mean infection was found in susceptible than a resistant hybrid. Mesocotyls was the specific plant parts most affected for the infection in all samples. For the rest of the segments, the percent of infection was similar in the resistant plant. Henceforth, the highest infection was evident in root, mesocotys and nodes and the lower infection was found in leaves and stems, in susceptible hybrids.

Discussion and Conclusion: We found that resistant and susceptible hybrids differed in terms of growth parameters. In fast-growing species as maize, in general, are expected that plants maximize resource acquisition. These species are characterized by a set of leaf traits enabling high carbon acquisition and roots by high specific root length, which is usually, associated with rapid rates of root elongation, high relative grown rate, high nutrient and water uptake capacities and high metabolic activities. Our result showed that the root systems of susceptible hybrid presented reduced development; therefore it is less efficient in terms of the acquisition of mobile resources than the controls and resistant hybrids. For the other hand, morphologic characteristic of infected resistant plant seems beneficial for the host, where the fungus could stimulate growth and development of maize plant, possibly due to the production of plant-growth promoting hormones.

One possible explanation for the symptom/symtomless association with fumonisin-producing strains of *F. verticillioides* could be the induction of potentials mechanism of action triggered by fumonisins in maize, in this case, the host could be suppress or no fungal pathogen. These issues deserve further study.

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P-9 *ASPERGILLUS FLAVUS Y* AFLATOXINAS EN GRANOS DE HÍBRIDOS DE MAÍZ BLANCO EN SIEMBRAS EXPERIMENTALES DE DOS LOCALIDADES DEL ESTADO YARACUY, VENEZUELA.

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Antecedentes. El maíz (*Zea mays* L.) constituye el alimento de mayor importancia en muchos países de América, es el cultivo anual mas valioso de EE.UU. y es el único alimento para muchos pueblos del mundo. En Venezuela se siembran alrededor de 600 mil ha casi exclusivamente con maíz blanco para consumo humano siendo el estado Yaracuy uno de los principales estados productores. En Venezuela se ha comprobado consistentemente elevada incidencia de *Aspergillus flavus* Link ex Fries y considerables niveles de aflatoxinas en granos maduros de maíz recolectados en el campo, con el consecuente riesgo que su presencia implica a la salud de humanos y animales. La resistencia genética es la alternativa más lógica y económica para prevenir este problema (Brown *el al.*, 2001). Anualmente son liberados por diversas casas productoras y distribuidoras de semillas, híbridos y otros cultivares los cuales han sido evaluados por características agronómicas y calidad de grano pero no por susceptibilidad a *A. flavus* y a las aflatoxinas. A la fecha, es la Fundación Danac el único ente cuyo programa de mejoramiento del maíz incluye dicha evaluación.

Objetivo. Evaluar la incidencia de campo de *A. flavus* (AF) y el contenido de aflatoxinas (AFLA) en granos de maíz blanco producidos bajo condiciones ambientales y manejos agronómicos diferentes en el ciclo de lluvias del año 2009.

Materiales y Métodos. Se seleccionaron dos localidades, San Javier y Sabana de Parra, estado Yaracuy y se evaluaron los mismos doce híbridos en cada localidad. Durante la cosecha se tomaron muestras de 2-3 Kg de granos húmedos y se secaron hasta alcanzar 12% de humedad. La incidencia de AF fue determinada por siembra directa de cien granos sin daños mecánicos ni por insectos, desinfectados con hipoclorito de sodio al 3.5 % durante 30 seg., lavados tres veces con agua destilada estéril, secados en placas conteniendo papel de filtro estéril, sembradas sobre el medio malta-sal-agar e incubadas durante siete días a 24± 2ºC. El porcentaje de granos colonizados fue determinado por examen bajo lupa estereoscópica. La incidencia se calificó como baja (<15%), intermedia (16-30%) y alta (>30%). El contenido de AFLA se cuantificó usando columnas de inmunoafinidad (Aflatest P, Vicam Sci. Tech) y fluorometría.

Resultados y Discusión. AF se identificó en el 87.5% de las muestras evaluadas en las dos localidades, mientras que todas resultaron contaminadas con AFLA. En las muestras colectadas en la localidad de San Javier la incidencia de AF fue mayor con valores desde 4% en 'D2A-223' y 'DK-357' hasta 28% en 'D1B 293' clasificándose de

baja hacia intermedia y el contenido de AFLA varió desde 1 ng/g (ppb) 'DK-357' hasta 460 ng/g ('D2A 092') y seis de las muestras excedieron la tolerancia de 20 ng/g. En las muestras colectadas en la localidad de Sabana de Parra la incidencia de AF fue baja, desde 0 en 'D2A-223', 'D1B 366', 'DK-357' y 'D 3273' hasta 6 % en 'D1B 293' y 'D2A-091'. También el contenido de AFLA fue menor aun cuando tres híbridos excedieron el límite de tolerancia. Los valores de AFLA variaron desde 1 ng/g ('D2A-223', 'D1B 366', 'D1A 842', 'D2A-091', 'D2A-086' y 'D1B 380') hasta 45 ng/g en 'D1B 293'. El híbrido 'D2A 092' resultó ser el más consistente para las dos localidades con baja incidencia de AF y alta contaminación con AFLA. En líneas generales, se observó que altos contenidos de aflatoxinas se correspondieron con bajas a intermedias incidencias de AF lo cual corrobora la ocurrencia natural de aislados de AF con alta capacidad de producción de aflatoxinas y la existencia de mecanismos genéticos distintos e independientes que actúan en la resistencia al moho y a la síntesis de la toxina. Asimismo, se confirmó la influencia de las condiciones ambientales particulares de cada localidad dentro del mismo estado sobre el crecimiento de esta especie de moho y la consecuente síntesis de la micotoxina.

Conclusión. Se confirmó la vigencia de AF como la especie de moho potencialmente toxigénico más importante en campos comerciales de maíz en la localidad de San Javier, estado Yaracuy y que elevados contenidos de aflatoxinas, que superan la tolerancia aceptada de 20 ng/g para maíz en Venezuela, son frecuentes en muestras de campo (Luzón *et al.*, 2007). La evaluación rutinaria de la incidencia de AF y otros mohos, y de la contaminación con AFLA y otras micotoxinas, sea a nivel experimental como a escala comercial, permite el seguimiento de los nuevos híbridos presentes en el mercado para conocer el riesgo de los productores de obtener cosechas contaminadas.

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P-10 SCREENING FOR AFLATOXIN IN CORN IN SONORA

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Background: Aflatoxins mean a sanitary problem for man and animals around the world. The problem of corn grain contamination with aflatoxin has induced enormous economic loses in several corn regions due to its high toxicity and carcinogenic effects Sweeney *et al*, (2000). Because of the conditions under which aflatoxins are formed in the grains, be it in the plant in the field or during further stages, harvest, transportation, storage and utilization the presence of aflatoxins is unavoidable in some corn lots and it is not limited to a determined climatic or geographic region Payne (1992).

Aims: To determine the presence of aflatoxins in different white national corn lots stored and distributed in the state of Sonora.

Materials and methods: One hundred and thirty three national white corn samples representative of the corn transported and stored in the state of Sonora during one year sampled at weekly intervals during 42 weeks, were analyzed following an official AOAC International method, (method 991.31. Immunoaffinity Column (Aflatest) method) AOAC International (2005). Ninety eight of the samples were taken from warehouses, 20 from silos, 12 from piles and, three from transportation facilities.

Results and discussion: Forty five percent of the samples were found with some level of aflatoxin contamination, traces to 18μg/kg, all of them below 20μg/kg which is the maximum limit approved by the limits and regulations, established by the Norma Oficial Mexicana for corn for human consumption Diario Oficial de la Federación (2002). From all of the storage facilities contaminated samples 46 of the 98 samples taken from warehouses were found contaminated, 5 out of 20 from silos, 9 out of 12 from piles. Two out of the 3 samples drown from transportation facilities.

Conclusions: Sixty out of 133 samples contaminated with aflatoxins as well as the low contamination levels have to make the decision takers cautious to avoid misconceptions and mistakes at compromising to making decisions related to what to do with contaminated corn lots for human consumption, particularly in Mexico where corn is the basic staple for its population's diet, considering the enormous poverty and undernourished levels.

P-11 SCREENING FOR AFLATOXIN IN CORN IN SINALOA

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Background: Aflatoxins are a sanitary problem for man and animals around the world. The problem of corn grain contamination with aflatoxin has induced enormous economic loses in several corn regions due to its high toxicity and carcinogenic effects Sweeney *et al*, (2000). Because of the conditions under which aflatoxins are formed in the grains, be it in the plant in the field or during further stages, harvest, transportation, storage and utilization the presence of aflatoxins is unavoidable in some corn lots and it is not limited to a determined climatic or geographic region Payne (1992).

Aims: To determine the presence of aflatoxins in different white national corn lots growing in the field, transported or/and stored in the state of Sinaloa.

Materials and methods: Two hundred and twenty national white corn collected in Sinaloa were analyzed following an official AOAC International method, (method 991.31. Immunoaffinity Column (Aflatest) method) AOAC International (2005). One hundred and eighty six of the samples were obtained, weekly, during a year, from storage or transportation facilities and seventy four were hand picked at harvest in different “municipios” of the state of Sinaloa.

Results and discussion: One hundred and two of the stored grain samples were aflatoxin contaminated. The aflatoxins levels detected in 101, 99%, of the stored or under transportation samples were contaminated, aflatoxins levels: <1 – 20 µg/kg, in one sample, 1%, 50 µg/kg aflatoxins were detected, a level that is above the legal levels allowed by the Mexican sanitary legislation Diario Oficial de la Federación (2002). The aflatoxins levels determined in this work, for the stored or under transportation grain were rather low based on the limits and regulations, 20 µg/kg, established by the Norma Oficial Mexicana for corn for human consumption Diario Oficial de la Federación (2002). Relative to the corn collected in the fields, 18 samples, 24%, were aflatoxins contaminated with levels below the limits established by the Mexican legislation.

Conclusions: The present inspection indicates that 46% of the analyzed corn was contaminated with aflatoxins. Being the corn the basic staple on the Mexican peoples diet, the frequency in which aflatoxins were detected, in spite of the low levels of contamination, almost all of them below the legal limits, it should make us to worry due to the characteristic cumulative behavior of the aflatoxins.

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P-12 SCREENING FOR AFLATOXIN IN CORN IN TAMAULIPAS

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Background: The state of Tamaulipas used to be one of the most important corn producing regions, mainly under irrigation conditions, of this country. However, several environmental and plant sanitary conditions as well as cyclic aflatoxin contamination have made the corn growing unstable and unpredictably, risky enough to make the corn producers to switch to growing other products Rodriguez del Bosque *et al*. (1995), Rodriguez del Bosque *et al*. (1992). The problem of corn grain contamination with aflatoxin has induced enormous economic loses in several corn regions due to its high toxicity and carcinogenic effects Sweeney *et al*, (2000).

Aims: To determine presence of aflatoxins in different white national corn, yellow national corn and yellow imported corn stored in the state of Tamaulipas, as well as to determine the presence of aflatoxins in white national corn growing in the fields of seven northern "municipios".

Materials and methods: Seventy seven corn samples collected from warehouse, silos and boats or from the fields at harvest in the state of Tamaulipas were analyzed following an official AOAC International method, (method 991.31. Immunoaffinity Column (Aflatest) method) AOAC International (2005). Fifty nine samples came from storage or transportation facilities and, 18, were hand picked in the fields at harvest.

Results and discussion: Thirty three of the fifty nine samples that came from storage facilities were aflatoxin contaminated. Twenty five out of 42 samples of white corn were found aflatoxin contaminated; 35 of them were taken from warehouses, and 18 were aflatoxin contaminated, 3 of the 3 samples collected from silos were found aflatoxin contaminated. The four white corn samples taken from boats were also aflatoxin contaminated. From the 17 samples of yellow corn 8 were aflatoxin contaminated. Of the yellow corn 10 samples were yellow national corn and 2 were aflatoxin contaminated all of them were taken from warehouses, while 6 of the 7 samples of yellow imported corn were aflatoxin contaminated 4 of the 5 that came from warehouses and 2 of the 2 that came from silos. All of the aflatoxin contamination detected was rather low from traces to ca.8 µg/kg. The Mexican legislation limits are 20 µg/kg, established by the Norma Oficial Mexicana for corn for human consumption Diario Oficial de la Federación (2002). All of the hand picked at harvest samples were aflatoxin contaminated in 72% of them the levels were 20 – 233 µg/kg, that is above the Mexican legislation limits.

Conclusions: In the case of the stored or under transportation grain, it was considered good for marketing; however 56% of these samples were contaminated as well as the fact that the aflatoxins have a cumulative effect on all the studied biological systems, should induce all agencies, public as well as private, involved on the sanity of feeds and foods to be prudent related to innocuousness of foods and feeds.

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Poster Section I: Mycotoxins in field crops.

Monday June 28

Group 3 (P-13 to P-17): Each presentation in 10 min.

P-13 DEVELOPMENT OF BRAZIL NUTS SHELLING MACHINE *VERSUS* FUNGI DETERIORATION AND QUALITY IMPROVEMENTS

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Background: In-shell Brazil nuts collected in the Brazilian Amazon forest have not been sold to the European Union (EU) since 2007 due to detection of aflatoxin (AFL) levels higher than the EU strict regulation. Thus, most of the in-shell Brazil nut production has been sold to Bolivia at much lower prices. That country, in turn, de-shells them and sells to EU at high prices and currency.

It is physically obvious that, fungi deterioration and cotoxins cannot be visually detected in the nut with the shell on (in-shell). Only after de-shelling consumer can discard contaminated nuts and toxin analysis can be made. De-shelling is necessary for further segregation of the moldy, spoiled, deteriorated nuts in the factories for good quality and safe dry nuts final product for consumption (Cartaxo, 2004; Pacheco and Scussel, 2006).

The methodology for de-shelling Brazil-nuts is still operated by hand (which gives jobs for many Amazon natives) in the Amazon region of Brazil. It is necessary the development of automatic machines to speed up shelling step as well improving nut quality and productivity for commercialization. Automatic processes for peeling/shelling grains and nuts are usually developed based on the withdrawal of the rind/shell by cracking, applying (a) centrifugal force, (b) pressure or (c) friction (Buhler, 2009). The mechanical processes of Brazil nut shell confront with some limitations, which demand research for adjustments on how cracks have to be made regarding the Type (size) and characteristics (tree faces, triangle shape) of the product to be de-shelled.

Aims: To develop an automatic process for shelling Brazil nuts, able to be used in the Amazon Forest, that can keep nut integrity (no cracks, broken or de-shellerd nuts) and allow further deterioration sorting for a safer high quality final product.

Material and Methods: *In-shell Brazil-nuts:* large (Type I > 50 mm), medium (Type II 40-50 mm lengh) and small (Type III < 40 mm) sizes obtained from the Renmero Factory, Cameta, Para State, located in the Eastern of the Brazilian Amazon region. *Material:* metal plates/sheets and tools for building the prototypes; scales (analytical and semi-analytical); CAD software for design and calculation; HPLC Gilson with fluorescene detection for aflatoxin analysis. *Prototype building:* two prototypes of de-sheller machines were built based on the following processes: (a) centrifuging (with “v” and “=” output shape) from 400 to 1000 rpm and (b) friction (conical and parallel-double cylinders). *De-shelling efficiency measurement:* each prototype process, had in-shell and shelled nuts evaluated for edible part percentage of cracks, bruises and broken as well as the best speed and economy/electricity/power saving.

Deterioration sorting: depending on the shelled nut deterioration degree, an ultra violet cabinet (λ 365 nm) was used for deterioration/aflatoxin presence visualization. *Aflatoxins analysis:* it was used the method developed by Sobolev (2007). *Moisture content (mc):* mc was controlled/adjusted (AOAC, 2005) prior the de-shelling process to get shell easier to crack without affecting their integrity.

Results and Discussion: *De-shelling process:* from the two processes applied, the best quality edible nut -final product- obtained was that based on friction with previous whole nut controlled mc. Centrifuging produced broken and bruised nuts, dispite of the rpm applied. *Sorting process:* regarding segregation of fungi deterioration, that machine allowed nut to be segregated just after nuts came out of the de-sheller as a sorting apparatus was built right next to it. Also a re-checking manual sorting conveyor was conected following the process and provided a final -de-shelled and segregated- safer and with better quality nut dry product. *De-shelling x deterioration sorting x AFL: segregated* (off-standard) and healthy (standard) shelled nuts were analyzed for AFL and results corroborated to the sorting process i.e., standard nuts presented very low AFL levels when comparedto the original in-shell batch.

Conclusions: The process based in friction utilizing double cilinders system allowed to obtain better quality shelled Brazil nut batches. Some nuts characteristics were important to take into account for best efficiency of the de-shelling process such as their irregular geometry; variation of nut sizes and moistures.

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P-14 COMPARISON OF PRECOLUMN AND POSTCOLUMN DERIVATIZATION SYSTEMS FOR THE LIQUID CROMATOGRAPHIC DETERMINATION OF AFLATOXINS IN PEANUTS

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Background: The monitoring of aflatoxins in food commodities as a consequence of the regulations established by many countries depends on the availability of adequate analytical methods. The most widely used method for the determination of aflatoxins in food and feed uses HPLC coupled with fluorescence detection. However, since aflatoxin B_1 and aflatoxin G_1 present less natural fluorescence, in order to improve the signals during analysis, various pre or postcolumn derivatization methods are used for signal enhancement.

Aim: To compare the method recovery when precolumn and postcolumn derivatization systems are used in the determination of aflatoxins in peanuts by liquid cromatography with fluorescence detection.

Materials and Methods: 25g samples of raw shelled peanuts and free of aflatoxins contamination were spiked with a pool of aflatoxin standards (2.02µg/Kg for B_1 and G_1 aflatoxins and 1.02µg/Kg for B_2 and G_2, 6.13 µg/kg total aflatoxins). The recovery tests were performed in triplicate.
The extraction and cleanup of the extract, using immunoaffinity column (Aflatest, Vicam Somerville, MA, USA), were done according to the AOAC Official Method 991.31 (AOAC, 2005a).
The precolumn derivatization was based on the AOAC Official Method 994.08 (AOAC, 2005b). A solution of trifluoroacetic acid, acetic acid and water 20:10:70 (v/v/v) was prepared to catalyze the conversion of aflatoxin B_1 and G_1 in B_{2a} and G_{2a}, respectively. The mixture of the extract and the derivatizing solution was heated to 65°C for 9 minutes to complete the derivatization reaction. Chromatographic conditions: mobile phase was methanol : acetonitrile : water in a gradient elution mode, starting composition 10:10:80 v/v/v, reaching 15:25:60 v/v/v in 3 minutes at a flow rate of 1.2 mL/minute; the C_{18} column, 150 mm x 4.6 mm, 5µm (XTerra® Waters) was maintained at 40˚C; the fluorescence detector operated at 364 nm excitation and 440 nm emission; 10 µL of the derivatized extract were injected.
Postcolumn derivatization was carried out as described in AOAC Official Method 999.07 (AOAC, 2005c), using an electrochemical cell (Kobra cell® - Rhône Diagnostics Ltd, Glasgow, UK). Chromatographic parameters: the mobile phase consisted of methanol : acetonitrile : water (for each 1L mobile phase 120 mg of potassium bromide and 350 µL of 4M nitric acid were added) in a gradient elution mode, starting composition at 20:20:60 v/v/v reaching 20:30:50 v/v/v in 3 minutes at a flow rate of 1.2 mL/minute; a C_{18} column, 250 mm x 4.6 mm, 5µm (XBridge® Waters) was kept at 40˚C; the fluorescence detector operated at 364 nm excitation and 440 nm emission; 30µL of the extract were injected.

The aflatoxins quantification was carried out based on a calibration curve established by the external standard method with seven concentrations within the working range of 0.0004 to 0.0203 µg/mL, for B_1 and G_1 aflatoxins, and 0.0002 to 0.0103µg/mL, for B_2 and G_2 aflatoxins.

Results and Discussion: The recovery rates for the precolumn derivatization method (95%, 88%, 89%, and 59% for B_1, B_2, G_1, and G_2, respectively) were within the acceptable recovery range (70 to 110%) indicated by the European Union Regulation n° 401, 2006, for concentrations between 1 and 10 µg/Kg of total aflatoxins, except for aflatoxin G_2, that shows inherent difficulty in recovering when it is purified by immunoaffinity columns. High recovery rates (90%, 85%, 100%, and 69% for B_1, B_2, G_1, and G_2, respectively) were obtained for the postcolumn derivatization.
Recovery is an important validation parameter of analytical methods, since both derivatization modes showed recovery values within the acceptable range, the viability of each method can be considered. The procedure for precolumn derivatization with trifluoroacetic acid promotes the aflatoxin detection and quantification at much lower cost when compared to the postcolumn method, since it is not necessary to purchase additional equipment. However, the analyst's cost must be considered. Also, the time required for the sample derivatization is an addicional step in the analysis, resulting in further human exposure to a solution composed of toxic substances such as acetic acid and trifluoroacetic acid. The postcolumn derivatization of aflatoxins, using the Kobra Cell®, occurs rapidly at room temperature. Moreover, it is not necessary to prepare the derivatizing agent daily and the maintenance is simple and easy. The derivatization reaction becomes part of the chromatographic run. The automation of the derivatization step increases the repeatability of results (results not shown).

Conclusion: Both derivatization methods have shown good recovery rates in the determination of aflatoxins in peanuts.

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P-15 DEOXYNIVALENOL CONTENT OF CEREALS IN ROMANIA BETWEEN 2005-2008

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Background: Deoxynivalenol (DON) is one of the most frequently found mycotoxine belonging to the trichothecene group, which was traced in raw materials in concentrations generally lower than 1000 ppb (Curtui et al., 1998), but which can get as high as 4000 – 5000 ppb. Under the climacteric conditions of Romania, cereals represent a substrate potentially favorable for the development of *Fusarium* moulds that can synthesize DON.

Aims: A survey of the DON contamination level in cereals from different regions of Romania between 2005-2008.

Material and Methods: 143 samples (wheat 100, 17 barley, maize 17, oats 4, two-rowed barley 2, rice 1, soya 1 and rye 1) from Southeast, Centre, West and North West of Romania were analyzed for the DON content. The quantification was realized through ELISA using a Veratox kit.

Results and Discussion: The quantification of the DON concentration in the cereal samples showed an important contamination with DON in the interval 2005-2008, with different percentage of contaminated samples depending of the year : 84.6% in 2005; 94.6% in 2006, 53.3% in 2007 and 87.9 % in 2008. The level of the contamination was sometimes higher than 1000ppb; the highest level was more than 2000 ppb DON. Despite this, most of the samples were contaminated with DON in a concentration between 0-50 ppb (76 samples) and 72.03% of the samples had a contamination degree under 200ppb. Only 2.09% of samples had a contamination degree higher than 2000ppb DON. The presence of DON in the cereals samples was identified since 1997, when Curtui et al. (1998) found that DON could be found in samples of maize and wheat in concentrations up to 880ppb. The present results could be added to other researches realised in our laboratory during the years 2002-2004 concerning the pattern of mycoflora and mycotoxin present in cereals from the southeastern Romania (Tabuc et al., 2009). In this study it was shown that Romania has a special pattern of mycoflora and mycotoxin contamination of cereals, DON being among the most frequently found mycotoxines.

Conclusion: In conclusion, DON was identified in most of the investigated samples. The DON concentrations varied between years and this fact was corelated with the variation of the temperature and with the region; the year 2006 was the most favorable to the DON production.

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P-16 NATURAL CONTAMINATION WITH NIVALENOL AND DEOXYNIVALENOL IN DURUM WHEAT GERMPLASMS IN ARGENTINA

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Background: In Argentina durum wheat (*Triticum durum* Desfontaines) is manly used for the elaboration of dried “pasta”. The national production of “pasta” in 2008 reached 369,600 ton and the consumption per capita was estimated in 8.5 kg/year. The total production is devoted to the local market, since an important decrease in the cropping area, in the last decades, made impossible the international market. This decrease can be explained by different factors such as: a) the demand of varieties with differentiated quality, b) the advance of new commodities with best profitability and c) decrease in the yield and grain quality due to fungal diseases, mainly *Fusarium* head blight (FHB). Previous studies in our country have shown that the main pathogen associated with FHB is *F*. *graminearum* Schwabe. Mycotoxins are frequently associated with the growth and invasion of cereal grains by FHB fungi, being the trichothecenes the most important ones. FHB is the disease that more challenges present for its control. The search for resistant germplasms is one of the strategies to reduce the impact of this disease.

Aim: The aims of this work were to evaluate the incidence of *F. graminearum* and *Fusarium* spp. and, the contamination with nivalenol (NIV) and deoxynivalenol (DON) in cultivars and lines used in a program of breeding of durum wheat in Argentina.

Materials and Methods: From varietal trials carried out at Experimental Station in La Dulce, in Buenos Aires Province, 26 durum wheat seed samples (cultivars and lines) were collected during the 2009 harvest season. To determine the percentage (%) of kernel infection by *F. graminearum* and *Fusarium* spp., 100 kernels per sample were placed onto Nash Snyder modified pentachloronitrobenzene medium (PCNB). The plates were incubated at 25°C for 7 days with a 12/12 photoperiod under cool-white and black-light fluorescent lamps. Single-spore cultures were obtained. Identification of *Fusarium* species was done on carnation leaf agar (CLA) and potato dextrose agar (PDA) according to Leslie and Summerell (2006).
The analysis of NIV and DON was performed using the methodoly proposed by AOAC (1995). The HPLC system consisted of a Hewlett Packard model 1100 pump (Palo Alto, CA) connected to a Hewlett Packard 1100 Series variable wavelength detector and a data module Hewlett Packard Kayak XA (HP ChemStation Rev. A.06.01). Chromatographic separations were performed on a Luna™ C18 reversed-phase column (100 x 4.6 mm, 5 µm particle size) connected to a guard column SecurityGuard™ (4 x 3.0 mm).

Results and Discussion: All the durum wheat germplasms evaluated showed 100% of contamination with *Fusarium* spp. *F. proliferatum*, *F. equiseti*, *F. semitectum, F. oxysporum* and *F. graminearum* were the most frecuently species isolated.
The contamination was variable dependent on the cultivars or line evaluated.

F graminearum recorded *a* low level of contamination (8%), this result, could be explained because the dry climate (especially during heading) does not allow *F. graminearum* infection. The meteorological conditions have an influence from the beginning of heading to early grain development. Factors such as RH affect *F. graminearum* infection and fungal invasion, *F. graminearum* grows ideally at or above 92-94% RH.
Although the *F. graminearum* incidence was low, both NIV and DON were detected in 10 and 11 samples, respectively. The levels of NIV, in positive samples, ranged from 132.00 to 752.00 µg/kg. The levels of DON were low, compared with the level accepted by European Community (1,750.00 µg/kg), except in one cultivar that showed a level of 7,719.00 µg/kg.

Conclusion: There was different behavior in the durum wheat germplasms in relation with incidence of *F. graminearum* and DON and NIV levels. Some cultivars were very susceptible, since in a year with climatic conditions not inductive for FHB, showed high levels of mycotoxin contamination.

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P-17 NATURAL OCCURRENCE OF MYCOTOXINS IN RAPE AND FLAX SEED IN LITHUANIA

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Background: Oil crop produce, characterised by good nutritive qualities, like other crop produce intended for food and feed, is subject to contamination with various micro-organisms, including microscopic fungi. Oil crop seed is an excellent medium for them to spread and produce secondary metabolites – mycotoxins. With increasing demand for oil crop produce mycotoxin research is becoming increasingly relevant, especially with the scientific evidence on these issues being rather limited.

Aim: Assessment of winter and spring rape seed and linseed contamination with microfungi and mycotoxins deoxynivalenol (DON), zearalenone (ZEA), T-2 toxin (T-2), ochratoxin (OCH A+B), aflatoxin (AFL B_1+B_2+G_1+G_2) and analysis of risk factors.

Materials and Methods: Mycotoxins DON, ZEA, T-2, OCH A+B, AFL B_1+B_2+G_1+G_2 were determined in oil crops' seed grown during the period 2007-2009. Analyses were done by ELISA technique (Wilkinson et al., 1992), using NEOGEN diagnostic test kits. Reading of immunoenzyme reaction optical density was done using a Multiskan Ascent photometer with 650 nm wavelength light filter. The data were processed using Ascent Software. Assessments of the levels of mycotoxins DON, ZEA, OCH, AFL were based on the regulations of the EU Commission, and those of T-2 toxin on the recommendations of the word research.

Results and Discussion: Mycotoxin contamination level of oil crops' seed was found to vary between years depending on the plant species and seasonal peculiarities. In 2007, DON, ZEA and T-2 contamination was found on 100 % of winter rape and oil flax seed samples analysed, and the highest contamination level with AFL B1+B2+G1+G2 was identified in 2008 in the samples of winter and spring rape seed (on average from 4.12 to 6.47 μg kg^{-1}). The quantities detected in most cases exceeded the limits allowable for food products (4.0 μg kg^{-1}). Higher OCH A+B quantities were also identified for spring and winter rape seed samples. Previous research suggests that air temperature and precipitation exert a marked effect on seed contamination with microfungi and on their diversity (Brazauskienė et al., 2006; Gruzdeviene et al., 2006). As a result, the risk of mycotoxins occurrence is increasing and their diversity is changing annually.

Conclusion: Assessment of the seed quality of oil crops intended for food and feed (spring and winter rape and oil flax) should involve ochratoxin and aflatoxin monitoring, since research findings exhibit a high risk of their emergence.

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Poster Section II: Mycoflora and Mycotoxin production. Groups 4 and 5.

Monday June 28

Group 4 (P-18 to P- 23): Each presentation in 10 min.

P-18 MYCOFLORA ASSOCIATED TO BEE POLLEN COLLECTED BY DOMESTICATED BEES.

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Antecedents. Pollen collected by domestic bees are harvested with special traps placed in the beehives, then is eliminate the excess of humidity and store by several months until their consumption. The contamination of bee pollen with fungi can be given in all their process, from its production in the field until its consumption, including its storage. Bee pollen is consumed by the people as a nutritional complement, and also by their therapeutic properties (Gutierrez, 2004), so for guarantee its innocuity must be free of mycotoxins produced by fungi able to colonize it. In field, the presence of *Penicillium*, *Aspergillus* and *Fusarium* in the stomach of the bees, as well as in the honey and other provisions of these insects has been demonstrated (Batra *et al*, 1973). During his storage, the contamination can continue as was demonstrated in a study where eight samples of bee pollen from different stores in Irapuato, Gto., Mexico were analyzed, resulting several from them highly contaminated with *Penicillium*, *Mucor*, *Alternaria*, *Rhizopus*, *Fusarium*, *Helminthosporium* and *Aspergillus*, including within this last genera to *A. flavus* and *A. parasiticus* (Bucio-Villalobos *et al*, 2007), and being the present research an extension of that study.

Objective. The objective of this reserach was to quantify the contamination with fungi in 19 samples of pollen collected by the domestic bees, obtained from natural products stores, markets, supermarkets and beekeepers of the city of Leon, Gto., Mexico.

Materials and Methods. 19 samples of pollen were obtained from natural products stores, markets, supermarkets and beekeepers of the city of Leon, Gto., Mexico. Their commercial presentations were of two types: without packages (plastic bags or small buckets) and handled in small glass bottles (with and without seals in its covers). All the samples of bee pollen were dry and were kept to room temperature until their process. After their perfect homogenization, by triplicate, 100 grains of bee pollen were separated of each sample and accommodated in five petri dishes with Potato Dextrose Agar medium. Petri dishes were incubated during seven days to 25°C. Then the number of grains of bee pollen colonized by the different fungi were quantified, expressing the results in percentage. The fungi were identified using taxonomic keys, using microscopic and colonial characteristics. The data were analysed with Kruskal-Wallis non-parametric test, that proves the null hypothesis of equality of the medians.

Results and Discussion. The results showed that the three samples with greater contamination of fungi (98, 100, and 100%) were handled without packages, similar result obtained in a previous study realised in 2007 with samples collected in the city of Irapuato,

Gto., Mexico. On the other hand, the contamination of samples handled in small glass bottles (with and without seals in its covers) tended to be low, in contrast to the found in the previous study already mentioned, where there were samples handled in glass bottles with more of 90% of contamination; this indicates that the degree of contamination is not consequence only of the form to package pollen. General average of fungi incidence was relatively low: *Aspergillus* (3,6%), *Alternaria* (3,6%), *Mucor* (3,1%), *Fusarium* (2,9%), *Penicillium* (2,9%) and *Rhizopus* (0,7%), including within the genera *Aspergillus* to *A*. *flavus* specie, which can include strains able to produce aflatoxins. *A*. *flavus* was detected in 4 of 19 analyzed samples, with incidences of 27, 14, 10, and 1%, which were higher than a previous study done in 2007 where the incidence was lower that 2%. This results showed that the presence of potentially toxigenic fungi can vary from place to place or in different years. It is important to insist about the necessity to realise future studies to determine the natural contamination with mycotoxins in bee pollen, or evaluate in laboratory the capacity to produce toxin of the fungi found in this research, as in other studies already the capacity to produce ocratoxin A and aflatoxin B_1 and B_2 were demonstrated in fungi isolated from bee pollen (González *et al*, 2005). It must be considered that bee pollen is a substrate that stimulates the ocratoxin A production when it is added to culture medium where *Aspergillus ochraceus* grow (Medina *et al*, 2004), phenomenon that could happen of natural way in pollen stored under bad conditions. The Kruskal-Wallis test indicated that there were not differences statistically significant between medians a 95,0% level, neither for the fungi contamination in the different packages forms, nor for the different identified fungi.

Conclusions. In the present descriptive study, contamination with fungi of bee pollen samples were from 0 to 100 %, demonstrating the presence of potentially toxigenic fungi. Future researches are recommended for evaluate the natural mycotoxins contamination of bee pollen, as well as determining strategies of mycotoxins control during production process, drying, packaging and commercialization of bee pollen.

Thanks. To De La Salle Bajío University by the economic support, thanks to which the present project could be developed. Also to the University of Guanajuato by the complementary support.

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P-19 FUNGAL CONTAMINATION OF BEE POLLEN STORED BY ONE YEAR UNDER DIFERENT CONDITIONS.

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Antecedents. Bee pollen is stored by beekeepers, industrialists, retailers or consumers by several months until it is consumed by the people. Diferent forms of the storage are doing and can be with or without a packing process. Contamination with fungi can happen during the storage; that was demonstrated in a research done by Bucio-Villalobos *et al* (2007) where several samples of bee pollen collected from different stores from Irapuato, Gto., Mexico, were highly contaminated with *Penicillium*, *Mucor*, *Alternaria*, *Rhizopus*, *Fusarium*, *Helminthosporium* and *Aspergillus*, including within this last genera to *A*. *flavus* and *A*. *parasiticus*. Both last species have strain with capacity to produce aflatoxins, as it was demonstrated in a study with bees, where ocratoxin A, aflatoxin B_1 and aflatoxin B_2 were identified (González *et al*, 2005), for this reason is necessary to carry out studies of natural contamination of pollen with mycotoxins. Storage conditions are important for avoid the contamination of foods with mycotoxins, since it has been demonstrated in many different substrates (Aidoo, 1991). Several storage conditions done during the manipulation in the commercialization of bee pollen, were evaluated in the present research.

Objective. The objective of this research was to quantify the contamination with *Fusarium* and other fungi, of pollen collected by the domestic bees after storing it during one year under different treatments of humidity, temperature and forms of packaging.

Materials and Methods. Pollen collected by domestic bees in November of 2008 in an experimental apiary located in Irapuato, Gto., Mexico, was divided in the following treatments: humid pollen versus dry pollen, pollen stored under freezing (-15°C) versus pollen stored to room temperature (15-20°C), pollen packaged in crystal bottles with hermetic cover versus pollen stored in open plastic packages. At the beginning of storage and 12 months later, by triplicate, 100 grains of bee pollen taken at random were separated of each treatment and accommodated in five petri dishes with Potato Dextrose Agar and Nash Snyder mediums. Petri dishes were incubated during seven days to 25°C. Then the number of grains of bee pollen colonized by *Fusarium* and the total contamination by other fungi were quantified, expressing the results in percentage. The fungi were identified using its microscopic and colonial characteristics. The data were analysed with analysis of variance procedure and Tukey test.

Results and Discussion. In order to know the original fungal contamination of bee pollen just harvested (considered this moment as the begining of storage), the pollen was processed microbiologically. 100% of fungal contamination was found in all bee pollen treatments (based on total population of fungi evaluated on Potato Dextrose Agar medium), whereas for *Fusarium* (evaluated on Nash Snyder medium), the fungal population fluctuated between 67

and 84%. The results found indicate that the fungal contamination of bee pollen was of origin, that is to say from the field where the pollen was collected. Before bee pollen was collected from the field, it was temporarily stored by seven days in the special boxes of the traps placed in the beehives; this place could be a fungal contamination source. Other researchers, working with bees, have found the presence in field of *Penicillium*, *Aspergillus* and *Fusarium* fungi (Batra *et al*, 1973). After 12 months of storage, total fungal contamination of bee pollen was not smaller in comparison at the beginning of storage, remaining in 100% for all treatments. However the population of *Fusarium* fell until a rank between 30 and 63% of incidence; the Tukey test detected significant differences between humid pollen and dry pollen, as well as between pollen packaged in crystal bottles and pollen stored in open plastic packages. Bee pollen stored to room temperature in open plastic packages, dry or humid, had visible fungal colonization at the end of storage, and was completely unsuitable for the human consumption. In counterpart, the best one of the treatments was the bee pollen stored within glass bottles and dry form, as much to room temperature as in freezing. In conclusion, the beekeeper can preserve by long time his pollen, if he immediately after the harvest it eliminates its humidity and store it in hermetic containers; under these conditions bee pollen will be able to remain to room temperature by several months, being even better if it is stored under freezing temperature. *A. flavus* was identified and found it in its greater incidence after stored the pollen for four months, especially in pollen stored with humidity and in open plastic packages.

Conclusions. Fungi of genera *Fusarium* reduced their population after storing bee pollen during 12 months, not however the total fungal population which remained high throughout the mentioned period. Bee pollen stored in open plastic packages, dry or humid, was the worse one of the treatments mainly when it was stored to room temperature where was destroyed at end of storage period. We concludes that the best way to store the bee pollen is within hermetic containers and without humidity; it is better if do it under freezing.

Thanks. To De La Salle Bajío University by the economic support, thanks to which the present project could be developed. Also to the University of Guanajuato by the complementary support.

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P-20 CHARACTERISATION OF *ASPERGILLUS* STRAINS ISOLATED FROM GROUNDNUTS IN BURKINA FASO

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The aim of this study was to isolate and characterise local aflatoxin-producing and aflatoxin-non-producing strains of *Aspergillus flavus* and *Aspergillus parasiticus* from groundnut samples. Strains were isolated and purified on bean broth agar and Czapek yeast extract agar slant was used for further purification and identification using the systematic classification of *Aspergillus* strains based on morphological characters described by Hocking, 1982; Cotty, 1993 and Christensen, 1981. The aflatoxin-producing ability was carried out based on fluorescence under UV light at 254 nm on coconut agar medium and by fluorescence HPLC on coconut broth. All experiments were performed along with reference strains (aflatoxin-producing and aflatoxin-non-producing) obtained from USDA Illinois and CDC Atlanta, USA. The use of the above different methods allowed the identification of two local isolates (one being an aflatoxin-producing strain (BfaS1) and the other a non-aflatoxin-producing strain (BfaS5)) as members of *Aspergillus flavus* or *Aspergillus parasiticus* species. BfaS1 showed detectable levels of AFB1 and AFB2 only and was comparable to the reference strain CDC 5333. The levels of aflatoxin production by BfaS1 were 0,59 ng/ml and 0,011 ng/ml respectively for AFB1 and AFB2 and were 3,55 10^{-3} and 10,05 10^{-3} folds lower than those of CDC 5333 (165,73 ng/ml and 1,74ng/ml AFB1 and AFB2 respectively). The results are discussed in relation to the use of affordable methods and techniques in the isolation and differentiation of toxigenic from non toxigenic isolates of Aspergillus species.

P-21 IDENTIFICATION OF AFLATOXIN PRODUCING ISOLATES OF *ASPERGILLUS FLAVUS*THROUGH COMMON CULTURE MEDIA MODIFICATION

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Background: The importance of aflatoxins has leaded a great deal of research on fast and efficient methods to detect these toxins in different feeds and foods. Besides those methods, research has been conducted to detect *Aspergillus flavus* isolates capable to produce aflatoxins Jaimez Ordaz *et al*. (2003), Fente *et al*. (2001), Pallavi et al. (1997), Davis *et al*. (1987), Hara *et al*. (1974).

Aims: To evaluate the ability of *Aspergillus flavus* to produce aflatoxins in some culture media used to growing the mold*,* with different intentions, adding and non adding methyl β cyclodextrin.

Materials and Methods: Four aflatoxin producing *Aspergillus flavus* isolates (A7, A9, G15 and K9), and one not producer (E7) were grown in: Czapek solution agar (Cz), Czapek yeast extract agar (CYA), coconut extract agar (CAM), yeast extract sucrose agar (YES), malt extract agar (MEA), 25% glycerol nitrate agar (G25N), coconut extract agar medium (CEM), potato dextrose agar (PDA), Sabouraud dextrose agar (SDA) and, modified Czapek agar medium (APA). Each of these culture media were also prepared adding methyl-β-cyclodextrin.

Results and discussion: Among the culture media, added and not added methyl-β-cyclodextrin, those who showed fluorescence were: APA, CYA, CAM, CAM, YES y PDA. The isolates in which fluorescence (and probably aflatoxin produced) was clearly observed were A9 and G15.

Conclusions: The addition of methyl-β-cyclodextrin improved the fluorescence of the aflatoxins produced by A*. flavus* en YES; in APA this improvement was not noticeable. The addition of cyclodextrin allowed the detection of aflatoxin production by aflatoxin producing isolates in the following culture media: CYA, PDA, CEM and, CAM.

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P-22 FUNGAL MICROBIOTA OF STORED *OGI* - A FERMENTED MAIZE GRUEL IN NIGERIA.

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Background: *Ogi* is a popular breakfast meal and the most common weaning food obtained from fermented maize, sorghum or millet. Its consumption cuts across different ethnic and economic classes along the West African coastal region. The traditional production of *ogi* from maize is a 2-stage process: steeping and souring. During steeping, dry maize grains are soaked in water for 3 days, washed, wet milled and wet sieved. Thereafter, it was allowed to settle and sour for 2-days. *Ogi* is usually stored at room temperature and the sour water decanted and replaced with fresh water one at 3-5days interval.

Aim: This study shows the fungal microbiota during storage of *ogi*

Materials and methods: *Ogi* was produced using the traditional method (fig 1) Samples of wet *og*i was stored at room temperature ($28^oC \pm 2$) for 28 days and samples were taken at interval for physiochemical, microbiological and sensory analysis. Proximate analysis was as described by AOAC, 1990.
The spoilage indices consisted of pH, total titrable acidity, total reducing sugars, dissolved hydrogen sulphide and ammonia level (Teniola and Odunfa, 2001). The odor changes were obtained by the means scores of a 25-man panel. Yeasts and moulds were isolated, purified and identified using standard tests and classification schemes.

Results: During storage at room temperature, initial yeasts counts in wet *ogi* increased from 3.51 ± 0.25 log cfu/g on day 1 and peaked at 7.33 ± 1.00 log cfu /g on day 14, then reduced thereafter. Moulds were not isolated until the 8^{th} day of storage and the count increased steadily to peak at 7.01 ± 1.00 log cfu/g on day 20. On the 8^{th} day of storage only *Aspergillus niger* was isolated, however, by the 20^{th} day of storage, the moulds isolated were *A. niger, A. flavus, Rhizopus nigrican,* and *Penicillium sp* while the yeasts were *Saccharomyces cerevisiae, Candida krusei, C. tropicalis, C. vini* and *Geotrichum candidum.* The observed discoloration of the stored *ogi* coincided with the isolation of moulds on the samples. Increases in the levels of dissolved hydrogen suphide (H_2S) and ammonia were noted until the 16^{th} day of storage after which there were declines. During the storage period more hydrogen sulphide (H_2S) was produced than ammonia (NH_3).

Conclusion: Considering the delicate health position of some *ogi* consumers and the current knowledge of some toxic metabolites (mycotoxins) produced by fungi; the presence of some of the identified mould in stored *ogi* is significant health wise.

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P-23 STUDY OF THE ENVIRONMENTAL FUNGAL FLORA IN SALAMI DRYER´S ROOM

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Background: During the maturation process of dry sausage and other meat products, bacteria, molds and yeasts are growing on their surface. Fungi presence has enormous biotechnological importance, since they provide taste, flavor and aromatic characteristics to the final product. But uncontrolled development of these microorganisms can result in adverse effects in those products such as: abnormal appearance, less nutritional value, technological properties, incorporation of allergens metabolites and mycotoxins.
Objectives: To characterize the environmental mycoflora in dryer's room by "Salame tipo Colonial", a dry sausage developed and produced exclusively by COFRA.
Materials and Methods: The study of environmental mycobiota present in dryer's room by "Salame tipo Colonial", was made in COFRA, a meat processor located in the southern province of Misiones (NE of Argentina).
Environmental sampling was conducted by the gravimetric method, based on the deposition of particles suspended in the air by the action of gravity on plates with culture medium. The sampling was performed in triplicate to 6 different levels depending on the arrangement of salami in the dryer´s room; Level 1 is closest to the roof and level 6 near to floor.
For the fungal growing were used Agar plates with dichloran-Rose Bengal-Chloramphenicol (DRBC, Oxoid) and incubated at room temperature for 5-6 days. Individual colonies were isolated for pure strains in Malt Extract Agar (Merck) and Czapeck Agar (Merck). The genus level identification of isolated colonies was performed by observing the macro and microscopic morphological characteristics.
Results and Discussion: 915 fungal colonies were isolated from a total of 36 plates open at six levels. The predominant genera found were: *Aspergillus spp* (42.8%), *Penicillium spp* (17.2%) and *Candida* type yeast (10.9%), less frequently, *Aerobasidium spp.* (2.1%), *Acremonium spp* (1.0%), *Geotrichum spp.* (0.7%), *Fusarium spp.* (0.7%), *Alternaria spp.* (0,5%), *Curvularia spp.* (0.4%), *Thermomyces spp.* (0.2%), among others.
Conclusion: The presence of "desirable" mold strains, it is necessary to develop sensory and functional characteristics of this dry sausage, the fact that other species are present in addition to the desirable, may pose a risk, since many may potentially toxigenic, so it should continue with the identification of strains to species level and the mycotoxigenic capacity of them.

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Poster Section II : Mycoflora and Mycotoxin production .

Monday June 28

Group 5 (P- 24 to P-29): Each presentation in 10 min.

P-24 CHEMOTYPES AND GENOTYPES CHARACTERIZATION IN *FUSARIUM GRAMINEARUM* SPECIES COMPLEX ISOLATED FROM SOYBEAN IN ARGENTINA.

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Background: *Fusarium* rot of soybeans is described in the literature and different *Fusarium* species have been isolated from this commodity (Pitt and Hocking, 1997). These strains are known to produce a broad spectrum of toxins including trichothecenes of A- and B-types. Among B-type trichothecenes, deoxynivalenol (DON) and nivalenol (NIV) are important mycotoxins produced mainly by *F. graminearum* Schwabe [teleomorph = *Gibberella zeae* (Schwein.) Petch]. Strains of *F. graminearum* usually express one of three sets of trichothecene metabolites either: nivalenol and acetylated derivatives (NIV chemotype), (ii) deoxynivalenol and 3-acetyldeoxynivalenol (3-ADON chemotype), or deoxynivalenol and 15-acetyldeoxynivalenol (15-ADON chemotype) (Ward et al. 2002). Surprisingly, *Fusarium* isolates that produce both DON and NIV (NIV/DON chemotype) have been reported and described as “unknown” chemotypes (Ward et al. 2002; Quarta et al. 2006). Due to the toxicological differences between NIV and DON, it is important to survey the presence of different chemotypes in a given region and different crops. With the identification of the genes responsible for this toxin production, PCR has become increasingly popular to discriminate between different genotypes. More recently, primers pair based on derived form the *Tri3*, *Tri5* and *Tri7* genes of the trichothecene gene cluster were designed to differentiate between the three genotypes.

Aim: to characterized the chemotype and genotype diversity among the *Fusarium graminearum* species complex isolated from soybean.

Materials and Methods: For determination of trichothecene chemotype, *Fusarium graminearum* complex strains were cultured on Erlenmeyer flasks (250 ml) containing 25 g of rice. Ten ml of distilled water was added before autoclaving for 30 min at 121 ºC, twice. Each flask was inoculated with a 3-mm diameter agar disk taken from the margin of a colony grown on synthetic nutrient agar at 25 °C for seven days. Flasks were shaken by hand for 1 week and incubated for 28 days at 25 ºC in dark. At the end of the incubation period the content of the flask were dried at 50 °C for 24 h and then stored at –20 °C until analyzed for toxin. Toxin

analysis was done by using a modified version of that originally reported by Cooney et al. (2001). For Determination of trichothecene genotype, multiplex PCR experiments were conducted with 10–25 ng of fungal DNA in a total volume of 50 μL of 1x reaction buffer containing 1.5 mM $MgCl_2$, 2 U Taq DNA polymerase (Promega), 200 mM dNTPs, 0.2 mM each of the three *Tri*3 primers (Tri3F971, Tri3F1325 and Tri3R1679) and 0.1 mM each of the

remaining four primers (Tri7F340, Tri7R965, 3551H and 4056H) (Quarta et al. 2005, 2006). A negative control, containing all reagents but no DNA, was included with every set of reactions. PCR was conducted in a PTC-2000 Thermal Cycler (MJ Research Inc., Watertown, MA). The PCR conditions were: 94º C, 3 min then 35 cycles of 94º C, 30 s, 53º C, 30 s, 72º C, 1 min, followed by a final extension step of 10 min, 72º C. PCR products were separated by electrophoresis through 2% agarose gels. Gels were stained with 1 μg/ml ethidium bromide and photographed under UV light.

Results and Discussion: A total of 40 isolates belonging to the *F. graminearum* species complex were evaluated for DON and their acetylated derivatives. Chemical analysis showed that 15-ADON was the most frequent chemotype observed among the population (60% of isolates). A 30% of isolated produced only DON and no acetylated derivatives and 7.5% of the strains had an unusual pattern of mycotoxin production because they simultaneously produce DON and NIV. The NIV and 3-ADON chemotype was not observed. PCR assays showed also that 15-ADON was the most frequent genotype observed among the population followed by DON/NIV genotype (12.5%). Out of 5 strains that showed DON/NIV genotype by PCR analysis, 3 of them were able to produce DON and NIV and 2 isolates produced only DON by chemical analysis.

Conclusion: The *F. graminearum* species complex isolated from soybean consisted mostly of the 15-ADON with the remaining strains exhibiting DON/NIV genotype, based on PCR genotype and chemotype determination. We observed neither the NIV nor the 3-ADON genotypes among the member of the population evaluated.

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P-25 INCIDENCE OF FUMONISINS B_1, B_2 AND THEIR HYDROXILATED COMPOUNDS HFB_1 AND HFB_2, IN SORGHUM GRAINS FROM THE STATE OF MORELOS, MEXICO.

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Introduction

Grain mold of sorghum is a worldwide disease that affects grain quantity by decreasing its size and quality by reducing the nutritional value. One of the causal fungi of this disease in Mexico is *Fusarium thapsinum* Klittich, Leslie, Nelson & Marasas, found as predominant fungi in sorghum grain mold in the State of Morelos, Mexico. Grain mold is a problem of early maturing sorghum in tropical regions (28 to 37°C), and the amount of fumonisins and aminopentols have not been well analyzed. Fumonisin B_1 is known to cause leucoencephalomalacia in horses, pulmonary edema in swine and liver cancer in rats. The objective of this study was to identify and quantify Fumonisins B1 and B2 as well as the hydroxilates aminopentol 1 (AP1) and 2 A (AP2) in grains of sorghum from the State of Morelos, Mexico where *F. thapsinum* has been identified.

MATERIALS AND METHODS

Twenty five samples of sorghum grains were obtained from a previously described experiment (Montes-Belmont et al., 2003) in Tlayca, Jonacatepec County, Morelos State, Mexico.

Samples were dried at 60°C for 48 hs, ground, weight and extracted for fumonisins B1 and B2, with a known method (Scott y Lawrence, 1996;) and aminopentols AP1 and AP2.

The derivatizing solution was done with 20 mg of O-phthaldialdehyde (OPA) in 500 mL of methanol) with 2 mL of sodium borate 0.05 M, finally 25 mL of mercaptoethanol, one minute before injecting the sample in the HPLC pump. Five mg of fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) standards (Sigma Chemical Co.) were separately prepared adding them 10 mL of acetonitrile: water (2:8 v/v). Four calibration curves were done (FB_1, FB_2, HFB_1 and HFB_2) with 6 dilutions (0.3, 0.5, 1.0, 3.0, 5.0 and 10.0 µg mL^{-1}). FB_1 and FB_2 hidrolysis were done from 10 µg mL^{-1} of FB1 and FB2 standards with known method (Thakur and Smith,1996). The hydrolysis was stopped with HCL 2N pH 4.5. The formation of HFB1 and HFB2 was confirmed in mass thermo spray-spectrometry. Liquid chromatography was done in a Series 400 pump, fluorescence LC-10 detector and computarizad integrator LCI-100 all from Perkin Elmer. A Prodigy 5 ODS-2, 250 mm x 4.6 mm x 5 µm Phenomenex C_{18} HPLC column was used. The HPLC method from (Shephard *et al.*, 1990) was used with 1 mL per minute flow. Retention times were 7.10-7.76 min (FB_1), 14.3-16.75 min (FB_2); 12.08-14.57 min (HFB_1) and 14.91-15.88 (HFB_2).

Results and Discussion.

There were more amount of hydroxylates HFB_1 than fumonisin FB_1, the FB_2 and HFB_2 were in the same range of mg g^{-1}.

Fumonisins and hydroxylates in sorghum grains.

Samples	Fumonisins (µg g^{-1})		Hydroxilates (µg g^{-1})	
	FB1	FB2	HFB1	HFB2
1-5	112.90	39.84	557.36	32.93
6-10	305.52	47.29	436.84	14.85
11-15	189.72	41.36	416.89	121.93
16-20	145.96	39.95	662.35	24.93
21-25	39.14	35.49	104.24	22.36

*average of 5 samples with 3 replications each.

Samples 21-25 were only grains with the fungus, and they had the lowest amounts of fumonisins and hydroxilates. There is no relation between the presence of the fungus and the fumonisins or hydroxilates.

The ranges of FB_1 reported for Morelos, Mexico (11.0- 536 µg g^{-1}) were similar to sorghum samples from India 70 to 800 µg kg^{-1} (Shetty and Bhat, 1997), but less in comparison with Brazilian sorghum samples (120 to 5 380 µg kg^{-1}) (Da Silva et al., 2004), and from sorghum malt samples of Botwana (47 to 1316 µg kg^{-1}) (Nkwe et al., 2005).

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P-26 TOXIGENIC PROFILE OF *ALTERNARIA* SPECIES ISOLATED FROM SOYBEAN SEEDS.

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Background. Soybean (*Glycine max* L. Merr.) is an Asiatic leguminous plant cultivated in many parts of the world for its oil and proteins. Soybean is often attacked by fungal during cultivation, or post-harvest (in transit or in storage), significantly affecting its productivity. *Alternaria* and *Fusarium* species are the most commonly isolated fungi from soybean in Argentina and in other regions of the world (Boca et al., 2003; Broggi et al., 2007; Gally et al., 2006; Roy et al., 2000). *Alternaria* species are well known for the production of toxic secondary metabolites, some of which are powerful mycotoxins that have been implicated in the development of human esophageal cancer (Thomma 2003). Among these metabolites with mammalian toxicity are alternariol (AOH), alternariol monomethyl ether (AME) (Ostry 2008; Logrieco et al., 2009). Recently have been reported that AOH and AME posses cytotoxic, genotoxic, estrogenic and mutagenic properties *in vitro* (Brugger et al., 2006; Fehr et al., 2009; Lehmann et al., 2006; Wollenhaupt et al., 2008), and there is also some evidence of carcinogenic properties (Yekeler et al., 2001). In previous studies we have showed natural occurrence of AOH and AME on soybean seeds harvested in Argentina (Oviedo et al., 2009). Considering the extensive used of soybean in the manufacture of animal and human foodstuffs the risk of both populations arising from continuing low-level exposure to AOH and AME should be taken into account.

Aim. To determine the toxigenic profile of *Alternaria* species isolated from soybean seeds harvested in Argentina.

Materials and Methods. Toxigenic profile was determinated for *Alternaria* strains previously isolated from soybean seeds and identified as *A. alternata* (8), *A. infectoria* (49), *A. oregonensis* (8), *A. graminicola* (4) and *A. triticimaculans* (1). Petri plates containing ground rice-corn steep liquor medium were inoculated centrally with agar disk taken from the margin of a 7-day-old colony of each *Alternaria* strains grown on synthetic nutrient agar (SNA) (Gerlach and Nirenberg, 1982). The plates were incubated for 14 days at 25 ºC in darkness (Chulze et al., 1994). The extraction method used was based on a microescale extraction (Smedsgaard 1997) modified into a three step extraction procedure suited for *Alternaria* metobolites as described by Andersen et al (2001). Toxins were detected and quantified by HPLC whit UV.

Results and Discussion. It was observed that 100% of the strains were able to produce some of the analyzed mycotoxins (AOH, AME and TA). Within the 70 strains of *Alternaria* evaluated, variations were observed in the toxigenic profile of the isolates. The most common toxigenic profile found was to AOH-AME-TA produced by 95.7% of the strains, while the rest

of the strains (4.3%) produced only AOH and AME. Depending on the species under study, it was observed that *A. infectoria, A. triticimaculans* and *A. graminicola* strains were able to produced alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TA). Among the *A. alternata* and *A. oregonensis* strains different toxin profiles were observed: AOH, AME and TA or AOH and AME producers.

Conclusion. All strains of *Alternaria* were able to produce mycotoxins, presenting two different toxigenis profile (AOH-AME- TA and AOH-AME). The results suggest a potencial risk to found *Alternaria* mycotoxins in soybean.

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P-27 EVALUATION OF OCHRATOXIN A PRODUCTION BY THE MAIN *ASPERGILLUS* SECTION *CIRCUMDATI* SPECIES

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Background: Ochratoxin A is one of the most important mycotoxins contaminating foodstuffs and beverages because of its potent toxic properties. Since 2006, OTA levels in several products for human consumption are regulated by the European Union (EC No 1881/2006). The species belonging *Aspergillus* section Circumdati have been traditionally considered an important source of OTA in agro-products. However, due to recent changes in the taxonomy in this group (Frisvad et al., 2004), the relative contribution of each species to overall OTA contamination in context of food safety is far from be firmly established.

Aim: The objective of this work was to study the capacity of several strains of *A. steynii, A. westerdijkiae, A. ochraceus* and *A. elegans* isolated from several commodities to produce OTA.

Materials and Methods: 30 strains were analysed for OTA production (6 *A. elegans*, 7 *A. ochraceus*, 9 *A. steynii* and 8 *A. westerdijkiae*). They were isolated from different sources (coffee, grapes, cereals, dried fruit or spices among others) or obtained from Culture Collections. Spots of highly concentrated spore suspensions (10^7 spores/ml) were placed on the center of a plate containing CYA, an optimal medium for OTA production in this kind of fungi. OTA was extracted after 7 days of incubation at 28ºC by the method designed by Bragulat et al., 2001 and measured by HPLC.

Results and Discussion: The results showed that *A. steynii* produced the highest values of OTA (9068 µg/l), followed by *A. westerdijkiae* (203 µg/l). In contrast, *A. elegans* and *A. ochraceus* produced low, if any, toxin levels (15 and 2 µg/l, respectively). The ability of *A. steynii* strains to produce OTA must be emphasized because of the large amount of toxin detected in these conditions and the diverse substrates this species is able to colonize, as shown in our study. Due to its recent description, there are few works about this species reporting its occurrence in food matrices (Leong et al., 2007; Noonim et al., 2008).Some authors have suggested that *A. elegans* cannot be considered a OTA-producing species, and that detection of OTA production by isolates of this species might be attributed to incorrect identification. However, the correct identification of all the strains analyzed in this work, including *A. elegans* strains, was tested using species specific PCR assays developed in our group (Gil-Serna et al., 2009). Therefore, the ability of *A. elegans* to produce OTA cannot be disregarded, although the values were low.

Conclusion: On the basis of this study, *A. westerdijkiae* and, particularly *A. steyni,* represent the major risk for OTA contamination in terms of the high amounts of OTA produced and the diversity of food matrices that these species colonize. However, the contribution of *A. ochraceus* and *A. elegans* cannot be disregarded.

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P-28 Modelling of *A. parasiticus* growth under non optimal environmental conditions

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Background: Mycotoxins are chemical risks of biological origin which are of increasing concern in food products due to the wide range of food types where they can be found. They are associated to the presence of fungal inoculum on predisposed substrates. Despite the absence of direct correlation between mould growth and mycotoxins production, prevention of fungal growth effectively conduces to prevention of mycotoxin accumulation. In general, water activity (a_w) and temperature are regarded as the main controlling factors determining the potential for mould growth. Predictive models can be a tool to prevent mould development. Secondary kinetic models describe microbial responses in relation to environmental factors, providing estimates for parameters of growth: lag phase (λ) and growth rate (μ). These researches are generally built on synthetic media and they should be validated in food systems, where there are other factors that influence on the mould growth.

Aims: To model the growth of *Aspergillus parasiticus*, an aflatoxins producer, near to the growth/no-growth boundaries and validate the models in maize grain and groundnuts.

Materials and methods: In this research one isolate of *A. parasiticus* was used. A spore suspension of 10^5 spores/ml of this mould was prepared and inoculated centrally with a needlepoint load on malt extract agar (MEA) adjusted to five different water activities: 0.93, 0.91, 0.89, 0.87 and 0.85. Plates were incubated at 10, 15, 20, 25, 30, 37 and 42ºC. For each of the 35 conditions, 10 Petri dishes were inoculated. Validation was carried out directly in sterile peanuts and maize seeds under the following conditions: 0.85a_w-25ºC; 0.85a_w-30ºC; 0.87a_w-20ºC; 0.87a_w-30ºC; 0.87a_w-37ºC; 0.89a_w-15ºC; 0.89a_w-20ºC; 0.89a_w-37ºC; 0.91a_w-15ºC and 0.93a_w-25ºC. For both, culture media and food substrates, two perpendicular diameters of the growing colonies were measured daily until the colony reached the edge of the Petri dishes.

Results and discussion: The growth followed biphasic Baranyi's function without an upper asymptote. Growth rates (μ) and lag phases (λ) were estimated through this function. Under near marginal conditions, μ decreased and λ increased. No growth was observed under extreme suboptimal conditions (eg. <15ºC or <0.85 a_w). The higher μ was found at 0.93a_w-37ºC but the minor λ was at 0.93a_w-30ºC. Response of the moulds to the environmental conditions studied was examined with different secondary models. The square root transformation was introduced to stabilize the variance of the fitted values for the growth rate ($\sqrt{\mu}$). To model lag phase, natural logarithm transformation was used (ln λ). The general polynomial model published by Panagou *et al.* (2003) showed the better performance (R^2=0.78, MSE=0.039, for both models, for growth rate). Validation on maize grain led to poor bias and accuracy factors, due to decreased growth compared to that on MEA under these marginal conditions. For groundnuts, bias factors of 6 and accuracy factors of 1 revealed a better performance of both models. Additionally, a probability model was applied to results on

MEA which revealed good potential for prediction near the growth/no-growth boundary, according to validation results.

Conclusion: Probability models may be of interest to predict the probability of growth of mycotoxigenic moulds under suboptimal conditions which often occur in food substrates.

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P-29 Variability of mycotoxin production by *Alternaria* strains isolated from different substrates in Argentina

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Background:
The genus *Alternaria* includes both plant pathogenic and saprophytic species that may damage crops in the field or cause post-harvest decay of fruits and vegetables. *Alternaria* spp. are the most common fungal species invading tomatoes and are important pathogens of various pome and stone fruits, citrus fruits, grapes, persimons, mangoes, melons, squashes, peppers, carrots, beans and other fruits and vegetables. During their development in the host tissues, species of *Alternaria* are also known to produce a great number of secondary metabolites. Of these**,** only seven major mycotoxins belonging to three different structural classes are known as possible food contaminants with a potential toxicological risk. These include the following mycotoxins: alternariol (AOH), alternariol methyl ether (AME) and altenuene (ALT), which are dibenzopyrone derivates; altertoxins I, II, and III (ALTX I; ALTX II, ALTX III), which are perylene derivates; and tenuazonic acid (TA), a tetramic acid derivative.

Aim:
Te aim of this investigation was the screening of TA, AOH and AME production from Alternaria isolates from several crops grown in Argentina in order to evaluate a potential risk to human and animal health.

Materials and Methods:
Mycotoxin production: Flask were made up containing 12.5 g of autoclaved polished rice and were inoculated with one week cultures of Alternaria spp. isolates. The flasks were incubated in the dark at 25ºC for 21 days.
Mycotoxin extraction and detection:The rice samples were extracted with acetonitrile/4% KCl (9:1, 75 ml) for 30 min followed by the addition of 1N HCl (15 ml). The mixture was filtered, and 45 ml of the filtrate (equal to 7.5 g of wheat sample) was initially clarified with 90 ml of 0.05 M lead acetate and filtered again. The filtrate was divided into two parts. One part (75 ml) was extracted three times with 20 ml of chloroform. The organic phases were combined, evaporated to dryness, and dissolved in 4 ml of methanol for AOH and AME analysis by liquid chromatography (HPLC). Another part (36 ml, equal to 2 g of sample) was adjusted to pH 2 with 6 N HCl, filtered again, and extracted twice for TA with 25 ml of chloroform. TA was then partitioned into 15 ml of 5% sodium bicarbonate, acidified to pH 2 again, and extracted twice with chloroform (15 ml). The chloroform extracts were combined, washed with 13 ml of water, and evaporated to dryness. The residue was made up to 4 ml with methanol and analyzed for TA by HPLC. The HPLC system consisted of a Shimadzu LC-CA liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a Rheodyne sample valve fitted with a 20 □l loop and a Shimadzu SPD-M10Avp UV photodiode array detector. The analytical column was r 4.6 x 250 mm 5 □ C18. The mobile phase was methanol/water (80:20) containing 300 mg l^{-1} $ZnSO_4.H_2O$, for AOH and AME, and methanol/water (85:15) containing 300 mg l^{-1} $ZnSO_4.H_2O$, for TA. A flow rate of 0.4 ml min^{-1} was used. The wavelength for recording chromatograms was 258 nm for AME and AOH, and 280 nm for TA.

Results and Discussion:

196 strains isolated from different substrates were included (30 from tomato, 21 from blueberries, 115 from wheat and 30 from walnuts). A. alternata and A. tenuissima were the predominant species. A. tenuissima showed a higher incidence in tomato (55%) and blue berry (60%) isolates while A. alternata had an incidence of 26% and 24% in the same substrates respectively. There was almost the same proportion of isolates of A. tenuissima (50%) and A. alternata (44%) in wheat. In walnut A. alternata (84%) isolates were prevalent.

	A. tenuissima	A.alternata
Wheat	TA : 1- 14782 mg/kg AME : 8- 2589 mg/kg AOH: 5- 609 mg/kg	TA : 1- 9478 mg/kg AME : 21- 2352 mg/kg AOH : 4- 622
Walnut	TA : 1- 886 mg/kg AME : 18- 1055 mg/kg AOH : 20- 93 mg/kg	TA : 1- 1182 mg/kg AME : 5- 1410 mg/kg AOH : 1- 68 mg/kg
Blueberry	TA : 4- 1883 mg/kg AME : 6- 1178 mg/kg AOH : 4- 11 mg/kg	TA : 93- 989 mg/kg AME : 6- 28 mg/kg AOH : 5- 57 mg/kg
Tomato	TA: 1- 4021 mg/kg AME : 10- 600 mg/kg AOH : 10- 450 mg/kg	TA : 100- 2500 mg/kg AME : 2- 750 mg/kg AOH : 1- 350 mg/kg

Table: Mycotoxin production by Alternaria isolates from different substrates

TA was the toxin produced at the highest concentration in all substrates while AOH and AME yields were smaller. The maximum amount of TA (!4782mg/kg was produced by A. tenuissima in wheat, which also produced the maximum concentration of AME (2589mg/kg) detected. The maximum amount of AOH (622 mg/kg) was produced by A. alternata isolated from wheat.

Conclusions:

A. tenuissima seem to be highly toxicogenic and more frequently isolate in almost all substrates studied. The less frequently isolated species A. alternata was also toxigenic and produced TA, AOH and AME in amounts comparable to those produced by A. tenuissima. The toxicogenicity of isolates from the different substrates indicates a potential risk for contamination, thus systematic testing of Alternaria mycotoxins in these products is needed to increase their quality and safe

Poster Section III: Mycotoxins in Commodities. Group 6, 7 and 8.

Monday June 28

Group 6 (P-30 to P-35): Each presentation in 10 min.

P-30 MOLDS AND AFLATOXINS IN PEANUT BUTTER – SUGAR CANDIES (MAZAPÁN)

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Background: In spite of the popularity in México, mainly among children, of a peanut butter sugar candy known as mazapán we were not able to found information on its possible contamination with aflatoxins, in an earlier paper, Martínez & García (1987) did not find aflatoxins, however, they suggest that besides the deterioration risk, due to the big amount of *Aspergillus* and *Penicillium* the mold profiles could also represent a risk of mycotoxins contamination.

Aims: To have the mold profiles of different commercial brands of mazapán. To identify the isolated molds to the species level. To determine aflatoxins presence.

Materials and Methods: Three samples of each of 11 different mazapán commercial brands obtained from different small corner stores from southern Mexico City were analyzed. To determine the total amount of molds, the Warcup (1950) technique was followed the culture medium used was PDA, added with tergitol and aureomycin. The isolated genera were identified to species using the keys, culture media and growing schemes suggested by Raper & Fennel (1965) for *Aspergillus*, Pitt (1979) for *Penicillium*, Booth (1971) for *Fusarium*. *Absidia* and *Rhizopus* were confirmed as genera following Barnett & Hunter (1972).
Aflatoxins were determined following AOAC International (2005) Method 970.45 for aflatoxins in peanuts and peanut products using 5 duplicates of each sample.

Results and discussion: In none of the analyzed samples aflatoxins were detected.
Ninety isolates were obtained of the 33 analyzed samples. Of the total number of samples, 58% were contaminated with *Aspergillus*, 41 isolates, 33% with *Penicillium*, 23 isolates, 9% with Fusarium, 3 isolates. The Mucorales were found contaminating 55% of the samples 23 isolates. Most of the Aspergillus and *Penicillium* species identified have been reported as mycotoxins producers or bio deterioration indicators.

Conclusions: No aflatoxins risk was found, however, only aflatoxins were tested for. Since too many molds were found, other mycotoxins could be present. The bio deterioration risk is still present if the batches of peanut butter are not promptly processed and sold.

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P-31 MOLD PROFILES ON STORED GREEN COFFEE

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Background: Lots of exportation green coffees have been rejected from destination, mainly those from the European countries due to reportedly being contaminated with ochratoxins. Ochratoxin A is a nephrotoxic and carcinogenic mycotoxin produced by species of *Aspergillus* and *Penicillium*.

Aim: To know the mold profiles on green coffee recently harvested, processed and stored. To select the species reported as ochratoxin formers in any produce. To identify the selected species, following traditional methods as well as molecular biology techniques.

Materials and methods: Thirteen green coffee samples from three coffee south eastern producing areas, collected from different storage facilities at four different periods during one year were analyzed in order to know their mold profiles. The mold isolation was done on malt salt agar 2%/6%/2% and PDA Tuite (1969). The identification of the isolates was done following the traditional methods using the specific culture media and growing regime for each genus and species Domsch *et al* (1993), Klich (2002) Pitt (1979). The species of *Aspergillus* and *Penicillium* reported as ochratoxin producers were manipulated following the molecular biology techniques to confirm their identity.

Results and discussion: Twenty seven species, distributed mainly among *Aspergillus* 11362 isolates), *Penicillium* (809 isolates), and *Fusarium* (121 isolates) were identified; *Rhizopus* and *Chaetomium* were also found in lower amounts. *Cladosporium* and *Pestalotia* were isolated only occasionally. Among the species, *Aspergillus niger* (7116 isolates) was the most abundant of the all isolates *A. ochraceus* (342 isolates) and *A. melleus* (290 isolates), even not near so abundant, were also isolated frequently, *Penicillium viridicatum* (63 isolates) and *A. carbonarius* (30 isolates) not so often isolated are consider in this work because they have also been reported as ochratoxin producers The similarity percentage with the sequences deposited in the Gen Bank were 100% for *Aspergillus melleus, A. carbonarius A. ochraceus* and *Penicillium viridicatum*, and 99% for *A. niger*.

Conclusions: *Aspergillus niger*, the most abundantly isolated species is also the most frequently reported ochratoxin producing mold. This suggests that some green coffee lots even with not detected ochratoxin levels at the production area could end up contaminated if the conditions during transportation lead to such a contamination.

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P-32 ADVANCES IN DETECTING AFLATOXIGENIC *Aspergillus flavus* FROM SOIL SAMPLES OF CORNFIELDS IN JALISCO, MEXICO

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Introduction

Biological control of aflatoxin by applying atoxigenic strains of *Aspergillus flavus* and *A. parasiticus* are based on displacement principles of infection sources. Total fungal population producing aflatoxins in crop fields can be reduced, as has been shown to occur in cotton (*Gossypium hirsutum* L.), pistachio (*Pistacia vera* L.), maize (*Zea mays* L.) and peanut (*Arachis hypogaea* L.) with notable results (Leger *et al*., 2000). In Jalisco state, considering the results obtained in our preliminary study on the distribution of *Aspergillus flavus* and *A. parasiticus* in cornfields, the use of biological control and resistant cultivars against aflatoxin contamination can be contemplated.

The objective of the present study was to identify atoxigenic strains for use in biological control of *Aspergillus flavus* on maize.

Materials and Methods

Soil core samples were taken from fifteen sites in Jalisco. More than 300 isolates were obtained from each site and were probed on AFPA medium. *Aspergillus flavus* or *A. parasiticus* isolates tested positive in AFPA were then consigned to *nit* mutant selection process. The first step for obtaining *Aspergillas* fungi for biological control is the selection of *nit* mutants on selective media. To this end, all isolates were cultured in CYA medium at 35 °C for seven days, whereby five pieces were aseptically transferred to vials containing sterile distilled water and were then refrigerated at 4 ºC. Subsequently, 20 µL of the spore suspension were placed in the SEL medium at three positions in a Petri dish and were incubated at 30 °C until mycelial growth was observed in the medium. Once the mutants were grown, two pieces of each mutant were cut and transferred to MIT solid medium for incubation at 30 °C for three days and were stored in vials with sterile distilled water for further analysis of vegetative compatibility groups (VCG's).

Results and Discussion

We selected a total of 29 *nit* mutants some of which can be considered for use in biological control in cornfields in Jalisco. The origin of mutant isolates indicates that 48% of them were isolated from soil samples collected in Atotonilco area, 14% from Juanacatlan and 10% from Jamay (Figure 1).

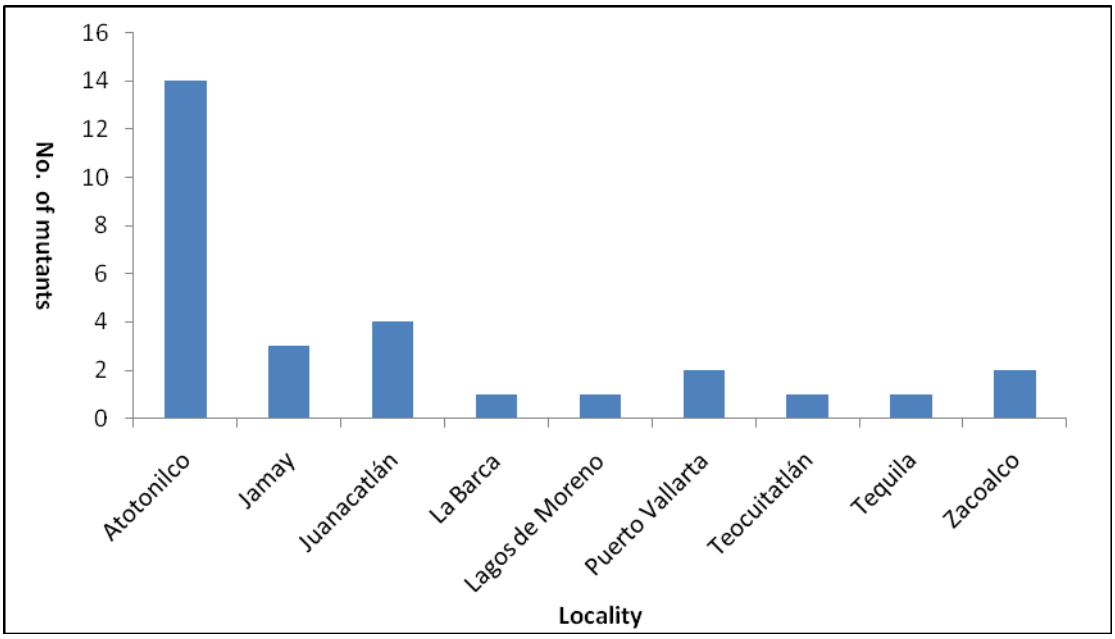

Figure 1. Distribution of *nit* mutants from soil cores in Jalisco, Mex.

Results obtained from samples in Atotonilco indicated a high number of toxigenic strains as being potentially atoxigenic strains. Similar research indicates that 4.6% of total isolates from a soil sample were atoxigenic (Das *et al*., 2008). In our case, genetic testing could identify more than 5 percent of atoxigenic strains, which is optimal for initiating biological control programs in conjunction with maize breeding programs for resistance to aflatoxin.

Twenty-nine *nit* mutants were obtained from fifteen soil samples of cornfields in Jalisco. Atotonilco, Juanacatlan, Jamay and Puerto Vallarta were municipalities where higher incidences of *nit* mutants were observed. Further study is needed to distinguish specific *Aspergillus* isolates in vegetative compatibility groups or VCG's.

Acknowledgement

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P-33 AFLATOXINS (B_1, B_2, G_1 AND G_2) IN MEXICAN AND SPANISH RICE AND THEIR APPLICATION IN THE MUTAGENICITY AMES TEST WITH *SALMONELLA TYPHIMURIUM* TA-98.

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Background: Rice is the cereal most commonly used in human diets; global consumption is ≈540 MTm, with an average of 58 kg/person/year. 90% of the world's rice is consumed in producing countries themselves. China, India and Indonesia are the largest consumers. Spain is second in production with 750,000 tons in the European Union, and in Mexico, rice production is 295 000 tons. Aflatoxins (AF) are secondary metabolites produced by strains of the fungi *Aspergillus flavus, A. parasiticus* and *A. nomius*, and they turn out to be a natural and dangerous contaminant of several cereal grains such as maize and rice.

Aim: Identifying and quantifying aflatoxins (B_1, B_2, G_1, y G_2) of 67 different samples of rice from Spain and Mexico.

Materials and methods: A total of 67 samples of rice were collected in local markets and supermarkets from Spain and Mexico. The rice samples were labeled and stored in plastic bags. To concentrate and purify AF, 25 g of rice were blended for 2 min with 2 g of NaCl and 120 mL of MeOH/H2O (80:20 v/v). Each mixture was filtrated, and 8 mL were taken and diluted in 25 mL of phosphate buffer solution (PBS), and poured into a Easi-Extract immunoaffinity column for total AF. Each sample was eluated with 1.8 mL of MeOH and it was dried at 40°C. Afterwards, each dried eluate was derivatized. The identification and quantification of AF was made by HPLC. The Ames test was applied to rice samples with positive result to AF presence. Samples of 20 and 50 g of rice were blended for 2 min with 5 mL of MeOH. Each mixture was filtrated and resuspended in 5 mL of MeOH, and evaporated again. Each sample was analyzed by thin-layer chromatography, having as mobile phase a mixture of toluene/acetone/ethyl acetate (60:20:40 v/v/v). In a dark chamber with a UV lamp long wave; the surface of each plate was scraped and resuspended in 100 μL of dimethyl sulfoxide (DMSO). The strain of *Salmonella typhymurium* TA98 (race) [hisD3052, gal, (chl, uvrB, bio) rfa, pKM101 (Muca / B Amp)] was thawed. The culture media were prepared in sterile and disposable petri dishes. A minimal Vogel-Bonner medium was used, added with an excess of L-histidine and ampicillin trihydrated. An aliquot of 0.1 mL of the strain of *Salmonella* TA-98 with 0.5 mL of S9 fraction and NADP were placed in petri dishes; 2.5 mL of culture medium containing L-histidine-biotin (0.5 mM) were added at temperature of 45°C. The Petri dishes were left to solidify, inverted and incubated at 37°C for 48 hours, and finally the number of mutant colonies was counted.

Results and Discussion: The total average amount of AF of the samples had levels of 13-53 ng g^{-1} of AFB_1, 2 ng g^{-1} of AFB_2, 5-78 ng g^{-1} of AFG_1 and 7-23 ng g^{-1} of AFG_2. Samples of rice from Spain were more contaminated than samples from Mexico. In Ames test had all the controls and tests, the AFB1 pure standard was very mutagenic but the AF extracted from rice samples did not behaved mutagenic, but they were cytotoxic to the bacteria.

Conclusion: The presence of the four AF in rice was confirmed, identified and quantified, and there is a need to evaluate a greater number of samples, distinguishing the origin of the rice grain.

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P-34 AFLATOXINS IN HOT PEPPER FROM THE CITIES OF PUEBLA AND MEXICO.

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Background: Aflatoxins (AF) are secondary metabolites produced by strains of the fungi *Aspergillus flavus, A. parasiticus* and *A. nomius*, and they turn out to be a natural and dangerous contaminant of several cereal grains such as maize and sorghum. They are a great risk for both human and animal health. There are four important AF: AFB_1, AFB_2, AFG_1 and AFG_2, from which AFB_1 is the most toxic, and it is considered a potent mutagen and carcinogen. Hot peppers are generally dried in the sun and open air that is why, hot peppers are more likely to be contaminated with AF because of their insufficient drying process.

Aim: To identify and quantify aflatoxins of dry hot peppers from Mexico and Puebla Cities, national and imported.

Materials and Methods:

The chemical analysis of Mexico City considered 99 samples of hot peppers of the trademark "Don Zabor" were sampled in a Comercializadora of chili, warehouse L-50 of the Wholesale Market of Iztapalapa, Distrito Federal (D.F.), which is the most important, because it has hot pepper from Mexico and countries as Chile, Peru and China. One type of chili was obtained in the market of San Angel, Alvaro Obregón, D.F. To purify the AF (AFB_1, AFB_2, AFG_1 and AFG_2) from the samples of hot peppers, the method of AF extraction of Joint FAO/WHO (1986) was used; and concentration of AF was made with Easi-Extract immunoaffinity columns for total AF. The identification and quantification of AF was made by HPLC. Four calibration curves were done, one of each AF, with nine different dilutions (2, 5, 10, 15, 20, 30, 50, 100 y 200 ng g^{-1}) with pure standards, as of a solution of 1 μg mL^{-1}.

The study of hotpeppers in the State of Puebla, Mexico, analyzed 76 samples of 4 types of chilli (Árbol´, costeño, serrano and chipotle) of 9 different markets of Puebla (Parral, Carranza, 5 de mayo, and Libertad, Zapata, Xilotzingo, Defensores, C. Martínez and El Angel) to identify and quantify the 4 aflatoxins (AFB1, AFB2, AFG1, AFG2).

Results and Discussion:

In Mexico City, 94% of the samples had traces of AF of ≈10 μg kg^{-1} and only 6% had a higher contamination. The samples with higher total AF were: N° 15 chilli “Chipotle Meco” (19.311 μg kg-1) from San Angel market, D.F.; N° 53 chilli “Mulato” from El Fresno, Zacatecas (21.027 μg kg^{-1}); N° 70 chilli “Morita” from Chihuahua (10.556 μg kg^{-1}); N° 80 Chili “de árbol” from China (16.099 μg kg^{-1}); N° 89 chili “Cascabel” from the State of Hidalgo (19.907 μg kg^{-1}) and the most contaminated sample, N° 94 chilli “Ancho” from Fresno, Zacatecas (39.841 μg kg^{-1}).

The results of the a total AF amount in the 72 samples of hotpeppers of Puebla were AFB1 (5730.10 ng g^{-1}), AFB2 (418.45 ng g^{-1}) , AFG1 (2999.52 ng g^{-1}) and AFG2 (9223.66 ng g^{-1}). The average values were: AFB_1 (79.60 ng g^{-1}), AFB_2 (5.81 ng g^{-1}), AFG_1 (41.66 ng g^{-1}) and AFG_2 (128.11 ng g^{-1}). The two most contaminated sample in Puebla was number 69 of "De árbol" chilli type from El Angel market with: AFB_1 (5429.60 ng g^{-1}), with AFB_2 (287.60 ng g^{-1}), AFG_1 (1305.72 ng g^{-1}) and AFG_2 (4962.84 ng g^{-1}). The second most contaminated sample was number 68 with AFB_1 (161.37 ng g^{-1}), AFB_2 (69.91 ng g^{-1}), AFG_1 (1493.12 ng g^{-1}) and AFG_2 (3462.10 ng g^{-1}) from C. Martínez market in Puebla and it was "Serrano" hotpepper type.

Conclusion: 94% of hot peppers had traces of AF, and only 6 % of samples had a severe contamination. This represents a risk for human health.
The hotpepper samples from the market "Central de Abastos" of Mexico City were cleaner of AF they have only traces, but 2 of the markets in Puebla had the most contamnated samples showing that although the Mexicans eat chilli all the time it means a risk for health.

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P-35 AFLATOXINS IN INDUSTRIALIZED HOT PEPPER CHILLI SAUCES.

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Background: Aflatoxins (AF) are secondary metabolites produced by strains of the fungi *Aspergillus flavus, A. parasiticus* and *A. nomius*, and they turn out to be a natural and dangerous contaminant of several cereal grains such as maize and sorghum. They are a great risk for both human and animal health. There are four important AF: AFB_1, AFB_2, AFG_1 and AFG_2, from which AFB_1 is the most toxic, and it is considered a potent mutagen and carcinogen. Chillis are contaminated with aflatoxins if they are stored in conditions which promote the growth of fungi. The distributor should check the conditions in which the chillis are stored before using them in their products.

Aim: To identify and quantify aflatoxins in industrialized hot sauces of different trademarks that are consumed in Mexico.

Materials and Methods: In order to properly represent the wide range of hot sauces found in the Mexican market, 52 different hot sauces were sampled. To concentrate and purify AF, samples of 40 mL were individually taken from hot sauces and blendered, with 5g NaCl and 120 mL of the acetonitrile/water solution, for about 1 minute. Each mixture was filtrated, and 4 mL were diluted in 36 mL of phosphate buffer solution (PBS). Later, each homogeneized and filtrated sample was poured into a Easi-Extract immunoaffinity columns for totals AF. Afterwards, it was eluated with 2 mL of MeOH and the eluate was dried at 40°C. Every sauce eluate was derivatized.The identification and quantification of AF was made by HPLC using as mobile phase. Four calibration curves were done, one of each AF, with nine different dilutions (2, 5, 10, 15, 20, 30, 50, 100 y 200 ng g^{-1}) with pure Sigma standards, as of a solution of 1 µg mL^{-1}.

Results and Discussion: Only 7 salsas had more than 10 ng g^{-1} of AF, they were Bufalo Tampico (16.14 ng g^{-1}), La Paisana Salsa Picante (36.03 ng g^{-1}), Crystal Extra Hot (43.27 ng g^{-1}), 21 Premium Salsa Picante 100% de Chipotle (14.77 ng g^{-1}), Tostitos Salsabritas Chamoy (115.6 ng g^{-1}), Tostitos Salsabritas Picante (17.47 ng g^{-1}) and Lol-Tun Salsa de Chile Habanero (20.83 ng g^{-1}). Also, two salsas had over 9 ng g^{-1} of AF, but less than 10 ng g^{-1}. Therefore, “La Costeña Salsa Picante para Botana” (9.14 ng g^{-1}) and “Dulce Ardor” made of Meco chillis (9.18 ng g^{-1}) should be taken with caution. Though these salsas technically can not cause a mutation in cells, if taken with another product that contains aflatoxins, it would increase the health risk and can cause mutations. Out of 52 hot sauces, only two were clean: “Bufalo tipo norteña casera” and “La Costeña salsa chamoy”.

Conclusions: It is very important to warn the public that some of most commonly used salsas (Buffalo, Tabasco, Valentina, etc.) are not very safe to eat. However, the best way to eliminate aflatoxins is to make sure the chillis do not suffer from dry periods during the growth in the field and have good methods of storage, with low humidities and cold temperatures.

Poster Section III: Mycotoxins in Commodities

Monday June 28

Group 7 (P-36 to P-40): Each presentation in 10 min.

P-36 AFLATOXIN CONCENTRATIONS IN MAIZE 'TORTILLAS', FROM MEXICO CITY.

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Background: Aflatoxins (AF) are secondary metabolites produced by several strains of the fungi **Aspergillus flavus, A. parasiticus**, **A. nomius**, *A. ochraceoroseus, A. rambellii, A. bombycis and Aspergillus pseudotamarii.* These fungi and their mycotoxins contribute to diminish food quality and represent a risk for human and animal health. The major AF of concern that are found in maize grains are designated B_1, B_2, G_1, and G_2. Maize and maize tortillas are the staple food of the Mexicans, and represent the main source of their energy for rural and urban areas. Maize 'tortilla' is subdued to 'nixtamalización' process, which consists of an alkaline cooking with calcium hydroxide added. This lime treatment causes anatomic, physical, and chemical changes in maize kernels, and there is the idea that it protects against AF. Several studies have been done to evaluate the degree of AF retention of grain during its industrialization. Mexico City receives all kinds of maize from the rest of the country, and it summarizes all the market, because it has the highest population density of 5871 hab/km^2 and represents 18.3% of the total Mexican population. Therefore, the study tortillas becomes meaningful in this city.

Aim: The purpose of the present study is to identify and quantify AF in Mexican tortillas.

Materials and Methods: A systematic random sampling of 392 'tortillas' samples was done in two yearly seasons (dry and wet) during 2006 and 2007. Mexico City is divided in 16 boroughs and within each of them 6 systematic random points were sampled, choosing 3 supermarkets and 3 'tortilla' shops, giving a total of 98 sampling points in all Mexico City, each one sampled 4 times. All tortilla samples, of 2 kg each one, were dried at 70 °C, for 48 hours, and ground in blenders. For recovery percentage studies, three clean subsamples were spiked with a mixture of the four AF (AFB_1, AFB_2, AFG_1 and AFG_2) in concentrations of 10, 20 and 30 ng g^{-1}. The AF extractions were done using immunoaffinity columns method. The AF were derivatized with TFA and analized by HPLC-FD. The results were analized by Wilcoxon non parametric statistical analysis.

Results and Discussion: From all the 392 samples, equivalent to 784 kg of tortillas, an average of 17.5% had one or several AF, and 82.5% of the 'tortilla' samples were free of AF. From the 67 contaminated samples, 13.43% exceeded the maximum tolerable limit in the Mexican legislation for maize 'tortilla' (12 µg kg^{-1}), and 86.57% were on rule. The presence of AFB_1 and AFB_2 is due to maize contamination by *Aspergillus flavus*, nevertheless the

detection of AFG_1 and AFG_2 means that *Aspergillus parasiticus* was present. *Aspergillus flavus* and *A. parasiticus* are the commonly recognized fungi capable of producing AF in maize, and not all the strains can do so (Diener & Davis, 1987). On the other hand, AFB_1 is the most mutagenic, teratogenic and carcinogenic AF (Richard, 2007), and was the one more frequently found in 'tortillas' (64.6%) in a range from 0.3 to 140.33 $\mu g\ kg^{-1}$. In accordance with the results of the present study, Torreblanca *et al*. (1986), found high levels (72 %) of AFB_1 in maize and 'tortillas' in Mexico, in a range from 5 to 500 $\mu g\ kg^{-1}$, in the 50 g samples from 'tortilla' shops from Coyoacan and Iztapalapa boroughs. Ten samples in the present study had high concentrations of AFG_1 (0.3 and 385.21 $\mu g\ kg^{-1}$), although AFB_1 was more frequent, both AF represent a high risk to human health.

Conclusions: Aflatoxins are present in maize tortillas of Mexico City. Alkaline treatment reduces AF levels in tortillas, but do not eliminate them completely. From 392 tortilla samples from tortilla shops and supermarkets of Mexico City, 17% were contaminated with aflatoxins; from them, 13% surpassed the maximum permissible limit established by Mexican law NOM-187-SSA1-2001 that permits $12\mu g\ g^{-1}$ in tortillas and maize by products. Although, 87% of the contaminated samples fulfilled the national normativity, there is a risk of developing cancer, caused by the accumulation of aflatoxins in DNA. AFB_1 was present in 64.6% of contaminated samples in a range from 0.3 to 140.3 $\mu g\ kg^{-1}$, and AFG_1 appeared in 14.92% of contaminated samples in a concentration range from 0.3 to 385.2 $\mu g\ kg^{-1}$.

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P-37 IDENTIFICATION AND QUANTIFICATION OF AFLATOXIN AND ITS HYDROXILATED METABOLITES IN GERMAN WINE. STUDY ON THE DETECTION OF A CARCINOGEN.

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Background: Wine is one of the most consumed alcoholic drinks in Europe (approximately 23 L per person per year). Aflatoxins AF are toxins produced by fungi of the genus *Aspergillus*: *A.flavus* and *A.parasiticus*. The widespread incidence and their metabolism helps them to grow in the cuticle of wine grapes by using fungal enzymes. Therefore aflatoxins liberated by the fungi flora of the grape could be founded in commercial wine, depending of the process of grapes selection and the quality of the production process which differs from company to company.

Aim: To quantify and identify aflatoxins inwhite and red wines of Germany with different methods.

Materials and Methods: The first part of this study was done in Germany where thin layer chromatography (TLC) was made before as a preliminary study, and to get rid of the pigments. Three samples (9, 13, and 14) were extracted chemically by vacuum-distillation and chloroform phases. The wine sample extracts in the TLC analysis, were compared with extracts from *Aspergillus*' cultures whose strains were collected in the vine cultural region of Meissen, Germany and identified through optical microscopy. These fungal cultures were extracted and helped as aflatoxin producer strain and some aflatoxin standard was produced.

The second part of the study was done in the Institute of Biology, UNAM in Mexico City with 16 samples of red and white German wines. The samples were from Germany and were bought depending on the kind of wine, trademark, region, and colour. The red wine samples were previously cleaned from pigments with the method of Stoloff (1971). The concentrates were saved in little bottles for their transportation to Mexico. Three of the samples: 9; 13; 14 were reduced from 300 mL. Six hundred mL of wine were evaporated, at 110 ºC, to a concentrated wine syrup free of water and alcohol. The quantification was done by Liquid chromatography (HPLC) analysis with a C18 column and a mobile phase of a mixture of toluene/acetone/ethyl acetate (60:20:40 v/v/v) and a 20 µL loop. Four calibration curves were done with pure Sigma standards, one of each AF (AFB1, AFB2, AFG1 and AFG2), with nine different dilutions (2, 5, 10, 15, 20, 30, 50, 100 y 200 ng g^{-1}) made up from a stock solution of 1 µg mL^{-1}.

The samples were dissolved in methanol to reduce de density. After evaporation at room temperature they were purified and concentrated by Easi-Extract total Aflatoxin immunoafinity columns.The un-pigmented extract was analysed by high performance liquid chromatography (HPLC) in a Agilent 1200 pump with autosampler and fluorescence detector. The results of the two methods were systematized.

Results: The wines showed aflatoxin in maximal concentrations of 0.1 μg L^{-1} which don't represent any danger for health. Aflatoxins AFM_1 and AFM_2 were the ones with the higher concentration, then AFL, AFB_1, AFB_2, AFG_1 and AFG_2 were founded in traces of a maximum of 0.004 μg L^{-1}. The TLC method presented green fluorescence possibly AFG which was founded in those samples (numbers 9, 12, 13) with HPLC. It was not possible to make an accurate quantification with TLC. From the microbiological analysis came out that other fungi like *Trichoderma* , *Penicillium* and *Alternaria* coexist with *Aspergillus* in the vine plant.

Conclusions: The best method was to eliminate the pigments by Stoloff method, a clearance and concentration by immunaffinity columns and quantification by HPLC. There was a biotranformation of AFB_1 to AFM_1 and AFM_2 during the production of wine. Therefore wines have principally hydroxilated metabolites of aflatoxins such as AFM_1, AFM_2.

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P-38 EFECTO DEL PROCESAMIENTO DEL TRIGO SOBRE LA MICOBIOTA Y NIVELES DE DON EN LA ELABORACIÓN DE PASTAS ALIMENTICIAS

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Deoxynivalenol (DON) también denominada vomitoxina es un metabolito tóxico del grupo tricotecenos producida por *Fusariun graminearum* principalmente en maíz, trigo, avena y cebada. Los granos contaminados con DON causan emesis, depresión del crecimiento de los animales y es muy estable al procesamiento.

Objetivo: Evaluar la incidencia de DON y el efecto del procesamiento sobre DON en muestras de trigo destinadas a la elaboración de pastas alimenticias
Las muestras fueron recolectadas en una planta productora de pastas alimenticias ubicada en la zona metropolitana de Caracas: Trigo en grano (7); harina de trigo (6), semolina (7) y pastas largas (6).

Metodología: Los niveles de DON se determinaron utilizando la metodología descrita por Trucksess y col modificada por Fernández y col (1994) usando TLC. Brevemente, la muestra es homogenizada con una mezcla de acetonirilo-agua (84+16) y luego de una filtración pasada a través de una columna que contiene carbón-alúmina y celite en la proporción (0.7g-0.5g-0.3g). El DON fue eluído con acetonitrilo y alícuotas conocidas fueron colocadas en placas de TLC y desarrolladas con cloroformo-acetona-2-propanol (8+1+1). Una vez desarrollada fue rociada con $AlCl_3$ y observada bajo luz UV (365nm).
Adicionalmente se evaluó la micoflora utilizando agar DRBC y PDA determinándose la Aw mediante el equipo Decagon CX-2.

Resultados: La incidencia de mohos varió entre 10^2 y 10^3 ufc/g en todas las muestras estudiadas. Los niveles promedio de DON en trigo fue de 0.7 ppm, en harina de trigo 0.43 ppm, semolina 0.21 ppm, pasta cruda 0,21 ppm y pasta cocida 0.2 ppm. La actividad de agua varió entre 0.45 y 0.61 mientras que los niveles de humedad entre 5.49 y 10,09%. Con respecto a las especies fúngicas colonizantes del grano de trigo tenemos: *Penicillium echinulatum* (20,17%), *Acremonium charticola* (17,65%), *Rhyzopus oryzae* (7,56%), *Cladosporium cladosporioides* (5,88%), *Ulocladium chartarum* (5,04%); también se encontraron especies de *Aspergillus,* tales como *A. fumigatus* (0,84%), *A. parasiticus* (0,84 %), *A.penicillioide* (0,84 %) , *A.tamarii* (0,84 %), *A.ustus* (0,84 %), *A. versicolor* (0,84 %). Entre las especies de *Fusarium* sp. se hallaron *Fusarium equiseti (*4,2%) y *Fusarium sporotrichioides* (2,52%).

P-39 DETERMINACIÓN DE FUMONISINAS DURANTE LA ELABORACIÓN DE HARINA DE MAIZ MEDIANTE LAS TÉCNICAS DE HPLC Y ELISA

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El maíz es uno de nuestros principales rubros agrícolas y comúnmente es atacado por algunas especies de los géneros Aspergillus y Fusarium productoras de aflatoxinas y fumonisinas las cuales pueden causar carcinogénesis, teratogenesis y mutagenesis.

Objetivo: Evaluar la incidencia de fumonisinas mediante HPLC y Elisa en 76 muestras a nivel de una planta procesadora de maíz para consumo humano: maíz a nivel de recepción (21), maíz acondicionado y almacenado en silos (22), concha y germen (10), harina precocida de segunda (11), harina precocida de primera (12).

Metodología: Técnica de ELISA y HPLC.

Resultados: Los resultados mediante la técnica de Elisa revelaron que el 38% de las muestras de maíz a nivel de recepción excedían los niveles de 4 ug/g) mientras que las muestras a nivel de silos, el 27,2% de las muestras excedían dicho límite. Los niveles de fumonisinas en las muestras de concha y germen así como en harina precocida el valor promedio fue de 6,99 ug/Kg. El porcentaje de recuperación usando la técnica de Elisa fue de 107,5%. El análisis mediante HPLC reveló que los niveles de fumonisinas en maíz a nivel de recepción fue de 2,84 ug/kg y para maíz almacenado de 2,24 ug/kg. El valor promedio para las muestras de concha y germen fue de 5,95 ug/Kg mientras que para harina de maíz el nivel promedio de fumonisina fue de 0.05 ug/Kg. La recuperación promedio usando HPLC fue de 84,3%. No se encontraron diferencias significativas entre ambos métodos en estudio obteniéndose una correlación de 0.9645 entre ambos métodos.

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P-40 MICROORGANISMS AND MYCOTOXINS ASSOCIATED WITH FOOD COMMODITIES IN CAMEROON

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Objective: Spoiled maize grains and numerous types of snacks that are consumed in Cameroon are infected by several mycotoxin producing fungi and some bacteria. The extent of contamination of these food commodities by secondary metabolites of fungal origin has not been well studied. This study aimed to identify the microorganisms that infect maize grains and some major snacks sold at road side ,markets and consumed in Cameroon and to sensitize the population on the health risks that are associated with consumption of contaminated commodities.

Methodology and results: Maize and snack samples were collected from various locations in Cameroon. Contaminating microorganisms were isolated and identified using conventional techniques. Staphylococcus and Salmonella species were the most frequently isolated bacteria found on snacks while *Fusarium* and *Aspergillus* species were isolated in highest frequency ranging from 20 to 100 % presence in the samples analyzed. Chemical analyses revealed the presence of fumonisins (50-26000 ng g-1), Deoxynivalenol (DON) (100-1300 ng g-1) and zearalenone (50-180 ng g-1) in the sampled maize.

Conclusion and application of findings: Contamination of agricultural products by microbial toxins is an important but often underestimated risk to public health and can have long-term health implications. Appropriate sanitary measures need to be taken to ensure that conditions for microbial contamination and toxin production are reduced or eliminated during the handling, transportation, packaging and storage and consumption of all agricultural products.

Poster Section III: Mycotoxins in Commodities.

Monday June 28

Group 8 (P-41 to P-45): Each presentation in 10 min.

P-41 MYCOTOXINS EVALUATION ON STORED CORN AND THEIR RELATION TO GRAIN CLASSIFICATION AND STORAGE ENVIRONMENT

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Background: Keeping quality of high amounts of grains in Cooperative Storage facilities is a challenge for all of those involved in grain preservation. To control and maintain the conditions for safe grain storage (fungi growth reduction wise) it is necessary to apply a series of procedures related to the environment and also to the grain stored. It is necessary to carry out specific studies on grain chracteristics standard quality including mycotoxin data for chekcking the efficiency of control conditions applied.

Aims: To correlate the toxin levels (fumonisins - FBs: FB_1 and FB2; aflatoxins – AFLs: AFB_1, AFB_2, AFG1 and AFG_2 and ochratoxin A – OTA) detected in bulky corn Storage Unities (250 tons) to the grain characteristics and storage environment in order correct and/or improve the control conditions currently applied (only for insects).

Material and Methods: Corn samples (29) were collected from different storage facilities located in the grain Storage Unities (GSU) of the States of Parana and Mato Grosso do Sul during 2008 and 2009. Corn characteristics: moisture content (mc), water activity (a_w), impurities, percentage of rotten, fermented and broken grains were evaluated weekly (AOAC, 2005.a). Toxins to be analyzed: FBs were determined by high performance liquid chromatography with fluorescence detection (AOAC, 2005.b) and the other toxins by thin layer chromatography and ultraviolet detection (Soares and Rodrigues-Amaya, 1989).

Results and Discussion:

The percentage of broken grain varied from 0.8 to 3.05% and impurities. varied quite widely from 0.5 to 11 % which correspond to insect fragments and other strange materials. Important to emphasize that those high percentages of impurities in silos can lead to difficulties on the system of grain aeration operation (air blockage). Air cannot pass through the grains (suroudend by dust, debris, etc) - thus there is no reduction of temperature and moist removing. That allows hot spots (grain cakes -by fungi and insect proliferation together with moist) to be formed. Regarding mc and aw, they were from 11.56 to 13.85% and 0.55 to 0.73, respectively. The temperature readings were from 15 to 25^{o}C. The highest mc was registered in the Sao Jose das Paineiras GSU which reached a percentage close to the maximum allowed (13.8%). That was registered in an old silo with no aeration system instaled and had high AFLs levels and it has been. That will be deactivated. Regarding FBs, FB_1 and

FB_2 were found in most of the samples and their levels varied from low 1.41 to 7.80 mg/kg. Six samples presented AFB_1 at levels varying from 1.4 to 12.0 µg/kg. OTA was not detected in any of the samples surveyed. As expected from the FBs data obtained, the highest contamination was related to the field conditions prior harvesting as the storage conditions of temperature, RH and grain mc were not enough to allow *Fusarium* to grow indoors. On the

other hand, storage condition together with the grain characteristics of impurities, broken and fermentation allowed AFB_1 formation in some facilities of tree GSUs, being necessary optimization and implementation of the aeration systems as well as improviment on grain quality prior storage.

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P-42 CO-OCCURRENCE OF AFLATOXINS, OCHRATOXIN A AND ZEARALENONE IN CAPSICUM POWDER SAMPLES AVAILABLE ON THE SPANISH MARKET.

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Background: Mycotoxins are a group of chemical substances produced by some fungal species that can cause illness or even death. There is a growing concern regarding mycotoxin contamination in foods and feeds because mycotoxins can be found in a wide range of commodities, including cereals, spices, dried fruits, apple products, wine and coffee. Poor hygienic conditions and deficient water activity (a_w) control during processing of peppers can lead to fungal growth, which can result in mycotoxin accumulation in paprika and chilli. Mycotoxins encountered in paprika and chilli includes aflatoxins, ochratoxin A, zearalenone, trichothecenes and sterigmatocystin. The co-occurrence of different mycotoxins in paprika and chilli increases the probability of interactions, such as additive or synergistic effects (Speijers & Speijers, 2004), which may increase the risk to human health. Within the European Union (EU) AFs in *Capsicum* fruits are regulated with a maximum tolerable limit of 10 µg/kg for total AFs (B_1+B_2+G_1+G_2) and 5 µg/kg for aflatoxin B_1 (Commission Regulation EC No.1881/2006). Legislation and levels for OTA in spices are currently being considered (Commission Regulation No.1881/2006).

Aims: The aim of this study was to determine the co-occurrence of aflatoxins (AFs), ochratoxin A (OTA) and zearalenone (ZEA) in paprika and chilli samples purchased in Spain, using high performance liquid chromatography (HPLC) with fluorescence detection.

Material and Methods: A total of 99 samples (64 of paprika and 35 of chilli) were collected during June to August 2008 from different retail shops in Catalonia (Spain). In all samples AFs, OTA and ZEA contamination was analysed. Mycotoxins concentrations were quantified using immunoaffinity columns for clean-up and HPLC with fluorescence detection for mycotoxins determination.

Results and Discussion: The occurrence of mycotoxins in paprika samples was 59% for AFs, 98% for OTA and 39% for ZEA, whereas in chilli samples the contamination was 40%

for AFs, 100% for OTA and 46% for ZEA. None of the samples contained levels of AFs higher than the legally allowable limits. Regarding the co-occurrence of mycotoxins, 75% of paprika samples and 65% of chilli samples contained more than one mycotoxin. Chilli samples generally contained lower concentrations of AFB_1, AFB_2, total AFs and OTA than paprika samples.

Conclusion: This study shows that *Capsicum* products could be a source of mycotoxin intake for Spanish consumers and clearly indicates the urgent need for additional regulation with regard to OTA contamination due to the high contamination values found. On the other hand, currently there is no legislation regarding the joint contamination of food items with more than one mycotoxin. This study confirmed a high rate of contamination with more than one mycotoxin. The joint contamination, especially when present at high concentrations, could pose an additional threat to human health.

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P-43 OCHRATOXIN A IN ROASTED COFFEE IN CATALONIA, SPAIN.

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Background: Ochratoxin A (OTA) is a toxic secondary metabolite produced by certain species of the *Penicillium* and *Aspergillus* genera, with the latter being the most frequent producer in coffee. This mycotoxin has been classified as possible human carcinogen (group 2B) by the International Agency of Cancer Research (IARC, 1993). It has also been considered in the etiology of the Balkan Endemic Nephropathy (Petkova-Bocharova and Castegnaro, 1991) and has been related to the outbreak of urinary tract tumours (Smith et al., 1994). Several kinds of coffee are available in the Spanish market, mainly roasted and instant, and the decaffeinated variant within each kind. Two types of roasted coffee can be found: the locally called "naturally roasted" coffee (roasted coffee) and the "torrefacto roasted" (sugar roasted coffee), i.e., coffee that has been roasted in the presence of up to 15% of sugar. Mixtures of both in different proportions are commercialized as final product. The European Union has set the OTA maximum level for roasted coffee at 5 ng/g (European Commission, 2006).

Aim: To assess OTA contamination of some brands and kinds of coffee available in Spain.

Materials and Methods:

Sampling: 45 samples of four nationally available brands of four types per brand (100% naturally roasted, mixture 50% naturally roasted and 50% torrefacto, decaffeinated 100% naturally roasted, and decaffeinated mixture 50% naturally roasted and 50% torrefacto) were analysed. Three samples per brand and per type were analysed, except for the decaffeinated mixture 50% naturally roasted and 50% torrefacto of one brand, of which there was no production and therefore no availability in markets. Samples were purchased in hypermarkets and supermarkets of twelve cities of the province of Catalonia, Spain, representative of the 72 % of the region's population.

OTA chemical analysis*:* OTA was extracted with a sodium hydrogen carbonate solution, filtered and mixed with a phosphate buffered saline solution. Afterwards, the mixture was cleaned-up by means of an immunoaffinity column (Ochraprep, R-Biopharm, Rhône LTD). Detection and quantification of OTA was performed by high performance liquid chromatography (HPLC). Limit of detection was 1.16 ng/mL and mean recovery rate was 85%. Positive samples were confirmed by derivatization of the toxin to its methyl ester (Trucksess, 2005).

Results and Discussion: 43 out of 45 samples (95.5%) presented OTA concentrations above the limit of detection of the method, and positive samples ranged from 1.30 to 5.24 ng/g. Mean OTA concentration of positive samples was 2.07$\pm$0.61 ng/g. No significant

differences were found between brands neither between coffee types. The maximum level found was similar to that found in another Spanish study (5.6 ng/g, Burdaspal and Legarda, 1998). Except this maximum value, all values found were below the limit established by the European Commission.

For a 70 kg weight adult, assuming the consumption of two coffee cups per day (5 g of roasted coffee per cup), an estimation of the daily intake by OTA would be 0.3 ng OTA/kg body weight/day. This data is below the Provisional Tolerable Daily Intake (PTDI) of 14 ng OTA/kg body weight/day set by the European Food Safety Authority (EFSA) Scientific Panel on Contaminants in the Food Chain (EFSA, 2006). Even for high consumers, daily intake of the toxin would be lower.

Conclusion: Incidence of OTA in coffee samples shows that, even though the levels were below the established limits, exposure to OTA by coffee consumption can be chronic. The incidence of OTA in a range of food products other than coffee should also be taken into account.

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P-44 ASSOCIATION OF CAFFEINE AND OCHRATOXIN A IN GUARANA (*Paullinia cupana*, Mart.)

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Background: The guaraná (*Paullinia cupana* H.B.K.) is an alkaloid source used in the production of beverages or as ingredients of different formulations (Kuskoski, . Beverage industries use guarana as a very important material in the soft drinks processes and to the consumers as an energetic source. The fruit grows especially in many regions in North of Brazil and It is dried and milled to powder, used in beverages production or commercialized toasted or grinded as ingredient to regional cuisine. The process chain has some similarity to the coffee. On the other hand, failures such as an inadequate storage can origin mould contamination and the ochratoxin A (OA) production.

Aim: To study their caffeine levels association to the ochratoxin A (OA) contamination in grinded guarana commercialized in Manaus- Am, Brazil.

Materials and methods: 30 samples of grinded guarana from retail were analyzed concerning caffeine, by spectrophotometry (AOAC, 2005) and OA by SOARES; RODRIGUEZ-AMAYA (1989) method (limit of quantification: 2.0 µg/kg).

Results and Discussion: the mean of caffeine in the studied samples, was 3.0 mg % (1.3-4.0), according to the expected for those samples kind. The results showed no statistics association between OA and caffeine in the guaraná samples analyzed, because no OA was detected in the samples.

Conclusion: Despite of no OA detected in the analyzed samples, further studies are necessary to explain the influence of alkaloids or other guaraná chemical compounds into the fungi metabolism and the OA production.

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P-45 DIETARY INTAKE OF AFLATOXINS BY CATALONIAN (SPAIN) POPULATION.

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Background: Aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2) are mycotoxins that can be produced by three moulds of the *Aspergillus* species (*A. flavus*, *A. parasiticus* and *A. nomius)* which can occur in a wide range of important raw food commodities including cereals, nuts, spices, figs and dried fruits. AFB_1 is the most carcinogenic mycotoxin known, and there is evidence from human studies that aflatoxins (AFs) are major risk factors for hepatocellular carcinoma. They have been classified in group 1 by International Agency for Research on Cancer (IARC, 2002; World Health Organization 1998).

Aims: The objectives of this study were, in one hand, to assess the occurrence of AFs in food marketed in Catalonia (North East part of Spain) and, in the other hand, to assess the exposure of its population to these contaminants through deterministic and probabilistic models.

Materials and Methods: During the months of June and July 2008, a total of 1310 samples of peanuts (n=212) , pistachio (n=169), dried figs (n=49), sweet corn (n=181), breakfast cereals (n=167), corn snacks (n=213), red pepper (n=165) and baby food (n=154) were obtained in six hypermarkets and supermarkets from twelve main cities of Catalonia, Spain, representative of 72 % of the population. From each supermarket or hypermarket, 3 samples (if present) of each product were randomly taken. The level of AFs was determined in a total of 550 composite samples obtained by pooling the 3 items taken from each store, if were available (12 cities x 6 stores / city=72 samples/category). Immunoaffinity columns were used for the clean-up extracts of AFB_1, AFB_2, AGB_1 and AFG_2 from all analytical samples. Quantification of AFs was performed by HPLC with fluorescence detector and photochemical derivatization.

Food dietary intake was assessed through a specific Food Frequency Questionnaire (FFQ) developed for Catalonian population. 79 elders, 720 adults, 236 teenagers, 68 infants and 166 baby parents were interviewed during 2008 (n=1269). Monte Carlo simulation was conducted with Microsoft Office Excel 2003® and Statgraphics Plus 5.1® for 10000 iterations, assuming Lognormal distribution.

Results and Discussion: Occurrence of AFs: low incidence of total AFs was found in all products, except in red pepper, pistachios and peanuts, the products with highest percentage of positive samples (56.9, 20.0 and 11.1 %, respectively). AFs were quantified in a low percentage of samples from the remaining categories, and they were below the limit of quantification in baby food samples. Mean levels were calculated from positives samples; highest values were found in pistachios, peanuts and red pepper. Two peanut composites and one pistachio composite were above EU safety levels of 4 µg/kg for total AFs, with values of 6.88 and 7.66 µg/kg for peanuts and 108.33 µg/kg for pistachio.
Exposure assessment: due to the low incidence obtained, left censored data was treated following GEMs/Food-WHO (1995) advices, with two estimates for non detected (ND)

samples. Given a scenario where ND=0, highest exposure was observed for high consumer teenagers, who showed probable daily intake (PDI) values of 0.39 ng/kg bw/day (39% of TDI). However, under the worst scenario (ND=LOD), babies were the age group the most exposed, with PDI of 1.39 ng/kg bw/day, above TDI of 1 ng/kg bw/day; this could contribute to risk cancer among healthy population (without hepatitis B).
Considering the results obtained through Monte Carlo simulation, the main group exposed to AFs should be expected to be the teenagers, either mean and high consumers, with respective AFs daily intake of 0.281 and 0.951 ng/kg bw/day, respectively, slightly higher than those values obtained with deterministic model.

Conclusions: The occurrence of AFs in food marketed in Catalonia was very low, with the exception of red pepper and nuts where their presence was more important, although only 3 samples showed contamination values above limits established by European Commission (2006). Regarding exposure assessment of the population, considering our results and tolerable levels proposed by authorities, no significant contribution to liver cancer risk should be expected from AF consumption among health consumers in Catalonia.

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Poster Section IV: Mycotoxins in poultry . Groups 9 and 10.

Tuesday June 29

Group 9 (P-46 to P-50): Each presentation in 10 min.

P-46 TWO CHEMICAL METHODS TO EXTRACT, QUANTIFY AND IDENTIFY AFLATOXINS IN BREAST OF LAYING HENS.

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Background: Aflatoxins (AF) are secondary metabolites produced by strains of the fungi *Aspergillus flavus, A. parasiticus* and *A. nomius*, and they turn out to be a natural and dangerous contaminant of several cereal grains such as maize and sorghum. They are a great risk for both human and animal health. There are four important AF: AFB_1, AFB_2, AFG_1 and AFG_2, from which AFB_1 is the most toxic, and it is considered a potent mutagen and carcinogen. AF are the most important mycotoxins in the broiler industry because they cause great economic losses, when poultry are fed with contaminated grains.

Aim: To quantify and identify AF in breast laying hen, and determine the best chemical method for their AF detection.

Materials and Methods: A total of 25 laying hens of 121-week old in a second cycle of egg production, were distributed at random in three experimental groups and placed in individual cages. The control group had 9 hens fed with 2 kg of milled and homogenized clean sorghum, without AFB_1, every day for one week; and of two treated groups of 8 hens each, fed with contaminated sorghum with a low (30 μg kg^{-1}) or a high (500 μg kg^{-1}) AF doses. After a week, the hens were killed by cervical dislocation and each breast was removed. The extraction method for AF was the one proposed by Qian & Yang (1984), and the chemical method with immunoaffinity columns given by Koeltzow & Tanner (1990). The quantification of AF was achieved by high-performance liquid chromatography (HPLC).

Results and Discussion: The breast had a high contamination and a great number of identified AF. The breast had small amounts of AFB_1 (0.7-9.7 μg kg^{-1}) and AFL (4.8-12.6 μg kg^{-1}), and it had high amounts of AFG_1 (78.7-121.0 μg kg^{-1}), AFG_2 (16.2-18.7 μg kg^{-1}), AFM_1 (10.3-591.8 μg kg^{-1}), and AFP_1 (96.2-771.3 μg kg^{-1}). The determination of the appropriate chemical method for the quantification of AF settled down with the percentage of recovery of both chemical methods, which was around 80%, and it is an acceptable recovery. Nevertheless, the method of Qian & Yang (1984) had a lower limit of detection (0.5 ng mL^{-1}), while the limit of detection of the chemical method of Koeltzow & Tanner (1990) was of 1 ng mL^{-1}.

Conclusion: The best chemical method for the quantification of AF in breast of laying hen was the proposed by Qian & Yang (1984), however, it requires a modification to obtain a recovery of 80 to 90%, which consists of the addition of both AF elutions, from the first and second Supelclean LC-18 SPE columns.

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P-47 METHODOLOGY OF EXTRACTION, QUANTIFICATION AND IDENTIFICATION OF AFLATOXINS IN GIZZARD OF LAYING HENS.

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Background: Aflatoxins (AF) are secondary metabolites produced by strains of the fungi *Aspergillus flavus, A. parasiticus* and *A. nomius*, and they turn out to be a natural and dangerous contaminant of several cereal grains such as maize and sorghum. They are a great risk for both human and animal health. There are four important AF: AFB_1, AFB_2, AFG_1 and AFG_2, from which AFB_1 is the most toxic, and it is considered a potent mutagen and carcinogen. AF are the most important mycotoxins in the broiler industry because they cause great economic losses, when poultry are fed with contaminated grains.

Aim: To quantify and identify AF in gizzard laying hen, and determine the best chemical method for their AF detection.

Materials and Methods: A total of 25 laying hens of 121-week old in a second cycle of egg production, were distributed at random in three experimental groups and placed in individual cages. The control group had 9 hens fed with 2 kg of milled and homogenized clean sorghum, without AFB_1, every day for one week; and of two treated groups of 8 hens each, fed with contaminated sorghum with a low (30 μg kg^{-1}) or a high (500 μg kg^{-1}) AF doses. After a week, the hens were killed by cervical dislocation and each gizzard was removed. The extraction method for AF was the one proposed by Qian & Yang (1984), and the chemical method with immunoaffinity columns given by Koeltzow & Tanner (1990). The quantification of AF was achieved by high-performance liquid chromatography (HPLC).

Results and Discussion: The gizzard had a low contamination and a few number of identified AF. The gizzard had AFG_2 (1.4-1.7 μg kg^{-1}), AFM_1 (11.1 μg kg^{-1}) and AFP_1 (9.8 μg kg^{-1}) in low amount; and AFG_1 (482.3-740.8 μg kg^{-1}) and AFL (68.5-456.1 μg kg^{-1}) in higher quantities. The determination of the appropriate chemical method for the quantification of AF settled down with the percentage of recovery of both chemical methods, which was around 80%, and it is an acceptable recovery. Nevertheless, the method of Qian & Yang (1984) had a lower limit of detection (0.5 ng mL^{-1}), while the limit of detection of the chemical method of Koeltzow & Tanner (1990) was of 2 ng mL^{-1}.

Conclusion: The best chemical method for the quantification of AF in gizzard of laying hen was the proposed by Qian & Yang (1984), however, it requires a modification to obtain a recovery of 80 to 90%, which consists of the addition of both AF elutions, from the first and second Supelclean LC-18 SPE columns.

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P-48 RECOVERY OF AFLATOXINS FROM FEED TO LIVER OF LAYING HENS.

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Background: Aflatoxins (AF) are secondary metabolites produced by strains of the fungi *Aspergillus flavus, A. parasiticus* and *A. nomius*, and they turn out to be a natural and dangerous contaminant of several cereal grains such as maize and sorghum. They are a great risk for both human and animal health. There are four important AF: AFB_1, AFB_2, AFG_1 and AFG_2, from which AFB_1 is the most toxic, and it is considered a potent mutagen and carcinogen. AF are the most important mycotoxins in the broiler industry because they cause great economic losses, when poultry are fed with contaminated grains.

Aim: To quantify and identify AF in liver laying hen, and determine the best chemical method for their AF detection.

Materials and Methods: A total of 25 laying hens of 121-week old in a second cycle of egg production, were distributed at random in three experimental groups and placed in individual cages. The control group had 9 hens fed with 2 kg of milled and homogenized clean sorghum, without AFB_1, every day for one week; and of two treated groups of 8 hens each, fed with contaminated sorghum with a low (30 μg kg^{-1}) or a high (500 μg kg^{-1}) AF doses. After a week, the hens were killed by cervical dislocation and each liver was removed. The extraction method for AF was the one proposed by Qian & Yang (1984), and the chemical method with immunoaffinity columns given by Koeltzow & Tanner (1990). The quantification of AF was achieved by high-performance liquid chromatography (HPLC).

Results and Discussion: The liver had a high contamination and the majority of AF were identified. The livers had AFB_1 (0.7-9.7 μg kg^{-1}), AFB_2 (0.06-0.6 μg kg^{-1}), AFG_1 (78.7-121.0 μg kg^{-1}), AFG_2 (16.2-18.7 μg kg^{-1}), AFM_1 (10.3-591.8 μg kg^{-1}), AFP_1 (96.2-771.3 μg kg^{-1}) and AFL (4.8-12.6 μg kg^{-1}), from which AFG_1, AFG_2, AFM_1 and AFP_1 were obtained in greater amount. The determination of the appropriate chemical method for the quantification of AF settled down with the percentage of recovery of both chemical methods, which was around 80%, and it is an acceptable recovery. Nevertheless, the method of Qian & Yang (1984) had a lower limit of detection (0.5 ng mL^{-1}), while the limit of detection of the chemical method of Koeltzow & Tanner (1990) was of 5 ng mL^{-1}.

Conclusion: The best chemical method for the quantification of AF in liver of laying hen was the proposed by Qian & Yang (1984), however, it requires a modification to obtain a recovery of 80 to 90%, which consists of the addition of both AF elutions, from the first and second Supelclean LC-18 SPE columns.

References:

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P-49 AFLATOXIN AND CYCLOPIAZONIC ACID PRODUCTION BY *ASPERGILLUS* SECTION FLAVI ASSOCIATED TO POULTRY FEED

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Background: Cyclopiazonic acid (CPA) is an indole tetramic acid produced by many *Penicillium* and *Aspergillus* species isolated from food and feedstuffs. It is well known that some isolates of *Aspergillus flavus* are able to produce CPA in addition to aflatoxins. Cyclopiazonic acid is toxic to poultry and may have contributed to the "Turkey X disease" in England in 1960. Some symptoms similar to those exerted by CPA have been observed in animals from several farms in Argentina and could be attributed to the presence of CPA in poultry feeds. This toxin may be co-occurred with aflatoxins since *Aspergillus flavus* strains capable of producing both mycotoxins have been found in agricultural commodities used as raw materials for poultry feeds.

Aim: to study the mycota in raw materials and poultry feeds elaborated in Río Cuarto (Argentina) and examine the distribution and toxigenicity of strains within *Aspergillus* section *Flavi*.

Materials and Methods: A total of thirty five poultry feeds and their corresponding raw materials (maize, soybean and meat meal) samples from a processing plant in Río Cuarto were analyzed. The quantitative enumeration of fungal propagules was determined by the surface spread method on DRBC and DG18 media. Each colony belonging to *Aspergillus* section *Flavi* were sub cultured on MEA for subsequent identification to species level, which was performed following the taxonomic keys proposed by Pitt and Hocking (1997) and Klich (2002). The strains were characterized as S or L according to the diameter and number of produced sclerotia. The ability to produce aflatoxins and cyclopiazonic acid by all strains was evaluated by the methods described by Geisen (1996) and Bragulat *et al*. (2001), respectively.

Results and Discussion: The mycological analysis of the samples indicated the presence of six fungal genera (*Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Eurotium*, yeast). *Aspergillus flavus* was present in 48.5% of the analyzed samples. Ninety one strains belonging to *Aspergillus* section *Flavi* were isolated; ninety of them were identified as *A. flavus* and only one as *A. parasiticus*. Fifty seven strains were capable to produce sclerotia, of which 41 were identified as L-type strains and 16 as type S. Fifty-seven percent of the strains produced aflatoxin B_1 (AFB_1) while the 86.8% were producer of CPA with levels that ranged from 0.5 to 137.8 μg g^{-1}. Strains L produced from 0.005 to 14.8 μg g^{-1} (mean 1.8 μg g^{-1}) of AFB_1 while the type S produced levels ranging from 0.005 to 1.65 μg g^{-1} (mean 0, 39 μg g^{-1}). No differences in CPA production among strains of S and L type were observed. The non sclerotia-producing strains showed levels ranging between 0.005 and 27.7 μg g^{-1} (mean 2.47 μg g^{-1}) and between 3.8 and 47.3 μg g^{-1} (mean 15.5 μg g^{-1}) of AFB_1 and CPA, respectively. *Aspergillus parasiticus* strain produced 13.1 and 1.6 μg g^{-1} of aflatoxin B_1 and G_1, respectively and was not producer of CPA.

Conclusion: *Aspergillus flavus* was the predominant *Aspergillus* species in all the analyzed samples and a great variability was observed in their mycotoxin producing potential. The high rate of strains capable of producing CPA represent a potential risk of contamination with this toxin in poultry feed.

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P-50 EVALUATION OF THE HISTOPATHOLOGICAL LESIONS IN THE DIGESTIVE TRACT OF BROILERS CONSUMING DIETS CONTAMINATED WITH 100 AND 200 μg/kg OF AFLATOXIN B_1.

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Background: The aflatoxins are mycotoxins identified as secondary metabolites toxins produced mainly by *Aspergillus* genus. They are being described as aflatoxins B_1 a compound highly hepatotoxic that can cause gastrointestinal injuries in broiler (Leeson et al., 1995), and hepatic carcinoma in human (FAO/OMS, 1999). Aflatoxins are detected in grains and cereal frequently, due to the fact that they are very thermostable and the processing of contaminated cereals during balanced feeding elaboration do not destroy them (Vaamonde, 1996); its presence in food for animal consumption is a problem of food safety (FAO/OMS, 1999), having economic negative impact in the poultry industry affecting health of broilers.

Aim: Histopathological lesions description of the digestive tract from broilers feed with contaminated diets (100 and 200 μg/kg) of aflatoxin B_1.

Materials and Methods: An essay with newborn up to 28 d-old chickens was carried out. The animals were kept in metalic crates with artificial heat from an electric source. They had free access to food an water. The chicken were randomly assigned to two experimental treatments receiving 100 and 200 μg/kg of aflatoxin B_1 in the diet, and a control group, which received an aflatoxina free diet. Each treatment had five replicates of five animals each for a total of 75 chickens in the experiment. At the end of the essay, the animals were slaughtered by bleeding, and tissue samples were obtained from esophagus, crop, proventriculus, gizzard, duodenum, jejunum, cecum for histopathological studies. Samples were fixed with formaldehide 10% and dye with the Hematoxilin- Eosin technique.

Result and Discussion: Results showed evident pathological effects observed as hypertrophy and hyperplasia of esophagus glands, fold thickness and mononuclear infiltrate in proventriculus, gland dilatation of gizzard, erosion, congestion and swelling of duodenum and jejunum. There was no changes either in crop or in cecum. These results are consistent with those reported by authors who performed similar experiments in broiler diets exposed to a contaminated diet with aflatoxins (Huff et al., 1986; Leeson et al., 1995)

Conclusion: Lesions were not observed either in crop or in cecum with levels of aflatoxin B_1 (100 or 200 μg/kg) administrated in the diet. A concentration response effect was found in levels of aflatoxin B_1 (100 or 200 μg/kg), meaning a severe lesion with a higher concentration of aflatoxin in esophagus, proventriculus, gizzard, duodenum and jejunum.

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P-50-A DETECTION AND QUANTIFICATION OF AFLATOXIN B1 IN MUSCLE AND LIVER BROILERS POULTRY FARMS

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ABSTRACT

A study was conducted in 14 poultry farms in Costa Rica, between the months of January and February 2000 to establish the degree of contamination of meat and chicken liver with aflatoxin B1. We collected 240 samples of liver and 240 of muscle (thigh) of chickens for human consumption and were analyzed by the technique of thin layer chromatography (TLC). 30 liver samples (12.5%) were positive for aflatoxin B1, with a concentration of between 1.5 to 2.5 ppb with a coefficient of variance of 0.4 to 0.5
ppb. Aflatoxin B1 was not detected in samples of muscle.

Introduction

Aflatoxin B1 is the major mycotoxin produced by fungi ***Aspergillus flavus*** and ***Aspergillus parasiticus*** (1). This substance is considered as one of the most potent natural carcinogen known to date (6) and also has mutagenic and teratogenic properties (3). Direct exposure aflatoxin B1, in both humans and animals, is given by the consumption of grains, dried fruits or other agricultural food contaminated with it. The indirect exposure can occur by consumption of tissues (viscera, meat) or other products of animal origin, contaminated with it or its metabolites (5). In Costa Rica there are no reported studies related to the detection of aflatoxins in animal products, so far. However if you have reported clinical consistent with aflatoxicosis in poultry and pigs. The aim of this study was to determine the possible contamination of meat and chicken liver with aflatoxin B1 in the Central Valley of Costa Rica.

Materials and Methods:

The study was carried out in 14 poultry farms, between the months of January and February 2000. 240 were collected 240 samples of liver and muscle (thigh) chicken for human consumption. The extraction and detection of aflatoxin B1 (AFB1) in liver and muscle was performed by TLC technique recommended by AOAC Official Methods Analysis (1995) (Aflatoxins B1 in Liver).

Results:

Of the 240 samples of chicken liver tested by the TLC, 30 (12.5%) were positive for aflatoxin B1 at a concentration of 1.5 +2.5 ng / g. (1.5 to 2.5 ppb) with a coefficient of variance of 0.4 to 0.5 ng / g.

No muscle samples were positive para aflatoxina B1 within the range of detection method used.

Table of Results

MUESTRAS	*n*	POSITIVAS A AFB1	% DE CONTAM.	CONC. EN PPB
HIGADO	240	30	12.5	1.5-2.5
MUSCULO	240	0	0	0

Discussion:

Of 240 samples of chicken liver analyzed, 30 (12.5%) were positive for aflatoxin B1, with a concentration of between 1.5 to 2.5 ppb. No toxin was detected in samples of meat. The concentrations of aflatoxin B1 found in this study are low compared with those reported in other countries (4).

This seems to be the result of the use currently in Costa Rica of maize with lower levels of aflatoxin than in previous decades was used in the preparation of feed.

Conclusion:

Aflatoxin levels found in this investigation are well within the permitted tolerances (35 ppb) by the Costa Rican health legislation (2).

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Poster Section IV: Mycotoxins in poultry.

Tuesday June 29

Group 10 (P-51 to P-56): Each presentation in 10 min.

P-51 RECOVERY OF AFLATOXINS FROM FEED TO EGG QUANTIFIED BY HPLC.

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Background: The egg represents one of the most powerful food industries, the cheapest way to obtain vitamins and valuable nutrients. Mexico is one of the first consumers of eggs Worldwide, and the people is eating also the AF present in the egg and by products.

Aims: To quantify and identify the AF separating the yolk from the White of the egg and in the whole egg. So we can have an estimation of the amount of AF that we eat in 8 days if he eat eggs daily.

Methodology: First we planned an experiment with 3 groups of hens on their second period of production . The first Group was the control and ate "normal " clean sorghum, and the other 2 groups ate sorghum that had been spiked previously with 30 or 500 µg kg^{-1} AFB1. During 10 days that last the experiment each hen was in an independent cage, where we collected the eggs and label them according to hen, date, etc. The eggs were taken to the lab, where they were weight , the yolk was separated from the white. Each sample was blended with MeOH / water, salt , and was filtrated, diluted and filtrated for a second time with glass cotton. The quantitation was done with a liquid chromatographer connected to a fluorescente detector. Afterwads each sample was passed through a immunoaffinity column, was eluted , dried and injected to the HPLC.

Results and Discussion.

Eggs had all AF. On respect to the yolk the daily averages were: AFB1 (4.2 µg kg^{-1}); AFB2 (0.2 µg kg^{-1}); AFG1 (14.8 µg kg^{-1}); AFG2 (1.5 µg kg^{-1}); AFM1 (74.0 µg kg^{-1}) , no AFM2, AFP1 (3.2 µg kg^{-1}) and AFL (1.12 µg kg^{-1}). About the white we had a daily average of AFB1 (10.5 µg kg^{-1}) ; AFB2 (0.03 µg kg^{-1}); AFG1 (15.3 µg kg^{-1}) and no hydroxilated metabolites such as AFM1, AFM2, AFP1 nor AFL. The daily averages of the whole egg had AFB1 (11.9 µg kg^{-1}); no AFB2, AFG1 (20 µg kg^{-1}); AFG2 (1.03 µg kg^{-1}) AFM1 (36.5 µg kg^{-1}), no AFM2 , AFP1 (53.9 µg kg^{-1}) and no AFL.

During 8 days the amounts of AF that passed from feed to the egg in the yolk were: AFB1 (33.43 µg kg^{-1}), AFB2 (1.42 µg kg^{-1}), AFG1 (118.3 µg kg^{-1}), AFG2 (12.32 µg kg^{-1}), AFM1 (591.6 µg kg^{-1}) , no AFM2, AFP1 (25.8 µg kg^{-1}) and AFL (8.96 µg kg^{-1}). The white had after 8 days or accumulation: AFB1 (83.68 µg kg^{-1}); AFB2 (0.23 µg kg^{-1}) , AFG1 (122.1 µg kg^{-1}) and AFG2 (18.31 µg kg^{-1}) with no hydroxilated metabolites. The amounts of AF from the whole egg in 8 days were AFB1 (94.99 µg kg^{-1}), no AFB2, AFG1 (159.8 µg kg^{-1}), AFG2 (8.21 µg kg^{-1}); AFM1 (292.0 µg kg^{-1}), no AFM2, AFP1 (431.5 µg kg^{-1}) and no AFL.

Conclusion

The yolk had a lot of AF mainly AFB1 and AFG1, from the cereal fed to the hens , but also had hydroxilated metabolites such as AFM1, AFP1 and AFL.

P-52 AFLATOXINS IN LAYING HEN FEED AND ITS RECOVERY IN POULTRY LITTER BY HPLC.

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Background: Aflatoxins (AF) are secondary metabolites produced by strains of the fungi *Aspergillus flavus*, *A. parasiticus* and *A. nomius*, and they turn out to be a natural and dangerous contaminant of several cereal grains such as maize and sorghum, and they are a risk for animal and human health. The poultry manure has been widely investigated and is used as a feed supplement for ruminants, because it reduces costs and is a sustainable strategy for livestock production. When hens are fed with AF contaminated feed, also the poultry manure is contaminated then AF passes to meat and milk consumed by humans.

Aim: To quantify and identify AF in poultry litter to evaluate the amount of residues that the metabolism of the hen transfers to livestock when they consume the contaminated excreta.

Materials and Methods: A total of 25 laying hens of 121-week old in a second cycle of egg production, were distributed at random in three experimental groups and placed in individual cages. The control group had 9 hens fed with milled and homogenized clean sorghum, without AFB1, every day for 10 days; and two treated groups of 8 hens each, fed with AFB1 spiked sorghum with two different dosages, one low (30 μg kg^{-1}) and another high (500 μg kg^{-1}). Hen´s feed and poultry litter were collected every day for 10 days, the litter samples were dried, weighed and kept in refrigerator for their chemical analysis. To concentration and purification of AF, from 40 g of feed samples and 40 g of poultry litter were independently blended for 2 min with 5 g of NaCl and 100 mL of MeOH/ACN/H_2O (65:10:25 v/v/v). Each mixture was filtrated and evaporated at room temperature. Each sample was redissolved in 40 mL of PBS pH 7.4 and it was applied into Easi-Extract immunoaffinity columns for total AF. It was recovered with 2 mL of MeOH and the eluate was evaporated to dryness at 40°C. Afterwards, each eluate was redissolved in 1 mL of HPLC MeOH. The identification and

quantification of AF was made by HPLC using H_2O/MeOH/ ACN (60:30:20 v/v/v) as mobile phase.

Results and Discussion: The data obtained shows that the percentage of added AFB_1 in feed is detected in 43% of poultry litter in the treatment of 30 μg kg^{-1} and 52% in the treatment with 500 μg kg^{-1}. AFM_1, AFM_2, AFP_1 and AFL had were no significant differences between treatments, and trace amounts were found. The AFB_1 is rapidly absorbed in the digestive tract of ruminants and affects their metabolism, reducing 67% digestibility of food. Prolonged exposure to AFB_1, in addition to other factors such as stress, can cause severe aflatoxicosis in these animals, and also contaminate their milk and meat by products that are consumed by humans. Although the AFL and other AF hydroxilates were found in traces, they are not as harmful, in long run, as the AFB_1 is.

Conclusion: The prevalence of AFB_1 in poultry manure is a risk to the animal feed, because it is used as a food supplement for ruminants and provokes economic losses in livestock industry.

P-53 OCCURRENCE OF TOXIGENIC FUNGI AND AFLATOXINS ABILITY OF *ASPERGILLUS* SECTION FLAVI SPECIES IN POULTRY FEEDS

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Background: Since the 60´s industrial poultry in Argentina has experienced sustained growth in production and consumption. In Argentina, the consuming on average is 100 kg of "meat" per year per person, of which 26% are chicken. Similar trend is observed all over the world. At the time, Argentina exports meat chicken (whole or quartered) to many countries around the world. The development of systems with short periods of breeding and fattening, driven by the high demand for these products, requires a special adjustment of factors affecting the efficiency of food (such us, ingredients, composition and quality of feed, genetics, prevention and disease control, and the environment of the farm). In particular, there are specific requirements relating to controlling risks arising from the presence of contaminants, additives and/or pathogens. The main ingredients in feeds are corn and soybeans. These grains are colonized by mycotoxigenics fungal species belonging to the genera *Aspergillus* and *Fusarium*. Aflatoxigenics producer species, *A. flavus*, *A. parasiticus* and *F. verticillioides* are the most frequently detected in these products.

Aim: The objectives of this study were to determine the presence of *Aspergillus* section *Flavi* and *Fusarium* species from poultry feed, and determine the ability of *Aspergillus flavus* isolated strains to produce aflatoxins (AFs).

Materials and Methods: The poultry feed samples were collected in a poultry industry located in Río Cuarto (Córdoba, Argentina). A total of 5 samples of feed were taken monthly throughout the year. A spread surface method was used to perform the quantitative enumeration of *Aspergillus* and *Fusarium* colony species. Two solid culture media were used: dichloran rose bengal chloramphenicol (DRBC) and dichloran chloramphenicol 18% glycerol (DG18). Plates were incubated for 5 days at 25ºC and the plates containing among 10 to 100 colonies were used for count the fungus, expressed as CFU g^{-1} of feed. Each colony belonging to section *Flavi* and *Fusarium* genera were subculture on malt extract agar (MEA) and in carnation leaf agar (CLA), respectively. These species were identified according to morphological characters following the taxonomic keys proposed by Klich, (2002) and Nelson et al. (1993). The methodology of Geissen et al. (1996) was followed to assess the ability of *A. flavus* strains to produce AFs. The strains were grown in MEA for 7 days at 28ºC. After this period, the mycelium was harvested and AFs were extracted with chloroform. The chloroformic extract was evaporated and the detection of toxins was carried out by TLC following the methodology proposed by Horn and Dorner (1999).

Results: Mycological examination of the samples showed that the percentage of contaminated samples with *Aspergillus flavus* and *Fusarium* spp. were 66% and 100%,

respectively. The specie *A. parasiticus* was not isolated in these samples. Three species from *Fusarium* genera were isolated. The predominant was *F. verticillioides* (100%) followed by *F. proliferatum* (33%) and *F. subglutinans* (16,6%). Total counts of *A. flavus* in DRBC ranged among $5x10^2$ to $1.1x10^6$ CFU g^{-1} (mean value: $1.1x10^5 \pm 3.3x10^5$) and DG18 ranged among $5x10^2$ to $1.5x10^5$ CFU g^{-1} (mean value: $3.5x10^4 \pm 5.3x10^4$ CFU g^{-1}) respectively. Counts of *Fusarium* spp. in DRBC ranged among $5x10^2$ to $9.7x10^6$ CFU g^{-1} (mean value: $1.5x10^6 \pm 2.4x10^6$ CFU g^{-1}) and DG18 ranged among 1.5 10^3 to 5.2 x 10^6 CFU g^{-1} with a (mean value: $4x10^6 \pm 2.2x10^6$ CFU g^{-1}). From 54 strains of *A. flavus* isolated from poultry feed, 45% were able to produce AFB_1 at levels ranged from 0.541 to 20.6µg g^{-1}. High levels of colony counts were found, which exceed the feed hygienic quality limit ($1x10^4$ CFU g^{-1}) (GMP 2005). These results are consistent with previous reports carried out on poultry feed that found counts over $1x10^5$ CFU g^{-1} (Dalcero et al. 1997, 1998, Rosa et al. 2006). In the present study, a high percentage of aflatoxin producers were found and important levels of AFB_1 were produced by these species. These results agree with Dalcero et al. (1997) and Magnoli et al. (1999) in poultry feed.

Conclusion: The results of this research indicate that the high prevalence of potential toxin-producing fungus is indicative of mycotoxin problems in poultry feeds. The high proportion of aflatoxicogenic species isolated suggests a potential risk of mycotoxins contamination of poultry feeds.

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P-54 EFFECT OF AN ORGANIC SEQUESTERING IN THE VALUES OF RED BLOOD CELLS, HEMATOCRIT, HEMOGLOBIN AND GROSS LESIONS OF BROILERS FED WITH DIETS CONTAMINATED WITH AFLATOXIN B_1 (90 μg/kg) FOR 28 DAYS.

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Background: The aflatoxins are mycotoxins identified as secondary metabolites toxins produced mainly by *Aspergillus* genus. They are being described as aflatoxins B_1 a compound highly hepatotoxic that can cause gastrointestinal injuries and hematological parameters changes in broilers, showing an negative economic impact at the poultry industry (Leeson et al., 1995). Aflatoxins are frequently detected in grains and cereals (Vaamonde, 1996;), due to the fact that they are very thermostable and the processing of contaminated cereals during balanced feeding elaboration do not destroy them, its presence in food for animal consumption is a problem of food safety (FAO/OMS, 1999). As a consequence of this situation, different methods and additives to reduce or prevent aflatoxins-contaminated rations to be fed at broilers it is common production procedure to avoid this toxin reach humans, this have been an effort worldwide. Among those additives, *Saccharomyces cerevisiae* culture (cell wall molecules derived) has been reported as having a cell wall that sequesters or binds aflatoxins present in the ration (Raju and Devegowda, 2000; Karaman et al., 2005)

Aim: with the purpose of determining the effect of an organic sequestrant diets contaminated with Aflatoxin B_1 (90 μg/kg) on values of red blood cells, hematocrit, haemoglobin and gross lesions of liver, spleen, Bursa and gizzard.

Materials and Methods: 240 broilers (Cobb) were males, distributed under a randomized design of 4 treatments (T), 6 replicate for T and 10 chickens per replicate, with a duration of 28 days. Where: T1: basal diet (BD) with no detectable levels of aflatoxins; T2: BD + 0.2% of Organic sequestrant (SO) [Mycosorb]; T3: BD + 90μg/kg of aflatoxin B_1 (AFB_1) and T4: BD + AFB_1 (90 μg/kg) + SO (0.2%). Blood samples and organs were collected weekly.

Result and Discussion: No significant effects were found in the haematological parameters for any of the treatments, however, it showed an ascendent tendency of the red blood cells in the treatment consuming food contaminated with AFB_1 (T3), and this effect could be due to hemoconcentration caused by electrolyte imbalance due to decreased synthesis of proteins (Leeson et al., 1995). It was noted that the inclusion of organic sequestrant diets contaminated with aflatoxin B_1 (T4), showed less obvious gross lesions than the treatment diet consisted by food contaminated with AFB_1 (T2). These results are similar with those reported by authors who performed similar experiments in broiler diets exposed to a contaminated diet with aflatoxins and molecules from wall of *S. cerevisiae* (Raju and Devegowda, 2000; Karaman et al., 2005).

Conclusion: No effects were significant (P > 0.05) in haematological parameters for any of the treatments. Broilers diet with organic sequestrant and aflatoxin B_1 contaminated showed less severe gross lesions, compared to treatment with contaminated food (90 μg/kg of AFB_1) without scavenger.

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P-55 The effects of 1.2 ppm T -2 Toxin on performance, lesions, and general health of male broilers and the efficiency of an organoaluminosilicate (mycotoxin binder).

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Background: general signs of trichothecene toxicity in broilers include weight loss, decreased feed conversion, feed refusal, vomiting, severe dermatitis, hemorrhage and death. Because T-2 toxin was one the first trichothecenes discovered, its effects on animals were early characterized. T-2 is a mycotoxin affecting broilers health and performance (CAST, 2003). The aluminosilicates do not adsorb T-2 Toxin (Brito et al., 2008).

Aim: a trial was performed to evaluate the toxic effect of a contamination (1.2 ppm) of T-2 and the efficiency of a commercial organoaluminosilicate (mycotoxin binder).

Material and Methods: Ninety Ross 308 male broilers were randomly allocated in five groups: group 1 (negative control group), group 2 positive control (1.2 ppm T-2 toxin), group 3 (1.2 ppm T-2 toxin + organoaluminosilicate binder low dose), group 4 (1.2 ppm T-2 toxin + organoaluminosilicate binder high dose), and group 5 (organoaluminosilicate binder).
The birds were fed their respective diets from 10 days of age, up to 39 days, date in which they were sacrificed. We take the individual weight of the birds at the beginning and at the end of the experiment. At day 39, consumption. weight gain, feed conversion and mortality were calculated. Lesions of the oral cavity, feces and feathers were scored. In the animals that were given the mycotoxin binder, parameters were obtained same that those of the group control, for what it is considered that the organoaluminosilicate is innocuous. This experiment was realized in the Instituto Internacional de Investigación Animal, located in the Municipio del Marquez, Qro. At 1800 msnm, being the semi-desert climate.

Results and discussion: the results shown, at the concentration of 1.2 ppm of T-2 Toxin, affects significantly: feed intake, weight gain, feed conversion and mortality. The presence of T-2 toxin in the diet of the animals caused lesions in the oral cavity. The effects of the T-2 toxin in the broilers were practically eliminated by the incorporation of any of both doses of the organoaluminosilicate in the experimental diet.

Conclusion: The T-2 toxin at 1.2 ppm in the diet of the birds affects the health and performance of the broilers. We observed that T-2 toxin is of dermal toxicity and that the oral lesions reduce feed intake. The organoaluminosilicate in the diet of the animals that were given T-2 toxin, reduced the negative effects caused by the aforementioned mycotoxin.

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P-56 DETERMINACIÓN DE FUMONISINA B1 Y AFLATOXINAS EN ALIMENTOS PARA AVES USANDO HPLC

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Introducción:Las fumonisinas y aflatoxinas son metabolitos tóxicos producidas por algunos miembros de los géneros Fusarium y Aspergillus respectivamente, encontrándose principalmente en granos de cereales y en alimentos concentrados para animales. Estas toxinas dañan el sistema inmunológico, causando leucoencefalomalacia equina, edema pulmonar porcino y se ha asociado al cáncer de esófago en humanos en el el caso de las fumonisinas, mientras que las aflatoxinas se han asociado además con cáncer hepático

Metodología: Se analizaron 51 muestras de alimentos para animales tomadas a nivel de la tolva de llenado. La detección de fumonisinas mediante HPLC se realizó mediante una extracción con metanol:agua seguida de su purificación a través una columna en fase sólida y su posterior derivatización con OPA y mercaptoetanol. La muestra fue luego inyectada en un cromatografo Waters usando una columna Novapack C18 y una longitud de onda de excitación de 365 nm. La detección y cuantificación de aflatoxinas se realizó de acuerdo al método de la AOAC (994.08) utilizando acetonitrilo-agua para su extracción. La purificación o limpieza de la muestra se llevó a cabo utilizando la columna Micotox-M2002) y su detección utilizando HPLC y una fase movil metanol-agua (60:40)

Resultados:

El 98% de las muestras analizadas mediante HPLC resultaron positivas para fumonisina B1 con un valor promedio de 1.7 mg/Kg mientras que por Elisa, lel valor promedio fue de 1.3 mg/Kg encontrándose diferencias significativas entre ambos métodos. El nivel de recuperación utilizando HPLC fue de 110%. El 100% de las muestras analizadas cumplían con lo establecido en las regulaciones. La totalidad de las muestras estaban contaminadas con aflatoxinas (100%) en el rango de 0.1-62.2 ppb. El 27,4% de las muestras tenían niveles > 20 ppb. Se encontró un 98% de co-ocurrencia de aflatoxinas y fumonisinas

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Poster Section V: Mycotoxins in swine. Group 11.

Tuesday June 29
Group 11 (P-57 to P-62): Each presentation in 10 min.

P-57 EFFECTS OF DIETARY TREATMENTS CONTAINING VARIOUS CONCENTRATIONS OF CORN NATURALLY CONTAMINATED WITH FUMONISIN (FB_1) ON PERFORMANCE OF NURSERY PIGS.

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Background: *Fusarium verticilliodes (=moniliforme)* has been shown to produce the fumonisins FB_1, FB_2, FB_3, and FB_4 (Bezuidenhout et al., 1988). Fumonisin B_1 and fumonisin B_2 are the two most toxic metabolites and cause leukoencephalamalacia (ELEM) in horses and pulmonary edema (PPE) in swine (Kriek et al., 1981;Ross et al., 1990). Fumonisin FB_1 has been shown to be very detrimental to the performance of swine. Swine fed culture material containing high concentrations of FB_1 (≥ 100 ppm FB_1) have reduced feed intake, average daily gain, and death due to pulmonary edema (Casteel et al., 1993;Colvin et al., 1993). Field cases involving swine, in the United States, have shown that corn screenings contaminated with 105-155 ppm FB_1 with poor nutritional content and palatability and caused poor body weight gain (Harrison et al., 1990).

Aim: To determine the effects of diets containing various concentrations of corn naturally contaminated with fumonisin FB_1 on nursery pig's performance.

Materials and Methods: A 21 d study was conducted using 30 two wk old post-weaned barrows individually penned with an initial weight of 6 kg (±2 kg) in a RCBD randomly allotted to 1 of 5 dietary treatments containing different concentrations of FB_1. Pigs were fed experimental diets prepared using, nutritionally equivalent, naturally contaminated fumonisin corn containing 135 ppm FB_1 and contained no other mycotoxins. Dietary treatments included a Phase 2 nursery pig diet supplemented with 0 ppm FB_1 (control), 20 ppm FB_1 , 30 ppm FB_1 , 40 ppm FB_1 , and 50 ppm FB_1 . Pigs were given feed and water ad libitum. Pigs were weighed on d 7, 14, and 21. On d 21, blood sampled was collected via anterior vena cava from each pig and serum was analyzed for albumin, total protein, globulin, total bilirubin, direct bilirubin , aspartate transaminase (AST), and gamma glutamyl transferase (GGT). In addition, on d 21, pigs were euthanized and tissue samples were collected. Liver was analyzed for sphinganine and sphingosine levels by high performance liquid chromatography using the modified procedure described by Hsaio et al. (2007).

Results and Discussion: Pigs fed dietary treatments had both a linear and quadratic reduction from 0 ppm to 50 ppm FB_1 in ADG and final BW ($P < 0.05$) during the 21 d study. Pigs fed dietary treatments had a linear and quadratic increase in AST concentration from 0 ppm to 50 ppm FB_1 ($P < 0.05$). Feeding dietary treatments from 0 ppm to 50 ppm FB_1 caused a linear increase in GGT concentration ($P < 0.1$). Other serum chemistries such as albumin, total protein, total bilirubin, and direct bilrubin were not altered by dietary FB_1 treatments ($P > 0.05$). Pigs fed increasing concentrations of FB_1 from 0 to 50 ppm showed a linear increase in the amount of globulin found in the serum ($P < 0.1$). Relative liver and lung weights were not affected by dietary FB_1 concentrations ($P > 0.05$). Histopathology results revealed no major

lesions or abnormalities in the pig's livers or lungs. Liver SA: SO ratio increased linearly in pigs fed increasing concentrations of FB_1 ($P < 0.05$) revealing liver damage.

Conclusion: In conclusion feeding a diet containing up to 20 ppm FB_1 did not adversely affect the growth performance of nursery pigs for 21 days. In addition, feeding nursery pigs diets containing fumonisin B_1 caused liver damage demonstrated by an increase in the liver enzymes AST, GGT and the liver SA: SO ratio.

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P-58 EFFICACY OF TWO ADSORBENTS IN AMELIORATING THE EFFECTS OF FUMONISIN (FB_1) SUPPLIED BY NATURALLY CONTAMINATED CORN ON THE PERFORMANCE, BLOOD, AND LIVER CHEMISTRY OF NURSERY PIGS.

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Background: *Fusarium verticilliodes (=moniliforme)* has been shown to produce the fumonisins FB_1, FB_2, FB_3, and FB_4 (Bezuidenhout et al., 1988). Fumonisin B_1 and fumonisin B_2 are the two most toxic metabolites and cause leukoencephalamalacia (ELEM) in horses and pulmonary edema (PPE) in swine (Kriek et al., 1981;Ross et al., 1990). Fumonisin FB_1 has been shown to be very detrimental to the performance of swine. Swine fed culture material containing high concentrations of FB_1 (≥ 100 ppm FB_1) have reduced feed intake, average daily gain, and death due to pulmonary edema (Casteel et al., 1993;Colvin et al., 1993). It has been shown that adding a non-nutritional adsorbent to diets containing mycotoxins such as aflatoxins reduces the deleterious effects of the mycotoxins on nursery pigs. Recently, adsorbents shown to work in reducing the adverse effects of aflatoxin have been used against other mycotoxins such as *Fusarium* mycotoxins in hopes of having the same positive affect.

Aim: To evaluate the efficacy of clay-based adsorbents, with high in vitro binding efficacy for FB_1, to reduce the toxic effects of FB_1 present in naturally contaminated corn used to produce diets for nursery pigs.

Materials and Methods: Exp. 1 and 2 utilized 30 and 32 two-wk old post-weaned individually penned barrows, respectively (6.4±0.9 kg and 6.7 kg±0.8 kg, respectively) and allotted to 1 of 5 or 6 dietary treatments in a 2×3 or 2×2 factorial arrangement with either 3 (0, 20, and 50 ppm) or 2 (0 and 50 ppm) levels of FB_1 and supplemented with or without 0.5% carbonized clay (CC) or clay-activated carbon (AC) mixture. Pigs were given feed and water ad libitum. The amount of feed not consumed was weighed back on d 7, 14, and 21 to calculate feed intake and feed efficiency. Pigs were weighed on d 7, 14, and 21. On d 21, blood sampled was collected via anterior vena cava from each pig and serum was analyzed for albumin, total protein, globulin, total bilirubin, direct bilirubin , aspartate transaminase (AST), and gamma glutamyl transferase (GGT). In addition, on d 21, pigs were euthanized and liver tissue samples were collected. Liver was analyzed for sphinganine and sphingosine levels by high performance liquid chromatography using the modified procedure described by Hsaio et al.(2007).

Results: ADG and FE were not affected ($P > .05$) by dietary treatments. Pigs fed 20 or 50 ppm FB_1 without CC showed a reduction in ADFI ($P < .05$) compared to pigs fed control, 20 or 50 ppm FB_1 + CC. Pigs fed 20 ppm FB_1 + CC had a numerical increase in serum GGT compared to all other treatments. Pigs fed control diet has lower liver sphinganine (SA) to sphingosine (SO) ratio 0.04 and 0.08 ($P < .05$) compared to pigs fed 20 (1.315 and 0.67) or

50 (1.2 and 1.35) ppm FB_1 with or without CC. Pigs fed 20 ppm FB_1 + CC had higher liver SA to SO ratio ($P < .05$) compared to pigs fed 50 ppm FB_1 + CC . In Exp. 2 pigs fed 50 ppm FB_1 and 50 ppm FB_1 + AC had a decrease in overall ADG (271) and feed efficiency (0.527) ($P < .05$) compared to pigs fed the control diet (520 and 0.751). Pigs fed 50 ppm FB_1 with or without AC had an increase in serum AST (385.15 & 251) and GGT (280 & 232) ($P < .05$) compared to control of 52.25. Pigs fed 50 ppm FB_1 with or without AC had an increase in liver SA to SO ratio (2.481 & 1.886) ($P < .05$) compared to pigs fed the control diet (0.167).

Conclusions: Dietary inclusion of carbonized clay and clay-based activated carbon at 0.5% did not affect growth performance of nursery pigs during the 21 d studies. Dietary inclusion of FB_1 did affect growth performance of nursery pigs during the 21 d study. SA: SO ratio were increased in those pigs fed 20 and 50 ppm FB_1 with and without the carbonized clay or clay-based activated carbon at 0.5%. The carbonized clay or clay-based activated carbon at 0.5% did not reduce the adverse affects of 50 ppm FB1 on performance, serum chemistry, and liver SA: SO ratio of nursery pigs fed diets for 21 days. The *in vitro* results of the carbonized clay or clay-based activated carbon at 0.5% did not translate into *in vivo* efficacy.

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P-59 COMPARATIVE EFFECTS OF ZEARALENONE AND ITS METABOLITES ON SEVERAL IMMUNE FUNCTIONS IN PORCINE BLOOD MONONUCLEAR CELLS.

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Background. Mycotoxins, natural contaminants of feed and food, are toxic metabolites produced by several fungi which are considered an important risk factor for human and animal health (Finks-Gremmels, 1999). Trichothecenes (deoxynivalenol, T-2 toxin), zearalenone, ochratoxins, aflatoxins, fumonisins are the mycotoxins of most significance for public health as well as for agronomic production (Galvano et al., 2001).
Zearalenone (ZEN) is a mycotoxin that can be produced by several field fungi including *Fusarium graminearum (Gibberella zeae), F. culmorum, F. cerealis, F. equiseti* and *F. semitectum* (Richard, 2007). The toxin is common in maize, soybeans, barley, wheat, oats, sorghum and sesame seeds, which are ingredients in many feed/food products for human or farm animals (Engelhardt et al., 2006; Tabuc et al., 2009). The presence of ZEN was also observed in hay and corn silage. Following oral exposure, zearalenone is metabolized in various tissues, particularly in the liver, the major metabolites being α-zearalenol and β-zearalenol (Dänicke et al., 2005; Zinedine et al., 2007). Its metabolites (α–zearalenol-α ZOL, β-zearalenol-β ZOL, zearalanone-ZEN, zeranol, and taleranol) can be also produced by *Fusarium spp*. in corn (Bottalico et al., 1985) and in rice (Richardson et al., 1985). The major toxic effect of ZEN is the alterations in the reproductive tract of farm and laboratory animals (mice, rats, guinea pigs) (Minervini et al., 2008). Numerous *in vivo* and *in vitro* studies have indicated that zearalenone binds to oestrogen receptors, generating an oestrogen-like response which demonstrated decreased fertility, reduced litter size, changed weight of adrenal, thyroid gland, etc (EFSA, 2004). Among the all domestic species, swine (especially female pigs) is considered to be the most sensitive animal species to ZEA, followed by ruminants.
In addition, ZEN has been shown to be immunotoxic (Pestka et al., 1987; Luongo et al., 2008). However to our knowledge few studies investigated the effect of ZEN on immunity and none of them on swine.

Aim. The aim of the present study was to assess the *in vitro* effects of ZEN and some of its metabolites (α-ZOL, β-ZOL, ZAN) on several aspects of cellular and humoral immune response in porcine peripheral blood mononuclear cells (PBMC).

Material and Methods. Blood samples were aseptically collected by jugular vein puncture from healthy swine, and PBMC were isolated and cultivated at a density of $2.5x10^6$cells/mL for proliferation measurement using MTT [3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide assay with absorbance at 570 nm (A570) as the endpoint indicator] or $5x10^6$ cells/mL for cytokine (IL-1α, TNF-α and IL-8, IL-4, IFN-γ) and immunoglobulin (IgA, IgG, IgM) concentrations measurement (ELISA immunoassay).

Results and Discussion. The results obtained in the present study showed that *in vitro* incubation of porcine lymphocytes with increased concentrations of ZEN, α-ZOL, β-ZOL and ZAN affected in a dose-dependent manner the cellular proliferation following mitogen stimulation. The low doses of the mycotoxins (between 1 and 10μM) exerted a stimulative effect on cell proliferation above the value of PMA/ionomicine induced proliferation, considered as 100%. By contrast the proliferative response of PBMC was inhibited by the higher doses of mycotoxins. A dramatically decrease of the cells proliferation was observed beginning with the dose of 25μM for all investigated mycotoxins (64.8%,

57.7%, 31.0%, 18.7%, for ZAN, ZEN, α-ZOL and β-ZOL, respectively). Between 0 and 25μM the less toxic effect was noticed in the case of ZAN (64.8%) and the highest one for β-ZOL (18.7%). Analogue observations were shown by Luongo et al., (2008), Abid-Essefi et al., (2009) who observed that ZEN as well as its metabolites (especially α-ZOL, β-ZOL) induced cell death and decrease proliferation in porcine PBMC and Caco-2 cell.

The treatment of porcine PBMC with ZEN and its metabolites induced a similar decrease of cytokine synthesis (IL-8, IL-1β and TNF-α) and imunoglobulins. Low doses of toxins (especially α-ZOL and β-ZOL) increased the production of these bioactive molecules while the higher doses reduced their production. Exposure of the thymoma cell line EL4.IL-2 (EL-4) used as a T-cell model to various concentrations of either zearalenone or alpha-zearalenol for 5 d resulted in a significantly elevated levels for both interleukin 2 (IL-2) and intereukin-5 (IL-5) (Marin et al., 1996).

Conclusion. Taken together all these results suggest that ZEA, α-ZOL, β-ZOL and ZEN altered the *in vitro* cellular and humoral immune response and this alteration was dose dependent.

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P-60 EVALUATION OF AN ALUMINOSILICATE TO DECREASE THE TOXIC EFFECTS OF AFLATOXIN B1 IN SWINE.

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Background: Aflatoxin B1 (AFB1) is a mycotoxin with carcinogenic, teratogenic, mutagenic and immunosuppressant properties; it is produced by several species of the *Aspergillus* genus (*flavus, parasiticus, nomius*y *pseudotamarii*). Its target organ is the liver (hepatotoxic). The effects of aflatoxins (AFs) in swine depend on the age, concentration in feed, and exposure periods. Problems related to contamination with these toxins have been reported in all stages of swine lifespan. Its most common effects are: weight loss, anorexia, ataxia, roughened hair, tremors, comma and death (Coppeck et al., 1989), other reported effects include decreases in feed conversion, hepatitis, nephrosis and systemic hemorrhages (Hoerr et al., 1983, Miller et al., 1982). Several aluminosilicates have been reported to reduce the effects of aflatoxins in swine diets (Ramos and Hernandez, 1997). Thus, the study proposal was to evaluate the reduction of the negative effects produced by AFB1 in contaminated feed by incorporating a phyllosilicate in swine diets.

Aim: to evaluate the efficiency of a mycotoxin binder AFUMTEK, to decrease the toxic effects of a diet contaminated with 680 µg/kg (ppb) of AFB1 in swine balanced feeds.

Materials and Methods: 18 swine were used, weaned at 21 days of age divided into 3 groups with 6 repetitions. They were housed in individual corrals with swine use floor. A week of contiditioning was considered. Commercial feeds were used, they were proven to be free of contamination with other mycotoxins (AFs, fumonisin B1, Ochratoxin A, DON and T.2). The treatments were: 1. Negative control diet, 2. Positive control diet with 680 ppb of AFB1, 3. Challenge diet with Afumtek adsorbent: 5 kg/t + 680 ppb of AFB1. The experiment took 21 days. Swine were weighed at the beginning of the experiment and individual weights were recorded weekly. Feed conversion was calculated weekly. At the end of the experiment all swine were sacrificed; during the necropsy, samples of tonsils, lungs, livers, gallbladders, kidneys, spleens and mesenteric lymph nodes. All organs were previously weighed. Samples were submitted to histopathology studies. Fat, iron, vitamin A and AFM1 were quantified in livers. Biochemical parameters were analyzed in sera (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyltransferase (GGT), glucose, local proteins, albumen, creatinine, triglycerides, cholesterol, urea, uric acid) and vitamin A. The information obtained was analyzed with the Tukey test using the SYSTAT statistical software; the significance value was based on a probability of 0.05.

Results and Discussion: significative statistical differences were observed in animal weight gain, total weight, feed consumption, alkaline phosphatase, GGT and total proteins. In animals in which damages produced by aflatoxins were observed, a recovery was obtained from the challenge diet that included the adsorbent. The decrease in weight gain of the

positive control group was of 31.2% compared to the negative control group. 52.3% of the weight gain difference was recovered in the challenge group. The rest of the evaluated parameters only showed numeric differences. The histopathological analysis showed the effects of consuming aflatoxins in livers and kidneys. Adding mycotoxin adsorbents in the diets of animals exposed to contamination with aflatoxins diminished economic losses attributable to these toxins, especially in large productive facilities.

Conclusion: AFB1 at the concentrations used in the experiment affected animals. The increase of the concentration of alkaline phosphatase and GGT enzymes indicate the effects of AFB1 in swine. The decrease of these enzymes in the group given a diet with adsorbent is another proof of the protection afforded by the adsorbent. An increase in profits is also expected in the group of animals that consumed the AFs and the binder due to weight recoveries.

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P-61 OCURRENCE OF FUMONISINS IN SWINE FEEDS FROM MISIONES (NE ARGENTINA)

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INTRODUCTION

Corn grain (*Zea Mays* L.) posses a high nutritional value and it is used for the preparation of diverse food products. Among different toxigenic fungi that may affect corn crops, *Fusarium verticillioides* and *Fusarium proliferatum* are relevant for producing Fumonisins (mainly FB_1, FB_2, FB_3).

Twenty eight analogs of fumonisins are toxic or potentially toxic to animal and humans. Swine are considered to be one of the most sensitive animal species to fumonisins, developing pulmonary edema and hydrothorax.

Guidance levels for FB_1+ FB_2+ FB_3 had been established by FDA in corn and corn by-products intended for swine feeds of 20 ppm (with corn being no more than 50% of the diet on dry weight basis).

Fumonisins are natural contaminants of cereals worldwide and there are several reports of FBs occurrence in Argentina.

The purpose of this work is to determine the fumonisin presence in swine feeds and the percentage of the samples collected between February 2007 and March 2008 from Misiones (NE Argentina) that falls under the tolerance limits established by FDA.

MATERIALS AND METHODS

A total of 49 representative samples of 10 different stages of swine feeds were collected between February 2007 and March 2008. This period was chosen in order to obtain feeds made with corn belonging to two different seasons.
Fumonisins B_1, B_2 and B_3 (LOD 10 μg kg^{-1}; LOQ 18 μg kg^{-1}; LOD 18 μg kg^{-1}; LOQ 30 μg kg^{-1}; LOD 10 μg kg^{-1}; LOQ 20 μg kg^{-1}, respectively), were analyzed according to AOAC method 955.15.

RESULTS AND DISCUSSION

In order to get values for comparing swine feeds prepared with corn from two seasons, Fumonisins (FBs) levels were evaluated in two groups: one from February until November of 2007, and another from December of 2007 until March of 2008.
All samples were contaminated with Fumonisins B_1, 89% with FB_2 and 78% with FB_3 showing maximum values of 9224 μg kg^{-1}, 3245 μg kg^{-1} and 490 μg kg^{-1} respectively.

A non parametric test (Mann-Whitney W test) was applied to compare medians between 2007 and 2008. The values found were 792 μg kg^{-1} and 1746 μg kg^{-1} for each year, respectively. Significant statistical differences were observed at a confidence level of 95%.

CONCLUSIONS

Occurrence of fumonisins in swine feeds in Misiones NE Argentina was demonstrated. Its presence was confirmed in two seasons, 2007 and 2008, even at levels close or above the FDA limits.

When comparing swine feeds prepared with corn from two different seasons, 2008 showed higher values than 2007 seasons.

These data could be related to some climate factors as rainfall, temperature and humidity for further studies.

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P-62 INTERANNUAL CHANGES IN AFLATOXINS CONTENT IN SWINE FEEDS IN MISIONES (NE ARGENTINA)

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INTRODUCTION

The aflatoxins AFB_1, AFB_2, AFG_1 and AFG_2 are produced mainly by two species of *Aspergillus*: *A. flavus* (also producer of cyclopiazonic acid - ACP -) and *A. parasiticus* which may grow in a variety of crops including corn, rice and wheat.

These molds are especially abundant in tropical regions, less common in temperate regions, and virtually not present in cold regions.

Occurrence of aflatoxins in corn (*Zea mays* L.) and corn products has been a world-wide problem since it is a basic ingredient in diets for pigs at all stages of production. The naturally occurrence of AF's in corn may develop from the planting to the harvest and it is strongly influenced by the storage conditions.

In pigs, the consumption of these toxins is characterized by reduced weight gain, changes in biochemical parameters and internal organ injuries.

The main purpose of this work is to determine the presence of aflatoxins in feedstuff for pigs, as well as its variation in time in Misiones (NE Argentina). The period studied ranged from February 2007 to March 2008.

MATERIALS AND METHODS

A total of 52 representative samples of 10 different stages of swine feeds were collected between February 2007 and March 2008. This period was chosen in order to obtain feeds made with corn belonging to two different seasons.

Aflatoxins B_1 (LOD 0.2 µg kg^{-1}; LOQ 1 µg kg^{-1}), B_2, G_1 and G_2, were extracted by MycoSep #226 (Romer® Labs) and with immunoaffinity columns clean-up (AflaStar® - Romer® Labs), analyzed by HPLC and confirmed by TLC.

RESULTS AND DISCUSSION

In order to compare two seasons, Aflatoxins (AFs) levels were evaluated by quarter, with a total of 5 quarters from February of 2007 until March 2008. Plus, by AFs values, G group was evaluated separately.

AFs were found in 80 % of the samples, with a maximum of 11.1 μg kg^{-1}. There was an increase in levels from first to fifth quarter. The average total AFs found were 3.57 μg kg^{-1} and aflatoxin B_1 (AFB_1) = 2.92 μg kg^{-1}.

Significant statistical differences were found for AFB_1 along the five quarters. The lowest value (0.8 μg kg^{-1}) was found in the first quarter and the highest value (6.3 μg kg^{-1}) registered in the fifth quarter. It could suspect that the 2008 corn harvest developed a higher level of mycotoxins.

All samples had AFG_2 content below LOD. Nevertheless, 36% of the samples analyzed were positive for AFG_1. This was registered during the 2nd, 3rd and 4th quarters.

CONCLUSIONS

In the period ranging from February 2007 to March 2008 the presence of growing levels of aflatoxins in swine feeds in Misiones (NE Argentina) was confirmed. Therefore, the second crops season surveyed, through the feed analysis, show higher levels of aflatoxin contamination than 2007 harvest season.

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Poster Section VI: Mycotoxins in bovines, rats and goats. Group 12.

Tuesday June 29

Group 12 (P-63 to P-66): Each presentation in 10 min.

P-63 CONTAMINATION OF CORN SILAGE USED AS FEED FOR BEEF CATTLE ASSESSMENT

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Background: Ruminants feed mainly on forage (silage) and grains that are in natural contact with fungal spores before, during and after harvesting, transportation and storage (Scudamore y Livesey, 1998). According to the Food and Agriculture Organization (FAO, 2007), 25% of food production in the world is subject to fungal contamination, in particular *Aspergillus spp, Fusarium spp.* And *Penicillium spp.*). Corn silage is an important component in beef cattle diet, since it is considered a preservation method with minimal losses of nutritional quality (Oudeelferink y Drienhuis, 2001). In fact, the decrease or loss of silage's nutritional value during storage is due to fungal development (Garon, 2006).

Goal: Assess the types of fungi contaminating corn silage used as beef cattle feed and the impact on yield and carcass quality.

Methods and materials: 12 corn silage sampling processes were conducted using W's technique (Bautista and Santos, 2004). A fungal culture was prepared per sampling, using selective Czapek-Agar and Potato-Dextrose-Agar cultural media. 20 male bovines were selected, with a mean weight of 300 ± 40 kg, distributed as homogenously as possible in two groups of 10 animals. The animals has free access (*ad limitum)* to water during the trial of 90 to 110 days. The animals were fed with the same diet and the intake per group was recorded. Weighing took place every 21 days. Production parameters such as Food Intake (FI), Daily Weight Gain (DWG), Conversion Ratio (CR) and Final Weight (FW) were assessed. The animals were slaughtered at the end of the trial and the yield and meat quality were assessed, according to USDA's reported technique (1996). The data were organized and analyzed through parametric and non-parametric statistical analysis, according to the variables under study.

Results and Discussion: Results obtained from the 12 sampling processes conducted for a year on corn silage showed 81.8% of *Aspergillus spp.* Contaminating presence and 63.6% of *Fusarium spp.* These results coincide with Garon's findings (2006), who reported that the main fungi genus associated to silage are: *A. flavus, A. fumigatus, F. verticillioides* and *F. proliferatum.* On the other hand, Uriarte *et al*., (2002) says that fungal development is frequently seen in silage where anaerobic conditions are missing. The current work was not able to find the fungal contamination impact on assessed production parameters, nor in beef quality. There isn't much information available on those variables and the results couldn't be compared against a baseline.

Conclusion: Corn silage is severely contaminated by *Apergillus spp*. and *Fusarium spp* fungi. We were not able to measure the impact of fungal contamination on the carcass yield and quality of beef cattle fed from fungi-contaminated silage.

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P-64 *Saccharomyces cerevisiae* STRAINS WITH AFB_1 BINDING ABILITY AND BENEFICIAL EFFECTS ON THE RUMINAL ECOSYSTEM

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Background. Mycotoxins are metabolites produced by fungi growing on foods or feeds and represent a serious hazard to humans and animals. They can be produced preharvest or during storage, transport, processing or feeding. Aflatoxin B_1 (AFB_1) is one of the most harmful mycotoxins in animal production and food industry (1). Among the developed strategies to eliminate mycotoxin, biological decontamination using microorganism is highly promising. *Saccharomyces cerevisiae* strains are among promising candidates that can be applied in animal feed promising candidates that can be applied in animal feed towards improved robustness of animals in the production environment (2,3).

Aim. To evaluate the ability of *S. cerevisiae* strains (RC008; RC009; RC016), isolated from animal environmental and with AFB_1 binding ability previously *in vitro* demonstrated, to survive and binding AFB_1 under ruminant gastrointestinal conditions. This study was conducted to the further selection of potentially probiotic strains to be included in animal feed.

Matherials and Methods. Rumen fluid (RF) was obtained by ruminocentesis from 3 healthy cows. The RF was strained through 4 layers of gauze and used as strained RF (SRF), or it was set for 1 h in cones tubes after which the middle layer was centrifugated for 30 min at 15000 rpm and the supernatant autoclaved (ARF). To evaluate the viability of yeast strains under gastrointestinal conditions, ARF was inoculated with each yeast strain ($1x10^6$ CFU/ml) for 24 h at 37°C in anaerobiosis (a). Yeast cells were washed and resuspended in simulated gastric juice and incubated for 60 min at 37º C under agitation (b). After centrifugation, cells were added to artificial intestinal fluid and incubated for 60 min at 37ºC under agitation (c). Aliquots of yeast cells after a; b and c conditions were taken and serial dilutions were carried out and spread on YPD agar. In order to study AFB_1 binding ability under physic-chemical conditions of gastrointestinal tract, yeast strains were someted to the same conditions described above. After that, yeast cells were washed twice with PBS and incubated with 1 ml of AFB_1 (50 ng/ml in PBS) for 1 h at 37ºC in a shaking bath. Cells were centrifugated and the supernatant containing unbound mycotoxin was collected and stored at -20ºC for HPLC analysis. Yeast cells without gastrointestinal passage were included as control group. To determine the effect of yeast strains on ruminal cellulolytic bacteria, SRF was used as inoculum and incubated in medium 10 of Caldwell and Bryant (4) with filter paper strip as only carbon source. The incubation was carried out in anaerobic conditions during 10 days at 39°C. The activity of cellulolytic bacteria was evaluated by the paper strip degradation and ruminal pH was measured at the end of incubation. SRF without yeast addition were included as control.

Results and Discussion. All yeast strains were able to survive under gastrointestinal conditions, but they decrese one log after rumen passage. However, cell viability was maintained after stomach and intestinal conditions. AFB_1 binding percentages varied among yeast strains: RC008 38.4%; RC009 48% and RC016 39%. After passage in simulated gastrointestinal conditions, the strains RC008 and RC016 increased their AFB_1 binding ability to 58 and 46% respectively. Aflatoxin adsorption is influenced by pH and phosphate concentration in an aqueous environment (5). The obtained results suggest that conditions in the gastrointestinal tract would enhance adsorption and not decrease the mycotoxin-adsorbent interactions. However, the strain RC009 decreased the percentage of AFB_1 binding to 36% after passage to simulated gastrointestinal tract. The number or activity of cellulolytic bacteria in SRF was increased in the presence of RC008 and RC016 yeast strains. This was assumed by the observation of total degradation of filter paper in comparision with control group (SRF without yeast strain addition) in which the paper strip was still present after the incubation period. These results were not obsserved with the strain RC009.

Conclusions. Strains RC008 and RC016 are promisory probiotic candidates to be included in animal feed. They are able to survive and binding AFB_1 under gastrointestinal conditions. They also affect beneficially the rumen microbiota.

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P-65 A Clinical-Laboratory Investigation of Systemic Mycosis in male goats due to *Penicillium* and *Aspergillus* spp, in mouldy maize and fodder in Kathmandu valley Nepal.

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Abstract:

An outbreaks of a syndrome of unknown etiology associated with the feeding of moldy maize grain and green fodder to the male goat in a herd of 2000 meant for sale for Dashahara festival during the month of October-2008 in Kathmandu valley of which 52 goats suddenly became ill with symptoms of anorexia, apathy, diarrhea and ruminal stasis .On clinical examination based on history these goats were provisionally diagnosed as sudden illness due to moldy corn/fodder poisoning were treated with liquid Toxol and bio-sel-e and liquid zist of which 34 male goat died. Necrosis of the fore stomach mucosa was the most characteristic gross pathological change. Clinical pathological findings included mild focal erosions to severe, diffuse, coagulative necrosis of the mucosa in the rumen, reticulum and omasum and congestion and hemorrhages in the abomasum.Liver with shrunken appearance pale to yellowish discoloration with bile filled distended bladder pin point hemorrhage in kidney, small intestine with excessive mucus. On mycological and microbiological examination of tissue samples from post-mortem of dead goat on respective medium revealed the growth of fungal pathogens like *Aspergillus* and *Penicillium* spp with E.coli.These results provide circumstantial evidence that feeding of moldy maize grain and green fodder leaves infected by *Penicillium* and *Aspergillus* spp may cause outbreaks of a systemic Mycosis in these goats.

Key words:

Moldy maize, green fodder,*Penicillium,Aspergillus* Spp,Fungus, Male Goat, Kathmandu valley,Dashahara.Toxol,zist.

Background:

During the Dashahara festival of year 2008 about 2000 male goats intended to supplied by Nepal Food Corporation to the customers in Kathmandu valley were being purchased from mid-western region of Nepal. In lirage these goats were being fed exclusively with whole maize grains and green fodder leaves. On clinical examination based on history these goats were provisionally diagnosed as sudden illness due to moldy corn/fodder poisoning were treated with liquid Toxol and bio-sel-e and liquid zist of which 34 male goat died were presented to Central Veterinary Laboratory Tripureshwor Kathmandu Nepal for investigation of cause of illness.

Material and Method:

1: Clinical examination of goats in lairage.

All goats present in lairage for sale in Nepal Food Corporation's compound on clinical examination were found having symptoms like Anorexia, Ataxia, Diarrhea, Dullness, Dysmetria, Generalized weakness, similar to reported by Dr. Maurice E. White 2008, , and ruminal stasis, Schneider DJ, Marasas WF, Collett MG, van der Westhuizen GC, 1985. R. W. Medd, G. M. Murray and D. I. Pickering,2008. : L. W. Whitlow and W. M. Hagler, Jr.2008.

2: Post-Mortem Examination of dead goats.

On Post-Mortem examination of all 6 male dead presented to Central Veterinary Laboratory Tripureshwor ,Kathmandu revealed the lesions which included mild focal erosions to severe, diffuse, coagulative necrosis of the mucosa in the rumen, reticulum and omasum and congestion and hemorrhages in the abomasum.Liver with shrunken appearance pale to yellowish discoloration with bile filled distended bladder pin point hemorrhage in kidney, small intestine with excessive mucus similar to lesions experimentally induced by Schneider DJ, Marasas WF, Collett MG, van der Westhuizen GC, 1985,and reported by DhamaK, ChauhanR S1,*, MahendranMahesh, SinghKP1, TelangAG1, SinghalLokesh1, TomarSimmi2 2007, R. W. Medd, G. M. Murray and D. I. Pickering,2008; Hussein S. Hussein, and Jeffrey M. Brasel 2001.

3: Microbial/Mycobial Culture examination of Post-Mortem Tissue samples.
On mycological and microbiological examination of tissue samples from post-mortem of dead goat on respective medium revealed the growth of fungal pathogens like Aspergillus and Penicillium spp with E.coli similar to the findings of Karki et.al.2008 C. Wendell Horne 2008, www.springerlink.com/index/q7g038v8x3m10026.pdf 2008 Sabreen, M. S. and Zaky, Z. M.* 2001 .Where as all nasal and rectal swabs from sick and dead animals tested for PPR with penside test turn out to be negative.

Result and Discussion:

As during warm humid climate of tropics and subtropics favors growth of mold and fungus in feed grains and fodder especially after heavy monsoon rain feeding of exclusively such grain to livestock and poultry seems to cause the detrimental effect in the health these animals. As in this investigation clinical signs of anorexia, apathy, diarrhea and ruminal stasis and Clinical pathological findings included mild focal erosions to severe, diffuse, coagulative necrosis of the mucosa in the rumen, reticulum and omasum and congestion and hemorrhages in the abomasum.Liver with shrunken appearance pale to yellowish discoloration with bile filled distended bladder pin point hemorrhage in kidney, small intestine with excessive mucus. On mycological and microbiological examination of tissue samples from post-mortem of dead goat on respective medium revealed the growth of fungal pathogens like Aspergillus and Penicillium spp with E.coli.These results provide circumstantial evidence that feeding of moldy maize grain and green fodder leaves infected by Penicillium and Aspergillus spp may have caused this outbreaks of a systemic Mycosis in these goats need to be thoroughly investigated in field areas from where these goats were bought.

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P-66 OXIDATIVE STRESS IN RAT SPLEEN MONONUCLEAR CELLS EXPOSED TO AFB1 AND FB1: TIME-COURSE ACCUMULATION OF ROS.

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Background: Aflatoxins and fumonisins are synthesized by fungi of the genus Aspergillus and Fusarium, respectively. Aflatoxin B1 (AFB1) and fumonisin B1 (FB1) are the most important in their groups for their impact on food and immunotoxic and carcinogenic effects (Guindon KA et. al., 2008; Silva LJ et. al., 2009). Co-exposure to AFB1 and FB1 is a situation that occurs frequently in nature, in relation to individual exposure to these compounds (González Pereyra ML et. al., 2008). Although the alterations induced by these mycotoxins, when administered individually, are partially characterised, little is known about the effects induced by both-toxins mixtures. Previously, we have observed different immunobiological effects (Theumer MG et. al., 2003). and alteration of cellular oxidative state after a long-time (24 h) exposure of male Wistar rats spleen mononuclear cells (SMC) to AFB1 and FB1 individually or as a mixture.(Mary VS et. al., 2009).

Aim: To study the early oxidative status changes induced in SMC exposed to AFB1 and FB1 individually or as a mixture.

Materials and Methods:

-Cell culture: SMC of male Wistar inbred rats, 8 weeks old, were cultured in the presence or absence of AFB1 (20μM), FB1 (10μM) and MIX (AFB1 20μM + FB1 10μM) for 20 or 50 min, at 37°C in 5% CO_2.
-Apoptosis: Assessed by the propidium iodide staining and the subsequent analysis by flow cytomety.
-Measurement of intracellular Reactive Oxygen Species (ROS) and superoxide radical anion (O_2^-): The reduced form of 2',7'-dichlorofluorescin (DCFH), and Hydroethidine (HE) were used to determine the ROS and O_2^- intracellular contents, respectively. The probes were added to the cultures along with (time 0) or 30 min after the toxins addition, and then the incubation lasted for another 20 minutes at 37°C in 5% CO_2. ROS and O_2^- generation was analyzed by flow cytometry. We determined the percentage of fluorescent cells (ie cells that incorporated the DCFH or HE probes and oxidized them), and the mean fluorescence intensity (MFI) as a parameter to estimate the extent of ROS or O_2^- accumulation per cell (Kang J. et. al., 2003; Zielonka J et. al., 2008).

Results and Discussion: Cytotoxicity was not evidenced up to 24 h of culture in presence of the toxins individually or as a combination.
The main alterations in the ROS accumulation were found in SMC exposed for 50 min to the mycotoxins, where increases in the percentages of fluorescent cells and MFI were observed in all the treatments, however, FB1 and MIX induced higher rise than AFB1. In a previous work we observed that the toxins individually or as in combination increased the ROS production, being the highest ROS accumulation observed in the later group. Together, these observations suggest that the initial changes in the oxidative status of SMC exposed to the

mixture of toxins are mainly driven by the FB1, and that longer time of exposure are needed to evidence the contribution of AFB1 to ROS accumulation.
MIX is the only condition that produced O_2^- increases (time 30min). In a previous study, no changes were observed in this parameter when SMC were incubated for 4 and 24 h with the toxins, probably as a consequence of the short half life of this metabolite.

Conclusion: These results show that AFB1 and FB1 induce oxidative stress in rat SMC, however, the mixture of both-toxins produces major changes than the toxins individually. Furthermore, a differential behavior was found for O_2^- and total ROS accumulation.

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Poster Section VII: Mycotoxins in Pets and their feed. Group 13 and 14.

Tuesday June 29

Group 13 (P-67 to P-71): Each presentation in 10 min.

P-67 AFLATOXINS NATURAL CONTAMINATION IN PETS COMMERCIAL FOODS

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Background: Mycotoxins are chemically poisonous to dogs in low concentrations. Nausea, fever and fatigue are often associated with exposure to toxic fungi. In addition, are able to cause immunosuppression which in turn may lead to repeated infections. Some factors that enhance mould growth and aflatoxins include moisture content above 15 C and prolonged storage time. The maximum level for aflatoxins in commercial dogfoods in UE is 4 ng/g, 10 ng/g for total aflatoxin (Codex) and 20 ng/g for USA and México.

Aim: Determine the level of contamination in dogfood during two years 2007 and 2008 in pets foods commercialized in Mexico city.

Materials and Methods. Thirty two dog foods were collected in pet stores. The samples were chemically analyzed to determine their concentrations of protein, fat, fiber and pH. The method analytical was an assay ELISA, *RIDASCREEN®FAST Aflatoxin,* is based in the reaction antigen-antibody. The process consisted in weight 5 g of each sample and a methanolic extraction, to apply 50 µl of each sample included the standards into the microplaque.

Results: All of the dogfoods contained adequate levels of protein, fat and fiber. However not one of them was free of aflatoxins. In 2007, twelve foods showed aflatoxins contamination in a range 2.58 to 13.01. and average of 7.87 ng/g. All they lowed to 20 ng/g. 25% of total samples were in a level higher that 10 ng/g. In the In 2008, twenty foods contained a range 2.03 to 5.70 (puppy) average in 3.69 ppb in adult pets food a range between 1.75 to 5.99; and average 3.11 ng7g. All samples into the Official allowed in Mexico.

Conclusions: We probe that the aflatoxins contamination is a particular problem in each year. And that the occurrence of aflatoxins is a risk to dog health.

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P-68 MYCOBIOTA AND AFLATOXINS IN RAW MATERIALS AND PET FOOD IN BRAZIL

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Comercial feedstuffs are a Basic element in modern pet husbandry in the World. In dogs, the effect of mycotoxins is severe and can lead to death. Few reports on the influence of dietary mycotoxins were found in the scientific literature. The aims of this work were to isolate and identify the mycoflora and to determine the aflatoxins (AFs) natural occurrence in raw materials and ready dry pet food. Therefore, the aflatoxigenic capacity of *Aspergillus flavus* species was investigated. *Aspergillus* was the prevalent genera (65-89%) followed by *Penicillium* and *Fusarium spp. Aspergillus flavus* was the most prevalent species, followed by *Aspergillus sydowii, Aspergillus fumigatus* and *Aspergillus versicolor. Aspergillus flavus* frequencies ranged from 58% to 86% except in sorghum meal. All samples assayed (except corn grains and ready pet food) showed *Fusarium spp.* contamination. Corn meal and corn meal and gluten samples had 100% *Fusarium verticillioides. Fusarium graminearum* was isolated from sorghum meal. *Aspergillus flavus* strains (75%) isolated from raw materials and 57% from pet food were able to produce AFs. All samples showed AFs contamination percentages over 70%; corn and shorgum meal obtained the highest AFs levels. Ready pet food did not show quantitative levels of the tested toxins. This is the first report of the aflatoxigenic capacity by *A. flavus* from Brazilian pet food.

P-69 MYCOLOGICAL SURVEY IN FEEDS INTENDED FOR PSITTACIFORMS AND PASSERIFORMS RAISED AS PET BIRDS.

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Background: Lately people's interest in have a pet bird has increased, raising the presence of these animals, frequently psittacines and passerines, in the domestic environment. Its feedstuffs and other agricultural commodities are susceptible to contamination by molds and mycotoxins. Mycotoxins are chemical metabolites produced by various species of fungi that can cause adverse effects such as carcinogenesis, teratogenesis, nephrotoxicity and immunosuppression, leading to numerous pathologies and consequent economic losses. *Aspergillus*, *Penicillium* and *Fusarium* are the most frequently genera involved in human and animal cases of mycotoxicoses. More than one mycotoxin may exist simultaneously in a particular commodity or ingredient. Generally, the effects of these toxins tend to add up in synergic response, increasing the risk and hazard to animal health and productivity. There are no specific antidotes for mycotoxicoses. It is easier to prevent exposure to mycotoxins than to attempt treatment following their ingestion. Aflatoxins (AFs) are mycotoxins produced by *A. flavus* and *A. parasiticus*. Aflatoxin B_1 (AFB1) is the most frequently detected and it has been described as the strongest biologically synthesized hepatocarcinogenic substance that can affect humans and animals. Ochratoxin is produced by some *Aspergillus* and *Penicillium* strains. The toxin has an immunosuppressive effect and has been associated with air sacculitis, nephrotoxicity and other symptoms. Checking the mycological quality, control of feedstuffs and commodities destined to birds' consumption is critical for improving animal production and performance.

Aim: The aim of this work was to determine the occurrence of *Aspergillus spp.*, *Penicillium spp.* and *Fusarium spp.* in birds' feedstuffs.

Materials and Methods: Forty-seven samples from different commercial feeds were randomly collected from different establishments located in Rio de Janeiro, from March to December 2009. Analysis of the mycobiota was made by the plate dilution spread method onto dichloran rose bengal chloranphenicol agar (DRBC), dichloran glycerol 18% agar (DG18) and Nash-Snyder culture media. Water activity (a_w) determinations were carried out with Aqualab Cx2 (Decagon, Devices, Pullman WA, USA). Total fungal counts were expressed as CFU/g. The isolation frequency (%) of fungal genera and relative density (%) of fungal species were determined. For the aflatoxigenic and ochratoxigenic species the determination of the toxigenic profile was carried out according to Geisen (1996) and Bragulat; Abarca; Cabañes (2001) respectively.

Results and Discussion: Fungal counts in DRBC ranged from 1.0×10^3 to 1.8×10^6 CFU/g and in DG18 from 1.0×10^2 to 2.9×10^6 CFU/g. *Aspergillus* (83%), *Cladosporium* (55%), *Penicillium* (36%), *Eurotium* (30%), *Wallemia* (17%), *Mucor* (11%) and *Fusarium* (2%) were the isolated genera. Among the genera *Aspergillus*, *Penicillium* and *Fusarium*, the isolated species and its relative density were: *A. niger* (34%), *A. fumigatus* (32%), *A. flavus* (14%), *A. oryzae* (8%), *A. carbonarius* (6%), *A. versicolor* (3%), *A. parasiticus* (1%), *A. foetidus* (1%), *P.*

citrinum (85%), *P. islandicum* (5%), *P. brevicompactum* (5%), *P. citreonigrum* (5%) and *F. verticillioides* (100%). The percentage of *Aspergillus flavus* and *A. carbonarius* toxigenic strains in these birds' feed samples were 15% and 60%, respectively.

Conclusions: The presence of aflatoxigenic and ochratoxigenic species shows the need of more research in this area; would be important to continue the study in an attempt to detect and quantify AFs and OTA. The determination of the micobiota is very important because it can provide data about mycotoxins that could be in the samples.

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P-70 TOXIGENIC FUNGAL AND MYCOTOXINS IN PETFOOD FOR WILD BIRDS

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Background: Mycotoxins are toxic products originating from the secondary metabolism of toxigenic fungi. The presence of those fungi and toxins in foods for humans and animals has represented a danger for these groups health. In the case of the pet animals, among them, the birds, the toxic effect can be fatal. (Boermans and Leung, 2007; Hussein and Brasel, 2001).The main toxins found in foods for birds are: aflatoxins (AFLs), fumonisins (FBs), ocratoxin A (OTA) and zearalenone (ZON) (Scussel et al., 2006; Simão and Scussel, 2008).

Aims: To determine the presence of mycotoxins and toxigenic fungi in foods for wild birds commercialized in Brazil.

Materials and Methods: Birds feed samples (36) were collected from the southern and southeastern regions of Brazil from May to July of 2009. The assays carried out for quality and safety of those feed were: (a) moisture content (AOAC, 2005); (b) total fungi and yeasts count (APHA, 1992); (c) determination of the toxigenic potential strains on *Aspergillus flavus* and *parasiticus* Agar (AFPA) (Pitt et al., 1983); (d) mycotoxins AFLs, OTA and ZON analysis by AOZ immunoaffinity columns (Vicam ®) clean-up and quantification by LC-MSMS (Xavier and Scussel, 2006). To determinate of FBs, the extract clean-up was carried through SPE ($+NH_4$, quaternary amino) cartridges followed by LC-MSMS (Xavier and Scussel, 2006 – modified).

Results and Discussion:
From 36 samples analyzed, 14 (39%) presented moisture content above of the limit of 12% stablished by legislation. This fact can result in the total counting of yeasts and moulds above of the expected and consequently can favor the presence of toxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus*. Those in turn, were found in some feeds. It is indicative of possibility of mycotoxins contamination.
In Brazil, the legislation established for animals feed that the AFLs levels do not exceed 50 ppb. However regulation for the other mycotoxins does not exist. In the case of the birds feed, the great variety of ingredients (maize, peanut, soy, rice, oats, wheat, fruits crystallized and/or dehydrated, *alpiste*, *painço*, among others) that are present in this type of food is a fact that can contribute for the contamination of a product for multiple toxins.

Conclusions: Evaluation of quality of foods for wild birds must be carried frequently in order to guarantee the safety of products and consequently, the health of the animals in question. Multi-toxins analysis should be recommended as multi-genera / species of fungi can be present in the feed.

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P-71 DETERMINATION OF THE PROFILE OF INGREDIENTS IN FEED FOR PET BIRDS *VERSUS* MYCOTOXINS CONTAMINATION

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Background: The product quality of pet feed must be investigated as a significant increase of the number of these animals being fed by commercial feed and the production of feed is on a rise (SIMÃO and SCUSSEL, 2008; SCUSSEL et al., 2006). Regulating agencies have to warantee to consumers, the quality of these products regarding to mycotoxins presence. Thus stablishing regulatory limits, mainly for aflatoxins (AFLs), ocratoxin A (OTA), fumonisins (FBs), zearalenone (ZON) and patulin (PTL), the most frequently observed in contaminated foods, is necessary. In the case of the pet birds, the complexity of ingredient in the feeds formulation make them possible for a higher occurrence of mycotoxins contamination (BOERMANS and LEUNG, 2007; SIMÃO and SCUSSEL, 2008). Thus, it is very important the certify the quality of ingredients, processing and manipulation of these feeds by the manufacturers. In addition, labels must corroborate with the real content in the packing.

Aims: To determine the profile of ingredients added in feed destined for pets birds, correlating the types of ingredients (composition) of these products to possible risk of contamination b mycotoxins.

Materials and Methods: 20 samples of birds feed were collected: 10 commercialized in sealed packs and 10 commercialized in bulk (500 packs of 200 g, respectively). The proportion (%) of each ingredient in the feeds were determined by means of manual separation and with microscope stereoscopic. After the separation, each group of ingredient was weighed and the respective ratios were calculated for 100 g. The data obtained were checked with the basic composition reported in the feed labels. Moisture content was also evaluated AOAC (2005).

Results and Discussion: The main ingredients present in the birds feed were: (a) feed in pellets, supreme biscuit and dried fruits (crystallized and/or plain dehydrated), (b) peanut (with and without rind), (c) (maize, rice, soy, wheat, triticale, *triguilho*, pea and oats), (d) others (bran of soy and wheat, *quirera* of maize and rice, canary seed, *painço*). The ingredients presented in larger amounts were: feed in pellets, seeds of sunflower, maize and peanut. It was observed variations in the ingredients composition ratio by the specification of different bird's species *versus* labels. In fact some of these feed presented moisture content above of the allowed, being able to favor the development of yeasts and moulds (also toxigenic species), being that about 50% of the samples (11 feeds - 6 closed and 5 in bulk). They had moisture content above of the limits stablished for the legislation for pet food, that is above of 12% (BRAZIL, 2003). This fact, together with the complex composition of the feed, can be a determinative factor for mycotoxin contamination. When the ingredients were correlated to the possible contamination by mycotoxins, it was observed that the peanut was present in 25% of the total samples (5 samples - 2 packed and 3 in bulk), being that component related to the

possible presence of AFLs. Also maize was present in 9 samples (45%), which can be related to contamination by AFLs and *Fusarium* toxins (FBs and ZON). Also dehydrated and crystallized fruits could be contaminated by OTA and PTL. In addition, many ingredients were found damaged and infested by insects, which could favor the proliferation of moulds and formation of mycotoxins.

Conclusions: It is necessary to pay more attention on pets food quality, especialy related to their composition & moisture content control, in order to reduce/avoid possiblility of mycotoxins contamination.

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Poster Section VII: Mycotoxins in Pets and their feed.

Wednesday June 30

Group 14 (P-72 to P-75): Each presentation in 10 min.

P-72 DOGS AND CATS PATHOLOGIES MYCOTOXINS RELATED - A SURVEY

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Background: Several pathologies affect pets. Some factors responsible for these pathologies and the increasing incidence of neoplasias are animal genetic predisposition, physical (radiation) and chemical intoxication (pharmaceuticals, heavy metals, pesticides), environmental (manners of breeding, stress, pollution) conditions and diet quality (unbalanced composition, lack of nutrients, mycotoxins contamination) and can be currently found in the everyday's life of these animals (Withrow, 2007). Among the factors related to diet, the mycotoxin contamination can play an important role on pets health, especially if the commercial feed has low quality ingredients and/or lack of storage quality (either, in the pet shops or in the animal owners homes) (Maia and Siqueira, 2002). Despite of the massive data reported in animals for meat industry, there is a lack of information on toxicity of mycotoxins or other contaminants for pets and their related pathologies. Due to the increasing of the pet food market, there is an urgent need of information on these pathologies and their relation to feed quality for pets, especially to find out the real factors, how to sort the problem and improve pet food safety.

Aim: The aim of this study was to evaluate the incidence of pathologies that affect dogs and cats in southern Brazil and their correlation with predisposing factors such as type of feeding, manners of breeding and living environment that they are exposed. The types of pathologies were selected according to their target-organs and systems affected when the possible contaminant (mycotoxins) is present in their diet.

Materials and Methods: Therefore, a survey was carried out in the Veterinary Clinics of the Santa Catarina State in the year 2009 on the following pathologies mycotoxin related: hepatic (aflatoxins and citrinin; ochratoxin A – secundary target-organ), reproductive (zearalenone), circulatory (trichothecenes), nervous (fumonisins), renal (ochratoxin A, citrinin) and the neoplasias (chronic mycotoxicosis). Eighty six casebooks were investigated for the type of pathologies affecting the animals, type of feeding, breeding environment and any other adverse condition registered that could may cause/lead to the illness.

Results and discussion: casebooks from the total cases investigated, 67 (78%) and 19 (22%) were of dogs and cats, and 45 (52%) and 41 (48%) of females and males, respectively. Among the pathologies, the major casuistic was of (a) hepatic portal system (28 cases/32%) with hepatic failure and hepatitis (21 and 7 cases, 75 and 25%, respectively), followed by the (b) renal system with 26 cases (30%), all diagnosed as renal failure. In the (c) nervous system (23 cases/27%) was observed convulsion (10 cases/43.5%), tremorgenia (muscle tremor and motor incoordination), loss of proprioception (8 cases/35%) and epilepsy (5 cases/21.5%). The (d) reproductive system had 5 cases (6%) registered with uterus infection-pyometra (2 cases/40%),

dead fetus (2 cases/40%) and one abortion (20%). Neoplasias corresponded to 4 cases (5%) of total pathologies: in the liver (2 cases/50%), the spleen and the adrenal gland. Regarding the factors that could be pathologies related, feeding: 68 (96%) and 3 (4%) animals were fed with commercial and commercial+homemade food (mixed diet), respectively and all dogs had mainly dry feed and cats, dry and moist food (50% each). As expected, the commercials feed composition was considered balanced (label: giving all the nutrients they need) different of the homemade food fed, as not control was made. Several ingredients utilized in pet food are known as good substrates for toxigenic fungi growth. In some cases their quality are bad and that can make the difference regarding toxins and animal safety (Boermans and Leung, 2007). Among the mycotoxins, the aflatoxins, fumonisins, zearalenone e ochratoxin A are more prevalent in Brazil. They may be found in pet food ingredients such as cereals (corn, wheat, millet, sorghum, oats, barley, ray), followed by pulses, seeds and nuts (peas, peanuts, sunflower, *alpiste*, among others) when stored in conditions that are allow fungi growth (Simão and Scussel, 2008; Scussel et al., 2006). In addition, corn is the main ingredient of most dry pet food (ca. 70-80% of total ingredients) depending on pet food type. Breeding environment: it was observed that 51 animals (72%) lived inside the houses and 19 animals (27%) lived outside; stress was observed in 84% and 10% of dogs (indoors) and cats, respectively. Animals under environmental stress may show the more pronounced signs. Important to emphasize that, animals living outside the house can get their feed moldy in rainy days and if not fresh can lead to fungi growth, and possibly toxigenic ones, thus toxin formation, apart from bacterias.

Conclusion: The clinical signs of mycotoxicosis are nonspecific and can confuse the veterinary final diagnostic. The epidemiologic preliminary data obtained in the present study is important tool to help elucidation of the current pet's situation reported in the veterinary clinics in Southern Brazil. They can indicate that mycotoxins may be clinically present, however, not usually considered as their final diagnostic. That has been resulting on few discussions and considerations about mycotoxicosis between vets of pets in Brazil. Recommendation on mycotoxins analysis of the sick animal feed, should be carried out together with enzymes related and also education of pets owners on how to choose and store to keep pet food safe – the same way as it is carried out when breeding zootecnic animals for meat industries (make sure feed ingredients quality and final feed products safe). "No treatment is effective if the cause of the illness is persistent."

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P-73 ANTIFUGAL ACTIVITY OF *ENTEROCOCCUS* SPP. ISOLATED FROM CANINE FAECES.

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Background. Food and feed spoiling and mycotoxicogenic moulds cause great economic losses worldwide. The reduction of mould and yeast growth in food and feed production and storage is thus of primary importance and there is great interest in developing efficient and safe strategies for this purpose. In this context, the biocontrol has received much attention in recent years. Lactic acid bacteria (LAB) such as *Enterococcus* spp., are known to produce different antimicrobial compounds (organic acids, bacteriocins, etc.,) and are important in the biopreservation of food and feed (1,2). LAB are of special interest as biopreservation organisms since they have a long history of use in food and are 'generally regarded as safe' organisms. The majority of the large numbers of reports on antimicrobial activity of LAB have focused on antibacterial effects, while there are only a few reports on antifungal effects (3). On the other hand, one of the effects identified on LAB is the protection against mycotoxins such as aflatoxins (AFs). Dogs are particularly sensitive to the acute hepatotoxic and carcinogenic effects of AFs. Anorexia and depression are the two most visible symptoms of aflatoxicosis in canine species (4; 5; 6; 7). In previous works aflatoxin producers strains and AFB_1 were detected from dog foods (8).

Aim. To evaluate the ability of *Enterococcus* spp., isolated from canine faeces to inhibit *Aspergillus flavus* and *A. parasiticus* growth and AFB_1 production.

Materials and methods. Well diffusion assay was done to determinate the inhibitory capacity of 13 *Enterococcus* spp., against 4 aflatoxigenic strains (two *A. flavus:* AF210 and AF281*,* one *A. parasiticus*: AP245 isolated from dog food and a reference strain: *A. parasiticus* NRRL 2999). *Enterococcus* spp. cultures (OD_{600} = 0.90) were plated (1 ml) in LAPTg agar by pour plate technique and incubated for 48 hs at 37ºC in microaerophilic conditions. After that, 2 wells were performed in the plates, and 40 µl of 10^5 esp. ml^{-1} suspension of each *Aspergillus* strain was inoculated. Plates were incubated for 7 days at 25ºC. Fungal growth was assessed every day during the incubation period and the plates were examined. Two diameters of the growing colonies were measured at right angles in two directions until the colony reached the edge of the plate. The radii of the colonies were plotted against time. The growth rate was calculated by linear regression as the slope of the regression line. Aflatoxin B_1 produced by each *Aspergillus* strain was extracted according to (9), detected and quantified by TLC according to AOAC TLC method (visual quantitation) (10).

Results and discussion. The results of the interaction among *A. flavus* strains and *Enterococcus* spp. show an increase in the fungal growth rate. No significant differences in growth rate between both *A. flavus* strains were found. Any *Enterococcus* spp. strain could

significantly reduce the AF210 growth rate, whereas GJ24 and GJ40 strains reduced the growth rate of AF81 ($p < 0.05$). Growth rate of the reference strain (AP2999) was significantly reduced by enterococci MF2 and MF5, but the rest of the bacterial strains increased this parameter ($p < 0.01$). *Aspergillus parasiticus* AP45 increased its growth rate in presence of all LAB strains, except on GJ19 strain. In terms of AFB_1 production, *A. flavus* strains significantly reduced the production, except the interaction with AF210 and GJ20, where the production significantly increased with respect to control ($p < 0.05$). In the interactions with the strain AP2999 the AFB_1 production was reduced, except to MF3 strain, where the levels increased from 4,5 to 7,73 µg/g ($p<0,01$).

Conclusion. This *in vitro* results show that although the growth rate of *Aspergillus* section *Flavi* strains is not reduced in presence of *Enterococcus* sp. strains isolated from canine faeces, these strains could potentially reduce AFB_1 production.

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P-74 AFLATOXIN B_1 ADSORPTION BY YEAST ISOLATED FROM DOG FOOD AND FAECES.

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Background. Aflatoxins, especially AFB_1, are a group of carcinogenic mycotoxins causing various acute and chronic intoxications in humans and animals and can occur in several foods and feeds. They are produced by *Aspergillus* section *Flavi* species. Its effects in dogs include liver cancer. Anorexia and depression are the most common symptoms (Leung, 2006). In the recent years, many strategies for prevention and detoxification involving microorganisms have been under investigation. Organic compounds such as several yeasts (*S. cerevisiae* among others) and bacterial cell walls have been studied for their ability to complex with several mycotoxins without harming the environment or reducing the bioavailability of certain nutrients. It is well known that yeast cell wall can bind AFB_1 (Bueno et al., Shetty et al., 2007) due its surface structure; in other hand it represents a nutritional additive in animal feed.

Aim. To evaluate the capability of yeasts isolated from dog food and faeces of binding AFB_1 *in vitro*.

Materials and methods. Yeasts were isolated from dog food and canine faeces in YPD agar by dilution plating. Preliminary identification of the strains was performed by Pitt y Hocking (1999). Aflatoxin B_1 binding assays was performed according to Peltonen et al. (2001), a work solution of AFB_1 at a concentration of 1µg/ml was prepared in phosphate-buffered saline (PBS, pH 7.4). Yeasts cultures in YPD broth of 24hs. were used. Each strain was harvest, washed twice with PBS and the cell concentration adjusted at (10^8 cel/ml). For adsorption assay, the cells were resuspended in 1 ml of the AFB_1 work solution and incubated for 1 h at 37ºC in a shaking bath. Then, the cells were pelleted by centrifugation at 5,000 rpm for 5 min at room temperature, and the supernatant containing unbound AFB_1 was collected and stored at -20ºC for high-performance liquid chromatography (HPLC) analysis. Positive (PBS + AFB_1) and negative (PBS + yeasts) controls were included for all experiments. Aflatoxin B_1 adsorbed by the analyzed yeast was calculated by the difference in the AFB_1 concentration in the work solution and each supernatant sample. The assay was performed by duplicates.

Results and discussion. Twenty four yeast strains were isolated from dog food free from added yeasts, and 5 from canine faeces. *Debaryomyces* spp., *Rhodotorula* spp. and *Candida* spp. were the principal isolated genera. From the total, 16 strains were randomly selected to perform the adsorption assay. All strains assayed adsorbed AFB_1 in percentage ranged from 79.4 to 92.1%. Four of the 16 strains analyzed, showed the highest adsorption capacity: LJ2 (92.1%), LD6 (90.6%), LD24 (88.8%) and LD22 (88.6%) ($p<0.01$).

Conclusion. Addition of yeast in dog foods may represent a useful method to prevent aflatoxicosis in pet.

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P-75 TOXIGENIC MYCOFLORA AND AFLATOXINS PRODUCTION ABILITY IN FEED INTENDED FOR BREEDING CHINCHILLA

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Background. Mycotoxins are secondary metabolites secreted by fungi, mostly belonging to the genera *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. They are produced in cereal grains as well as forages before, during and after harvest, in various environmental conditions. Due to the diversity of their toxic effects and their synergetic properties, mycotoxins are considered as risky to the consumers of contaminated foods. The presence of mycotoxins in feeds may decrease the feed intake and affect animal performance. Aflatoxins (AF) are a group of naturally occurring mycotoxins produced by *Aspergillus* species, especially *A. flavus* and *A. parasiticus*, which grow in a wide variety of improperly stored food commodities (Guengerich *et al*., 1998). Chinchillas (*Chinchilla lanigera*) are rabbit-sized crepuscular rodents native to the Andes Mountains in South America. They are farm raised and are currently used by the fur industry and as pets. The growing international demand for chinchilla fur makes the breeding of these animals a highly profitable activity in Argentina. Cases of acute aflatoxicosis have been reported worldwide in dogs, pigs, or cattle that consume naturally contaminated feed (CAST 2003; GMP 2005). Although chinchillas are known to be very sensitive to mycotoxins, few data about acute toxicosis have been reported (González Pereyra *et al*, 2008). The aims of present study were 1) to isolate and identify potential mycotoxicogenic mycobiota from chinchilla feeds and, 2) to determine the aflatoxigenic ability of isolated *Aspergillus* section *Flavi*.

Materials and Methods. A total of twenty feedstuff samples were collected from one factory (A) and one farm (B) during 6 months (10 samples from each place), located in Río Cuarto Córdoba. Three subsamples from different portions of each feedstuff bags to make representative laboratory samples were taken. Twenty grams of each milled sample were blended with 180 ml of peptone water 0.1% (w/v), diluted to final concentration of 10^{-1}, 10^{-2} and 10^{-3} and then, an aliquot was inoculated in triplicate on to Dichloran Rose Bengal Chloramphenicol agar (DRBC) and Dichloran 18% Glycerol agar (DG18). Plates were incubated at 28°C for a week. At the last day of incubation, *Aspergillus* and *Fusarium* colonies were picked and transferred to malt extract agar (MEA) and Nash–Snyder agar slants, respectively. They were allowed to grow at 28°C for 7 days for identification to species level. Identification for *Aspergillus* and *Fusarium* species was made according to macroscopic and microscope observation criteria in accordance with Klich (2002), and Nelson *et al.* (1983), respectively. Aflatoxin B_1 production ability was performed according to Geisen (1996).

Results and Discussion. Total feed mycobiota counts in farm were between 1.5 x 10^2 and 1.7 x 10^4 CFU/g in DRBC and 2 x 10^2 and 1 x10^4 CFU/g in DG18 for feedstuff A and between

1 x 10^2 and 3 x 10^3 CFU/g in DRBC and 1 x 10^2 and 7 x 10^3 CFU/g for feedstuff B. Counts from the factory samples were between1 x 10^2 and 2.5 x 10^3 CFU/ g in DRBC and 2 x 10^2 and 2 x 10^3 CFU/g in DG18 for feed A and between 2 x 10^2 and 2.8 x 10^3 CFU/g in DRBC and 7 x 10^2 and 7 x 10^3 CFU/ g in DG18 for feed B. Highest CFU counts were obtained from the feedstuff A (farm), which was over the recommended limit established by Good Manufacturing Practices (GMP, 2008). Mycological examination of different kinds of feeds indicated that *Fusarium* was the prevalent genus in feeds from farm and *F. verticillioides* and *F. proliferatum* were the most frequent isolated species. Prevalent genera and species were *Aspergillus* and *Fusarium*, and *A. flavus* and *F. verticillioides* for feed A and B, respectively. *Penicillium* was also present in all analyzed samples and it achieved a high isolation percentage. In the present study, the occurrence of the main toxigenic genera (*Aspergillus, Fusarium and Penicillium*) showed a potential risk of mycotoxins contamination. Some of the analyzed samples indicated the presence of AFB_1 and 46% of *A. flavus* strains were able to produce AFB_1 with levels that varied from 0.66 to 49.25 µg/g.

Conclusion. Feed samples containing toxigenic species and AFB_1 are potentially toxic for animals as chinchillas.

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Poster Section VIII: Mycotoxin Methodology. Groups 15 and 16.

Wednesday June 30

Group 15 (P-76 to P-81): Each presentation in 10 min.

P-76 RAPID HPLC METHOD FOR SIMULTANEOUS DETECTION OF AFLATOXINS AND CYCLOPIAZONIC ACID FROM *ASPERGILLUS* SECTION *FLAVI*

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Background: Mycotoxins are secondary metabolites produced by moulds and are an important world-wide food safety concern. Among the most relevant mycotoxigenic producer fungi are some *Aspergillus* species in particular those belonging to the *Aspergillus* section *Flavi.* These are known to produce the highly carcinogenic aflatoxins in agricultural commodities. Due to its impact in animal and human health, these species are among the most intensively studied ones, being well known producers of aflatoxins (AFB_1, AFB_2; AFG_1 and AFG_2) and cyclopiazonic acid (CPA). Aflatoxins are mainly produced by some strains of *Aspergillus flavus* and *Aspergillus nomius* and by most, if not all, strains of *Aspergillus parasiticus*. On the other hand, cyclopiazonic acid, which naturally occurs in a large variety of crop products as a co-contaminant with aflatoxins, is mainly produced by *Aspergillus flavus* strains. Together they have been shown to cause health problems in animals and humans, resulting in important economic losses. The production of CPA by *Aspergillus* section *Flavi* may also be routinely used for identification purposes since *A. parasiticus*, *A. flavus* and *A. nomius*, exhibit different mycotoxin profiles. The detection and quantification of both these mycotoxins is usually done separately by HPLC with UV detection for CPA and fluorescence detection after post-column derivatization for aflatoxins. There isn't a chromatographic method available to detect simultaneously CPA and the main four aflatoxins.

Aim: To be able to detect aflatoxins and cyclopiazonic acid in a single HPLC run.

Materials and methods: Twenty two strains belonging to *Aspergillus* section *Flavi* were tested for aflatoxins and CPA production in Czapek Yeast Autolysate agar medium (CYA). Strains were inoculated on 6 cm diameter plates and incubated at 25 °C for 12 days in the dark. Three 8 mm diameter plugs were extracted with methanol and filtered. Extracts were analysed using a HPLC system equipped with a Jasco FP-920 fluorescence detector (372 nm excitation wavelength; 462 nm emission wavelength) and a photochemical post-column derivatization (PHRED unit - Aura Industries, USA). Chromatographic separations were performed with a C18 column (Knauer eurospher 100-5, 4 mm x 250mm, 5 µm) and an amino column (Knauer, 4.6 mm x 250 mm, 5 µm), fitted with a precolumn with the same stationary phase. The mobile phase was pumped at 0.8 mL min^{-1} and consisted of an isocratic

programme as follows: methanol/4mM zinc sulphate (65:35, v/v), pH 5. The injection volume was 50 µL. Samples were taken as positive for each of the toxins when yielding a peak at a retention time similar to each standard, with a height five times higher than the baseline noise. CPA standard was supplied by Sigma (St. Louis, MO, USA). Aflatoxins standard was supplied by Biopure (Austria).

Results and Discussion: Under the tested conditions, the amino column generated a chromatogram where it was only possible to discriminate CPA from the total aflatoxins. On the other hand, the C18 column separated even further, allowing the separation of CPA from AFGs and AFBs. With this column the retention times of AFGs, AFBs and CPA were respectively 10, 11 and 16 minutes. The results obtained with the fungal extracts are consistent with the results previously obtained with the common methodology. Data from these assays will be presented and discussed.

Conclusion: This methodology can be used to detect simultaneously both mycotoxins (aflatoxins and cyclopiazonic acid) in fungi cultures using a single HPLC run, even though the separation of the four aflatoxins is still insufficient.

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P-77 Comparative Performance of Fluorometry and High Performance Liquid Chromatography in the Detection of Aflatoxin M_1 in two Commercial Cheeses.

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Background: Aflatoxins are the most studied group of mycotoxins because of their natural carcinogenic properties (Hussain and Anwar, 2007). Aflatoxin B_1 (AFB1) ingested from feed is metabolized to aflatoxin M_1 (AFM1). Quantitative carryover of AFB_1 to milk is around 3% of the concentration consumed and it is excreted into milk primarily as AFM_1 (Diaz et al. 2004). Aflatoxin M_1 (AFM_1) is frequently found in milk and dairy products. When contaminated milk is used to manufacture dairy products the toxins are transferred to the final product. The AFM_1 is unaffected by pasteurization or processing. Studies have demonstrated that AFM_1 binds mainly with casein increasing the concentration in the curd during cheese manufacturing (Galvano et al. 2005).

Aim: The present study attempts to demonstrate whether fluorometric analysis is comparable to HPLC and could be used to screening and predict AFM_1 from milk products.

Materials and Methods: Four Holstein cows in late lactation (> 220 DIM) were used. The cows were milked twice daily and fed a TMR diet. One group (high group) of two cows received 1.6 kg of contaminated corn (40 чg AFB_1/kg) and the other group (low group) received 0.125 kg of contaminated corn mixed with 1.475 kg of AF free steam rolled corn (to equals the matrix intake of the high treatment group) immediately after morning milking. Milk used for processing into cheese was taken from the 5 and 7 d morning milking after AFB_1 addition in the diet. The cheese production was during April of 2007. Two sets of cheeses, cheddar and fresco, with two AFM_1 concentrations, high and low, were made for each concentration of AFM_1 in milk. Aflatoxin M_1 was extracted from cheese using the method described by Dragacci et al. (1995) with some modification. Cheese was brought to room temperature and then cut into small pieces and minced thoroughly. A 10 g subsample of cheese was blended with 10 g of celite (Diatomaceous earth powder) 545 (Fisher Scientific Inc.), and 80 ml dichloromethane, for 2 min at high speed (Waring blender, Model No 35BL 64, Merck, Poole, UK). After washing further with 40 ml dichloromethane, the mixture was filtered (filter paper #1 circles 24.0 cm; Whatman) and pressed to release maximum amount of filtrate. Each cheese was analyzed in triplicate. The filtrate was evaporated at 40°C on a rotary flash evaporator (Buchler Instruments, Fort lee, NJ) with a precision stainless steel water bath Model 183. The residue was dissolved in 1 ml methanol, 30 ml water (1:30, v/v) and 50 ml n-hexane, and then transferred to a separating funnel. The lower (water phase) layer was collected and then the hexane phase was washed twice with 10 ml water. The water phase was collected after each wash. Bottles of the collected water fraction were wrapped in paper foil to avoid light contact and stored at 4º C until processed. The aqueous phase was passed through an AFM_1-HPLC immunoaffinity column (AFM_1 monoclonal antibody-based affinity chromatography of VICAM, Watertown, MA) at a rate of 2-3 ml/min. This process was performed in a solid phase extraction manifold (SPE manifold**,** Waters, Watertown, MA). The immunoaffinity column was washed twice with 10 ml of distilled water to eliminate impurities. The toxin was eluted using 2 ml methanol. Once the AF samples were

eluted, the 2 ml liquid was divided into 0.5 ml vials for HPLC measurement and 1 ml glass tubes for fluorometric measurement.

Results and Discussion: The presence of AFM_1 was detected in all samples (100%) by both methods. There were no significant differences between cheese type ($P<0.05$). Amounts of AFM_1 in feed resulted in difference of AFM_1 in cheese regardless of its type ($P < 0.01$). Interactions between concentration and cheese sample or cheese type were not statistical significant at ($P>0.05$). There were no differences between methods of detection with treatment ($P>0.05$). The regression analyses between HPLC and fluorometry shows that fluorometry is a good predictor to screen AFM_1 concentration in cheese. Comparing the cost of the equipment between the fluorometer and HPLC, the HPLC is almost 20 fold greater than the fluorometer. Considering the variable time, the HPLC took longer per sample. Although an automatic injection was use, time average for each sample is 10 min. Taking into consideration the variable cost of analysis, material for running the HPLC include HPLC quality solvents which are more expensed than regular solvents.

Conclusions: The reduced time to run a sample, the lower cost of the fluorometer machine and the lower variable costs of analysis make the fluorometry method more useful than the HPLC method for screening samples. A printable readout with the amount of AFM_1 can be obtained from fluorometer and eliminate some of variability due to determination at begin and end of HPLC curves.

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P-78 Multi-component analysis of the mycotoxins in food and feed by LC-MS/MS

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P-79 COMPARISON OF PRECOLUMN AND POSTCOLUMN DERIVATIZATION SYSTEMS FOR THE LIQUID CROMATOGRAPHIC DETERMINATION OF AFLATOXINS IN PEANUTS

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Background: The monitoring of aflatoxins in food commodities as a consequence of the regulations established by many countries depends on the availability of adequate analytical methods. The most widely used method for the determination of aflatoxins in food and feed uses HPLC coupled with fluorescence detection. However, since aflatoxin B_1 and aflatoxin G_1 present less natural fluorescence, in order to improve the signals during analysis, various pre or postcolumn derivatization methods are used for signal enhancement.

Aim: To compare the method recovery when precolumn and postcolumn derivatization systems are used in the determination of aflatoxins in peanuts by liquid cromatography with fluorescence detection.

Materials and Methods: 25g samples of raw shelled peanuts and free of aflatoxins contamination were spiked with a pool of aflatoxin standards (2.02µg/Kg for B_1 and G_1 aflatoxins and 1.02µg/Kg for B_2 and G_2, 6.13 µg/kg total aflatoxins). The recovery tests were performed in triplicate.
The extraction and cleanup of the extract, using immunoaffinity column (Aflatest, Vicam Somerville, MA, USA), were done according to the AOAC Official Method 991.31 (AOAC, 2005a).
The precolumn derivatization was based on the AOAC Official Method 994.08 (AOAC, 2005b). A solution of trifluoroacetic acid, acetic acid and water 20:10:70 (v/v/v) was prepared to catalyze the conversion of aflatoxin B_1 and G_1 in B_{2a} and G_{2a}, respectively. The mixture of the extract and the derivatizing solution was heated to 65°C for 9 minutes to complete the derivatization reaction. Chromatographic conditions: mobile phase was methanol : acetonitrile : water in a gradient elution mode, starting composition 10:10:80 v/v/v, reaching 15:25:60 v/v/v in 3 minutes at a flow rate of 1.2 mL/minute; the C_{18} column, 150 mm x 4.6 mm, 5µm (XTerra® Waters) was maintained at 40˚C; the fluorescence detector operated at 364 nm excitation and 440 nm emission; 10 µL of the derivatized extract were injected.
Postcolumn derivatization was carried out as described in AOAC Official Method 999.07 (AOAC, 2005c), using an electrochemical cell (Kobra cell® - Rhône Diagnostics Ltd, Glasgow, UK). Chromatographic parameters: the mobile phase consisted of methanol : acetonitrile : water (for each 1L mobile phase 120 mg of potassium bromide and 350 µL of 4M nitric acid were added) in a gradient elution mode, starting composition at 20:20:60 v/v/v reaching 20:30:50 v/v/v in 3 minutes at a flow rate of 1.2 mL/minute; a C_{18} column, 250 mm x 4.6 mm, 5µm (XBridge® Waters) was kept at 40˚C; the fluorescence detector operated at 364 nm excitation and 440 nm emission; 30µL of the extract were injected.
The aflatoxins quantification was carried out based on a calibration curve established by the external standard method with seven concentrations within the working range of 0.0004 to 0.0203 µg/mL, for B_1 and G_1 aflatoxins, and 0.0002 to 0.0103µg/mL, for B_2 and G_2 aflatoxins.

Results and Discussion: The recovery rates for the precolumn derivatization method (95%, 88%, 89%, and 59% for B_1, B_2, G_1, and G_2, respectively) were within the acceptable recovery range (70 to 110%) indicated by the European Union Regulation n° 401, 2006, for concentrations between 1 and 10 µg/Kg of total aflatoxins, except for aflatoxin G_2, that shows inherent difficulty in recovering when it is purified by immunoaffinity columns. High recovery rates (90%, 85%, 100%, and 69% for B_1, B_2, G_1, and G_2, respectively) were obtained for the postcolumn derivatization.
Recovery is an important validation parameter of analytical methods, since both derivatization modes showed recovery values within the acceptable range, the viability of each method can be considered. The procedure for precolumn derivatization with trifluoroacetic acid promotes the aflatoxin detection and quantification at much lower cost when compared to the postcolumn method, since it is not necessary to purchase additional equipment. However, the analyst's cost must be considered. Also, the time required for the sample derivatization is an addicional step in the analysis, resulting in further human exposure to a solution composed of toxic substances such as acetic acid and trifluoroacetic acid. The postcolumn derivatization of aflatoxins, using the Kobra Cell®, occurs rapidly at room temperature. Moreover, it is not necessary to prepare the derivatizing agent daily and the maintenance is simple and easy. The derivatization reaction becomes part of the chromatographic run. The automation of the derivatization step increases the repeatability of results (results not shown).

Conclusion: Both derivatization methods have shown good recovery rates in the determination of aflatoxins in peanuts.

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P-80 ANALYSIS OF T-2 AND HT-2 TOXINS IN SOYBEAN AND SOY MEAL USING IMMUNOAFFINITY CLEAN-UP AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Background: Soybean (*Glycine max* L. Merr.) is the main source of protein worldwide used both as food and feedstuffs. Hygienic safety of soybean and by-products depends on fungal contamination among other microorganisms. *Fusarium* rot of soybeans is described in the literature and different *Fusarium* species have been isolated from this commodity (Pitt & Hocking 1999). *Fusarium* species are known to produce a broad spectrum of toxins including type A- and B- trichothecenes. Among A-type trichothecenes, T-2 and HT-2 toxins are relevant mycotoxins produced mainly by *F. sporotrichioides, F. poae, F. equiseti* and *F. acuminatum* (Leslie and Summerell, 2006). In a previous study *Fusarium* species potential mycotoxins producers have been isolated from soybean in Argentina, however limited information about the occurrence of *Fusarium* mycotoxins in soybean and by-products is available.

Aim: to evaluate an HPLC analysis coupled with immunoaffinity column clean-up previously described by Visconti et al. (2005) for simultaneous determination of T-2 and HT-2 in cereal grains to be applied in soybean and soy meal samples.

Materials and Methods: Fifty grams of sample were extracted with 2 g NaCl and 100 mL of methanol:water (90:10, v v^{-1}) by shaking in a orbital shaker at high speed for 30 min. The extract was filtered through filter paper Whatman Nº 4. Ten milliliters of filtrate were collected and mixed with 40 mL of sodium chloride solution (2%, w v^{-1}). The diluted extract was left on the bench for 5 min. to allow precipitation to occur and then was filtered through a glass microfiber filter. Ten milliliters of filtrate (equivalent to 1.0 g of sample) were passed through the T-2 immunoaffinity column at a flow rate about one drop per second. The column was washed with ten milliliters of deionised water and the mycotoxins eluted from the column using 1.5 mL of methanol. The eluted extract was collected in a 4-mL screw-cap amber vial and evaporated under a stream of nitrogen at 50ºC in a heating block. The dried residue was derivatized with 50 μL of DMAP solution followed by 50 μL of 1-AN reagent as previously described for T-2 and HT-2 toxins by Visconti et al. (2005). Recovery experiments were performed in triplicate by spiking blank soybean and soy meal samples with T-2 and HT-2 toxins at levels of 500, 250 and 125 μg kg^{-1} by diluting aliquots of the stock solutions with the appropriate volumes of acetonitrile. Spiked samples were left overnight at room temperature to allow solvent evaporation prior extraction.

Results and discussion: Characteristics of this in-house method such as accuracy, precision and detection and quantification limits were defined by means of recovery test with spiked soybean and soy meal samples. Mean recoveries for T-2 within the spiking range 125-500 μg/kg, were 90.9 and 81.3% for soybean and soy meal, respectively with a within-laboratory relative standard deviation < 10%. Analysis of samples spiked with HT-2 in the

same range gave a mean recovery of 70.2 and 77.5% for soybean and soy meal, respectively, with relative standard deviations < 12%. The limit of detection (LOD) for the method was 25 *μ*g/kg for T-2 and HT-2, based on a signal-to-noise ratio 3:1 and the limit of quantification (LOQ) was established as three times the detection limit. In our study, the analysis of twenty soybean and soy meal samples obtained from an agricultural company located in Córdoba Province, Argentina, revealed that only one soybean sample showed T-2 contamination at level of 279 $\mu g\ kg^{-1}$ but HT-2 contamination was not observed.

Conclusion: The proposed HPLC method uses commercially available products, is simple to perform and shows good laboratory performance for determination of T-2 and HT-2. The method provides an alternative to GC as well as HPLC-MS/MS methods for the analysis of samples in those laboratories without mass spectrometry equipment.

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P-81 OPTIMIZATION AND VALIDATION OF A LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF OCHRATOXIN A IN WINES

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Background: Ochratoxin A (OTA) is a mycotoxin produced by several *Aspergillus* and *Penicillium* species. It is a potent nephrotoxin and hepatotoxin with teratogenic, mutagenic and immunosuppressive effects [1]. In 1993, the International Agency for Research on Cancer (IARC) classified OTA as possibly carcinogenic for humans (group 2B) [1,2]. Exposure to OTA mainly occurs via food chain, especially with cereals and cereals products. Following cereals, wine is considered the second source of human exposure to OTA in Europe. Most reported methods for OTA determination in wine use immunoaffinity columns (IAC) for sample preparation coupled to a liquid chromatography system, commonly with fluorescence detector. IAC are generally preferred over others columns, e.g. C_{18} columns, due to their higher selectivity, precision and accuracy as well as for producing chromatograms with lower background. The inconvenience of this kind of columns is the high cost. Although reverse phase columns with C_{18} sorbent have a lower cost, they are not commonly used due to the higher chromatographic background produced in comparison to IAC.

Aim: the objective of this work was to optimize and validate a liquid chromatographic method for a selective quantification of Ochratoxin A in wines after a clean up step with C_{18} columns.

Materials and methods: by means of a vacuum manifold the C_{18} cartridges were conditioned with methanol and ultra-pure water. After loading and washing, OTA was eluted with 0.5% v/v acetic acid in methanol. These elutes were evaluated for OTA presence by reverse phase chromatography, under isocratic mode, using a mixture of acetonitrile: water: acetic acid (59.5:39.5:1, v/v/v) as mobile phase. OTA was detected by fluorescence using an excitation wavelength (λ_{ex}) of 333 nm and an emission wavelength (λ_{em}) of 460 nm. Chromatographic conditions were optimized using design of experiment, specifically a face-centered central composite design with two central points.

Results and discussion: the optimal chromatographic conditions were established considering the three most relevant factors, i.e., percentage of acetonitrile in mobile phase, flow rate and column temperature. The responses of sixteen experiments fitted a second order polynomial model, which indicated the following optimal conditions, acetonitrile: water: acetic acid (59.5:39.5:1.0 v/v/v) as mobile phase, flow rate of 1.2 mL min^{-1} and a column temperature of 30°C. With these optimal conditions, an effective separation and quantification of the mycotoxin was achieved in less than 5 min using a C_{18} YMC-Pack ODS-A (150 mm x 4.6 mm, S-5 µm) column. Validation was established according to ICH guidelines; calibration fitted a linear regression model in the range of 0.5 to 50 ng mL^{-1} with a determination coefficient (R^2) of 0.9992. Repeatability (relative standard deviation, RSD) and intermediate precision (RSD) in matrix showed values of 1.3% (n=6) and 0.8% (n=3) respectively.

Recoveries of spiked samples at five levels ranged from 87.2 to 118.9% with a mean RSD of 7.4%. Considering an injection volume of 20 µL, the detection and quantification limits were 0.03 ng mL^{-1} and 0.1 ng mL^{-1}, respectively. The latter is much lower than the European limit of 2 µg kg^{-1} (ppb) established by the regulation (EC) N° 123/2005 [3], thus, the proposed method is completely useful for OTA determination in wines. Using a Food Analysis Performance Assessment Scheme (FAPAS) sample, the proposed method was statistically compared with the official method of the International Organization of Vine and Wine (OIV), which uses immunoaffinity columns. Statistically, F-test showed that the variances of the results obtained by the proposed and by OIV methods were not different ($F = 1.041$ $v_1 = 3$, $v_2 = 3$, $P = 0.9744$). Therefore the unpaired t-test was performed without Welch's correction. This test indicated that mean values calculated for OTA by the proposed and by OIV methods were not statistically different ($t = 1.051$, $v = 6$, $P = 0.3339$). Considering the results of both statistical tests the proposed method provided comparable results.

Conclusion: this work showed an optimized and validated method for a reliable quantification of OTA in wines. The optimization via central composite design allowed the use of C_{18} columns for sample preparation without matrix interferences as well as to obtain detection limits much lower than the European limit of 2 µg kg^{-1} (ppb). The proposed method demonstrated to be comparable with OIV official method, given similar results with similar precision but with a lower cost. From the 53 sample analyzed, the higher percentage (76%) showed undetectable OTA levels and can be considered safe according to the current limit.

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Poster Section VIII: Mycotoxin Methodology.

Wednesday June 30

Group 16 (P-82 to P-86): Each presentation in 10 min.

P-82 Characterization by molecular markers (AFLP) of *Aspergillus* section *Nigri* isolated from grapes in Argentina

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Background: Ochratoxin A (OTA) is a naturally occurring mycotoxin in vineyards and their presence cause important economic losses. *Aspergillus* section *Nigri* mainly *A. carbonarius* and *A. niger* aggregate produce this toxin in grapes and wines (Battilani et al. 2006; Belli et al. 2005, Cabañes et al. 2002). The correct identification of ochratoxigenic fungi occurring in grapes is important to determine the potential toxicological risk in the grape growing areas. Molecular markers like AFLP provide useful tool to help in the identification of black aspergilli.

Aim: To identify and determine the genetic variability within Aspergillus section Nigri isolated from grapes in Argentina.

Materials and Methods: A total of 562 *Aspergillus* strains, section *Nigri* isolated from grapes collected in 6 grape growing regions of Argentina during 2 vintage years were evaluated to determine their ability for OTA production. Out of these strains, 94 were selected for identification at species level by amplified fragment length polymorphism (AFLP) technique. Fungal DNA extraction was done using the cetyltrimethylammnonium bromide (CTAB) method of Murray and Thompson (1980) as modified by Kerényi et al. (1999). AFLPs reactions were performed as described by Vos et al. (1995), as modified by Zeller et al. (2000).

Results and Discussion: Among the strains isolated during vintage 2006/07, 32% were OTA producers, the levels ranging between 0.5 and 1,285 ng/gr. The strains were identified by classic morphology as *A. niger* aggregate (69%) and as *A. carbonarius*.(31%) In the 2007/08 vintage, A lower percentage of strains isolated during the 2007/08 vintage were toxigenic (18.7%) but the levels of ochratoxin A produced was higher ranging between 0.5 and 7,583 ng/gr. The OTA-producer strains were identified as *A. niger* aggregate (59%) and the remaining 41% as *A. carbonarius*. Uniseriate aspergilli species isolated in the two periods evaluated were not OTA- producers.

The AFLP analysis produced a complex fingerprint pattern. A total of 107 bands were generated following amplification with two different primer pairs: EcoRI + TG / MseI + CG, EcoRI + AT / MseI + CG. Based on cluster analysis, it was clear that the isolates can be readlly assigned into five main groups and three of them showed an intraespecific homology higher 50%, represented the same species: *A. carbonarius*, *A. tubingensis* and *A. niger*

aggregate. Regarding the cluster *Aspergillus* uniseriate showed low homology (42%) and high internal heterogeneity. In addition, high divergence was found between *A. aculeatus* and *A. japonicus* (similarity of 33%). The firth cluster not was assigned readily to any of the type strain of *A.* section *Nigri* evaluated. No genotypic differences could be established between the potential of the strains to produce ochratoxin A, the geographical origin and the year of vintage.

Conclusion: There was a concordance between morphological identification and separation up to species level using molecular markers (AFLP). The genome diversity of the black aspergilli in the vineyards of Argentina evidence the occurrence of two well defined species *A. carbonarius* and *A. tubingensis*, and the other species included in *A. niger* aggregate and uniseriate aspergilli.

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P-83 ALTERNATIVES FOR SAMPLE TREATMENT AND CHROMATOGRAPHY OF OCHRATOXIN A IN WINE USING FLUORESCENCE DETECTION

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The International Organization of Vine and Wine in its resolution OENO 16/2001 established as analytical method for Ochratoxin A (OTA) quantification in wine, concentration on immunoaffinity column (IAC) and HPLC separation with fluorescence detection (FLD). However, due to the high cost of immunoaffinity columns, there is a need of more economic alternatives, as direct injection to HPLC after filtering wine samples or concentrating OTA on cheaper SPE cartridges, like C-18. In this work the analytical parameters of OTA concentration on 3 types of IAC and on C-18 cartridges with 3 solvent combinations [1,2], using conventional packed and monolithic C-18 HPLC columns are contrasted.

Differences in analyte recovery were statistically significant for different IAC, being OCRA-STAR cartridges the better alternative, with an average recovery of 97.3 ± 3.3%. When C-18 cartridges were used, the higher recoveries (93.2 ± 3.3 %) were obtained using acetonitrile as extraction solvent, in comparison with methanol/acetic acid 99.5:0.5 % v/v. Repeatability and accuracy of immunoaffinity and C-18 pre-treatment were statistical comparable (p=0.05) and also their sensitivity, however, more favourable detection limits (LD) was obtained using the immunoaffinity pre-concentration (0.01 ug L^{-1}) in comparison with C-18 pre-concentration (0.09 ug L^{-1}), but considering that the maximal allowed concentration of OTA in wine is 2,0 ugL^{-1}, both methods are suitable for OTA quantitation in wine. They were applied to determine OTA in 58 real wine samples and for the quantitative results the statistical comparability was verified. These results were also confirmed from the qualitative point of view using a GC-MS method [3].

Recently the direct injection of wine samples on a monolithic HPLC column and FLD, without preceding OTA concentration was proposed [4]. In the present work this procedure was tested for real red wine samples, however, the results showed that this method was not appropriate at OTA levels usually found in wine, because a very low chromatographic selectivity was observed; the analytical signal of OTA was lost under a great interfering peak. Without OTA pre-concentration, a high efficiency 25 cm column is required to achieve a good separation between the interfering peak and OTA. On the other hand, with previous IAC pre-concentration, monolithic HPLC columns allow a much faster analysis (2 min) than those obtained with C-18 packed columns (10 min).

In conclusion, OTA pre-concentration is essential for HPLC with FLD at levels usually found in wine samples. In this context, the utility of a method without pre-treatment is discussed. For pre-concentration, C-18 cartridges are a more economic alternative with comparable analytical parameters than IAC if OTA levels are over 5 % of maximal allowed concentration in wine and their combination with conventional packed columns is the most efficient option.

Acknowledgements: To Fondo SAG, C4-94-14-31 and to Consorcio Tecnológico Vinnova

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P-84 DETERMINATION OF RESIDUES OF AFB1 IN THE LIVER OF BROILER CHICKEN BY HPLC CHROMATOGRAPHY-MASS / MASS

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Background: Aflatoxin B_1 (AFB_1), a mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus,* is known to be hepatotoxic and hepatocarcinogenic in several animal species, especially young poultry (Ortatatli and Oguz, 2001).
In vivo studies have shown that the liver is the predominant target organ for AFB_1 (Miller and Wilson, 1994). Aflatoxin contaminated feed are a severe threat to both livestock productivity and human health and thus, brings huge worldwide economic losses each year (Diaz, 2005). A large number of the observations have shown that hepatic metabolism plays a prominent role in determining the biological action of AFB_1. Conversion of AFB_1 to aflatoxin M_1, aflatoxin P_1, aflatoxin Q_1, aflatoxin B_{2a} and aflatoxicol are regarded to be detoxification steps,
because the toxicity or carcinogenicity of these metabolites are lower than those of aflatoxin B_1 (Larsson and Tjalve, 1992). However, AFB_1 in liver may remain unchanged even when exposure levels are relatively low. The present work deals with the evaluation of AFB_1 in livers of broilers fed diets contaminated with 50 ng of the toxin per g of feed

Aim: To develop a method for quantify AFB_1 residues in broiler liver.

Materials and Methods: The AFB_1 extraction from liver samples was performed following a methodology adapted from Tavčar-Kalcher et. al (2007). Five ml of citric acid (20%) and 10g of diatomaceous earth were added to 50g of crushed liver tissue. The toxin was extracted with 100ml of dichloromethane with mechanical stirring for 30 minutes at room temperature. The organic phase was subsequently filtered with the aid of a filter paper and dried with 5g of anhydrous sodium sulfate. Twenty ml of extract were evaporated to dryness at 60ºC. This extract was resuspended in 20mL ACN:H_2O 75:25 V/V and extracted with 10mL of hexane for fat separation, the mixture was thoroughly mixed, centrifuged, and 10ml of the ACN aqueous phase was evaporated to dryness. The solid was resuspended in 10mL of 80% of CH_3OH:H_2O, 90mL of water were added and passed through a previously conditioned SPE column (OASIS, HLB, 200mg) according to the methodology described by Sorensen and Elbæk (2005). The toxin was eluted with 7mL of methanol, evaporated to dryness and kept in freezer until the analysis. Them, the extracts were resuspended in 500µL of 20% CH_3OH/H_2O for quantification by means of HPLC-MS/MS.
Separation was performed in a X-Bridge™ C_{18} (3.5 µm, 2.1x100mm) using
solvents A: 0,1% aqueous formic acid and B: acetonitrile-0,1% formic acid. The solvent program was: t=0 20%B, t=3min 20%B, t=15min 100%B, t=25min 100%B, t=25.1 20%B, 35min 20%B. The flow was 0,2mL and the column temperature was 25ºC. The MS was operated in the positive mode. Quantitative analysis was carried out using multiple reactions monitoring (MRM) mode. The MRM observed transitions were 313>241, 313>285 (precursor ion MH^+). The last transition was used for quantification and the former for confirmation. The limit of quantification was 0.025ng g^{-1} and the detection limit of 0.0025ng g^{-1}.
The calibration of the toxin was down with pure standard. Recovery was as high as 99±13%

Results: No AFB_1 was recover from livers of poultry fed diets with toxin levels around of 2µg of toxin per Kg of feed, the results were below the detection limit of the technique (0.0025ng g^{-1}),

whereas in livers of poultry fed diets with 50µg Kg^{-1} of AFB_1 the residual toxin were of 0.4±0.1; 0.2±0.1 and 0.32±0.09ng per g of tissue. Results were not statistically different from each other. The toxin in the liver was also compared with the one recover in the presence of a non nutritive adsorbent.

Conclusion: These results indicate that a significant percentage of the toxin remains unchanged in the liver which is the target organ of the aflatoxicosis. The presence of AFB_1 in liver showed that the toxin can be found as such in the tissues even when the levels of exposure in the diets are quite low.

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P-85 EFFECT OF AFLATOXICOSIS ON HATCHING EGG QUALITY AND IMMUNE STATUS IN BROILER BREEDERS

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A study was conducted to investigate the effect of feeding diets containing different levels of aflatoxin B_1 (AF) on hatching egg quality and immune status of broiler breeders. The breeder hens aged 28 weeks were fed with four treatment diets containing AF viz., Control (0ppb), (300ppb), (400ppb) and (500ppb) for three periods, each with a duration of three weeks from 28 to 36 weeks of age. Feeding of AF did not affect the shell thickness, Haugh unit score and yolk color index. Inclusion of AF at all the three levels reduced the antibody titers against ND and IBD and the decrease in antibody titer was in dose dependent manner.

Keywords: Aflatoxin, Broiler breeder, egg production.

P-86 QUANTIFICATION OF AFLATOXIN METABOLITES IN BIOLOGICAL FLUIDS BY ISOTOPE-DILUTION TANDEM MASS SPECTROMETRY

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Background: Aflatoxin B_1 (AFB_1), a naturally occurring fungal contaminant found in foods such as maize, peanuts, soy sauce and fermented soybeans, has been identified as a potent hepatocarcinogen in many animal species (Busby & Wogan, 1985). Epidemiological and research studies have demonstrated a strong link between AFB_1 exposure and the development of hepatocellular carcinoma (Ross *et al.,* 1992). AFB_1 is classified as a human carcinogen by IARC (IARC, 2002). To better understand the multistage carcinogenic process, we have developed specific and sensitive isotope-dilution mass spectrometry methods to quantify important AFB_1 serum and urinary metabolites.

Aim: To develop and validate methods to accurately measure the metabolic disposition of AFB_1 (protein adducts in serum and DNA adducts and mercapturic acid conjugates in urine), to provide a platform for assessing individual human exposures and to measure the efficacy of planned interventions to reduce AFB_1-related liver cancer in high risk populations.

Materials and Methods: HPLC-ESI/MS/MS methods were developed on a triple quadrupole mass spectrometer using stable isotope-labeled internal standards: AFB_1-N^7-$^{15}N_5$-Guanine, AFB_1-$^{15}N_5$-FAPyr, AFB_1-D_4-lysine and $^{13}C_{17}$ AFB_1-NAC. The $^{15}N_7$-Guanine and $^{15}N_5$-FAPyr standards were prepared by coupling AFB_1-epoxide to algae DNA grown in a ^{15}N enriched environment (Egner *et al.,* 2006). The $^{13}C_{17}$ AFB_1 originated from cultures inoculated with *A. flavus* strain NRRL 3357 (ATCC 200026; SRRC 167) and grown in media containing ^{13}C-glucose (Johnson *et al.,* 2008). The synthesis of the AFB_1-D_4-lysine has previously been reported (Scholl *et al,* 2006).

Results and Discussion: Triple-quadrupole mass spectrometry using stable isotope-labeled internal standards provides accurate, precise, specific and sensitive measurements of AFB_1 metabolites. Aflatoxin metabolites measured in treated animals, found in human exposures and AFB_1-exposed populations participating in clinical trial interventions are presented.

Conclusion: Application of highly sensitive and specific isotope-dilution mass spectrometry will further enhance knowledge in the assessment of exposure and detoxification of the environmental carcinogen aflatoxin.

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Poster Section IX: Fungal and Mycotoxin Control. Groups 17,18,19 and 20.

Wednesday June 30

Group 17 (P-87 to P-92): Each presentation in 10 min.

P-87 *IN VIVO* DIRECT PATULIN-INDUCED FLUIDIZATION OF THE PLASMA MEMBRANE OF FISSION YEAST *SCHIZOSACCHAROMYCES POMBE*

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Bacground: Patulin [4-hydroxy-4H-furo(3,2C)pyran-2(6H)-one] was discovered as a broad-spectrum antibiotic but it later proved to be toxic to both animal and plant cells. It frequently occurs in commercially available fruit juices and it is a common feed pollutant. High levels of patulin have been found in conventional and organic rotten apple samples and the patulin concentration in these juices can reach 2500 μg kg^{-1}. There has been increasing interest in finding a safe and efficient detoxification process, and it is therefore very important to know how patulin affects cells.

Aim: In our experiments, we performed a general characterization of the effects of patulin on *S. pombe* cells, and studied its action on the plasma membrane by means of electron paramagnetic resonance (EPR) spectroscopy and investigated plasma-membrane structural perturbations as a possible participant in patulin-induced cell processes.

Materials and Methods: The *S. pombe* uracil auxotroph (ura4-D18) heterothallic (h^-) strain was used in all experiments. Mid-log phase cultures were used in all experiments. EPR measurements were carried out as described by Pesti et al. (2000). Detection of the leakage of substances absorbing at 260 nm: Since patulin absorbs at 260 nm (maximum at 276 nm), the loss of these substances from the cells was measured after lysis of the cells in boiling water (100 °C) for 30 min. Suspension (10^8 cells ml^{-1}) was treated with patulin with the indicated concentrations for 0, 30 or 60 min at 30 °C, and the sample was then centrifuged. The pellet was suspended in boiling (100 °C) water for 30 min. After repeated centrifugation, the OD_{260nm} of the supernatant was determined. The reported data are the means of the results of three independent experiments.

Results and Discussions: The phase-transition temperature (G) of untreated cells, measured by electron paramagnetic resonance spectrometry proved to be 14.12 ºC. Treatment of cells for 20 min with 50 μM, 500 μM, or 1000 μM patulin resulted in a decrease of the G volume of the plasma membrane to 13.93 ºC, 10.14 ºC or 8.73 ºC, respectively. This change in the transition temperature was accompanied by the loss of compounds absorbing light at 260 nm. Treatment of cells with 50 μM, 500 μM or 1000 μM patulin for 20 min induced the efflux of 25%, 30.5% or 34%, respectively, of these compounds. Besides its cytotoxic effects an adaptation process was observed. This is the first study to describe the direct interaction of patulin with the plasma membrane, a process which could definitely contribute to the adverse toxic effects induced by patulin.

Conclusion: The main targets of patulin are cellular nucleophiles (Fliege 1999), and most of its multiplicatory effects can be explained in terms of the following simultaneous processes: (i) the possible inactivation of cell wall-bound enzymes , (ii) the plasma-membrane perturbation effects (see this study), and hence the loss of essential cell constituents, and (iii) glutathione depletion, which induces the generation of reactive oxygen species, resulting in mitochondrial membrane depolarization, leading to apoptotic processes and the lipid peroxidation of the membranes.

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P-88 VACUUM AND OZONE GAS APPLICATION ON IN-SHELL BRAZIL NUTS BAGS FOR FUNGI AND AFLATOXIN DEGRADATION AND CONTROL DURING SHIPPING

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Background: As for other tree nuts, fungi and aflatoxins (AFLs) may develop in/on in-shell Brazil nuts (*Bertholletia excelsa* H.B.K.) under favorable conditions and methods for their prevention and detoxication need to be developed. Ozone (O_3) is a gas suitable for use in grain storage for fungi control. It is also indicated for other types of food as it leaves no residue due to fast decomposition to oxygen and in not toxic. Some papers have reported AFL degradation by O_3 gas (Giordano et al, 2008; Giordano and Scussel, 2010) and vacuum application has been used for reduction of microorganisms, either bacteria, yeast or fungi growth (Church and Parson, 1995; Farber, 1991).

Aims: To find out the effectiveness of O_3 gas and vacuum treatment on in-shell Brazil nuts fungi and AFL degradation for its application in long shipping distances (in bags) either, in the Amazon River or abroad. Also to evaluate treatment effect on the nuts lipid stability and consumers acceptance after 60 days of application.

Material and Methods: In-shell Brazil nuts from retail market were O_3 gas treated (at 31.5 mg/L, 5h), vacuum packed in low oxygen permeability bags, heat sealed and stored for a period of 60 days (Group I). Two Groups of nuts were kept as Controls: without O_3 gas treatment but with vacuum (Group II) and no O_3 and no vacuum at all (Group III). The nuts initial fungi load was 6.9×10^4 cfu/g, moisture content of 9.37% and aflatoxins 5.62 ug/kg. Any fungi load change (on MEA media), *Aspergillus* and *flavus, parasiticus* grow/inhibition (on AFPA media), AFL presence (analyzed by liquid chromatography with fluorescence detection), lipid oxidation (TBA test) and nut acceptance/rejection (sensory evaluation attributes: nut shell and edible part appearance, strange odor, residual taste, rancidity and firmness) were registered.

Results and Discussion: Right after O_3 gas treatment, no fungi (CFU) neither toxigenic species of *Aspergillus* (*A. flavus, parasiticus and/or nomius*) were detected in the nuts. Also no yeast growth was observed. The same persisted after 30 and 60 days of storage. Different behavior was observed in the Control Groups (with and without vacuum) that kept similar fungi count as the beginning of the experiment (slightly lower), probably due to lack of oxygen (micro-atmosphere) - Group II. That Control Group presented 9.8 x 104 cfu at the end of the storage. On the other hand, as expected for Group III, fungi load increased quite high. With the exposure of O_3 gas, AFLs were not detected neither in the 30^{th} and 60^{th} Day of storage up to the LOD of the method (0.26; 0.34; 0.24; 0.25 ug/kg). The same did not occur in the Control Groups, as AFLs were detected at 7.0 ug/kg. That contamination could be either due to toxigenic fungi growth or to the heterogenicity of the original nut contamination. The TBA test and sensory evaluation showed that nuts were still palatable and were accepted by the panelists, as no significant change ($p<0.05$) were found between shell sensory attributes. As far as the stability of lipids in the Brazil nuts O_3 gas treated and vacuum packed are concerned, the values of malonaldehyde were constant throughout the storage period indicating no detectable oxidation.

Conclusions: The O_3 –vacuum-packaging method can be a safer alternative for shipping batches of Brazil nuts (in-shell) in the Amazon River as well as abroad. It can prevent and control fungi and AFLs during the trip period, at the same time, it keeps nut sensory attributes of consumer acceptance. Trips to foreigner countries can be long, reaching 3 to 4 weeks thus, utilizing the current method developed, it keep nuts safe during the journey, despite also of environment temperature changes. This is the first study on O_3 gas and vacuum carried out on in-shell Brazil nut to date. A study on packaging material will be a future work to be carried out.

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P-89 DEVELOPMENT OF PROCEDURES FOR CONTROL AND PREVENTION OF FUNGI AND MYCOTOXIN CONTAMINATION IN THE GRAIN STORAGE UNITIES OF SOUTHERN BRAZIL

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Background: The technology for large scale storage of grains in Brazil is very well developed and that includes prevention of insect, fungi and mycotoxins. Those storage Unities are located mainly in the grain producing areas of the Southern, Center and Center Western regions of Brazil. However, a specific, comprehensive and practical / easy to apply methodology for control and prevention of fungi proliferation and mycotoxin formation is still necessary. Especially for training the in-house workers and their understanding of the importance of applying control procedures throughout all the segments that comprise the grain Receiving (GRU) and the Storage (GSU) Unities to accomplish with the Brazilian Regulation (Brazil, 2000). Those Unities will need to adequate their facilities to the National System of Certification for Storage Unities (GRU and GSU) which started from January the first, 2010 (Brazil, 2000).

Aims: To develop specific procedures for controlling and preventing fungi and mycotoxin contamination in GRU and GSU of Southern Brazil for further application to obtain the grain Storage Unities Certification.

Materials and Methods: The grain Unity utilized for the methodology development was the GRU of Coamo - Agro industrial Cooperative, located in the Dois Irmaos district, Toledo, Parana State, Southern Brazil. It is comprised of five buildings (segments) as follows: *1. Reception & Classification* (equipped with on-semi truck pneumatic sample collectors and laboratory), *2. Storage* (500 tones, with conveyor and elevator), *3. Lung Storage* (aeration system and elevator - 250 tones/hour), *4. Drying and Aeration* and *5. Expedition* (elevator - 250 tones/hour). Stored products: soya and wheat. Briefly, the materials utilized were questionnaires distributed to the workers, pictures (taken on each site/facility to evaluate and identify critical risk points), cleaning up and safety equipments (to be distributed in each segment of each Unity) and from cleaning up solutions (to be prepared and used throughout the facilities). The procedures developed set the right sites for distributing the equipments, timing for the cleaning up materials and chemicals solutions application. All for fungi prevention, aiming its efficacy for the whole year (despite of seasons changes) and in all the environments of the GRU facilities. The applicability of the procedures such as the effect of the cleaning methodology; critic points desactivation, effectiveness of the control actions set and the improvements of the grain quality was also checked.

Results and Discussion: The cleaning up and sanitation actions brought positive results for the COAMO-Toledo facilities, especially on the grain quality. As expected the implemented procedure lead to a cleaner and controlled (fungi spores, insects, dust) whole Unity.

The critical points for fungi and mycotoxin proliferation were identified at each segment of the GRU as follows: *Reception* (debris and grain residues with high moisture and spores load); *Storage* (tunnels with grain residues, insects and moist); *Drying* (moisture heterogeneity, debris, broken grains); *Transilage* (tunnels-ditto above); *Grain cleaning* (lack of efficiency and debris); *Lung storage* (drying and aeration need improvement of efficiency

control; elevators residues) *Expedition* and truck (debris, lack of truck cleaning and sanitation, time waiting for loading). After the fungi and mycotoxin critical points were identified, the methodology developed was applied their control and prevention in all facilities of each segment of the GRU.

It was possible to clearly observe a visual improvement of the GRU surrounding areas with no residues responsible for fungi proliferation as well as insects. It was observed also a reduction of rodents. In addition, the storage workers became more aware of the importance of their participation in the grain quality process. The data from the stored grain analysis improved with lower fungi load and toxin contamination throughout the year 2009.

All the methodology details were gathered in a Guide of Good Storage Practices for Fungi and Mycotoxins Control and Prevention for Safe Storage and distributed among the personnel which will be presented in the Poster Session together with mycotoxin data.

Conclusions: All optimization actions for grain conservation were established in a simple and practical way, so it could be applied in a continuous program of instructions and personel shedulles for cleaning and controlling conditions of grain storage. The procedures developed put together in a Manual is an important tool for guidance (a) on the conservation of grain quality and safety regarding fungi and mycotoxins and especially (b) to accomplish with the Brazilian Regulation regarding the Certification of Storage Unities that officially started in January, 2010.

Reference:

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P-90 FUSARIUM MYCOTOXIN CONCENTRATIONS IN STRAW, CHAFF, AND GRAIN OF SOFT RED WINTER WHEAT EXPRESSING A RANGE OF RESISTANCE TO FUSARIUM HEAD BLIGHT

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Background: Fusarium head blight (FHB) or scab, which is caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.), is an increasingly important problem in the north-central region of the United States. During years of heavy FHB infection, veterinary diagnostic laboratories have, on occasion, unexpectedly found unusually high concentrations of both deoxynivalenol (DON) and zearalenone (ZEA) in wheat straw, as well as FHB-infected grain. Because swine are sensitive to concentrations of DON and ZEA as low as 1 ppm, these mycotoxins are particularly problematic when wheat straw is used for bedding in less than optimal production settings. Similarly, where straw is used as a source of roughage for cattle in total mixed rations (TMRs), concentrations of mycotoxins, to which ruminants are fairly resistant, might be found at clinically relevant, high concentrations in straw. Although there is a large body of literature on mycotoxin content in FHB infected grain, little is known of both the range and concentrations of mycotoxins in wheat straw[1-4].

Aim: To investigate mycotoxin concentrations in the straw, chaff, and grain of 60 soft red winter genotypes from the 2008 Northern Uniform Winter Wheat Scab Nursery.

Materials and Methods: The 2008 Northern Winter Wheat Scab Nursery (Fig. 1) was grown at the Bradford Research and Extension Center near Columbia, MO and spray-inoculated at 75% heading with a macroconidial suspension of *F. graminearum* concentrated to 50,000 macroconidia/mL. The nursery was maintained under overhead mist irrigation through heading and evaluated for incidence and severity 18 - 21 d after inoculation. At harvest, a 3-meter long sample of each genotype was cut at ground level, dried, and separated into the spike and straw. Straw samples were ground and the spikes were threshed using a wheat head thresher to separate wheat grain from the chaff. Grain and chaff were collected and ground. Ground samples were extracted with acetonitrile/water (86/14), passed over SPE clean-up columns, and the eluants taken to dryness. Samples were derivatized with TMSI/BSTFA/Pyridine/TMCS 1/1/1/1 and analyzed by GC/MMS in the SIM mode with an Agilent MSD 5975B MSD for 17 *Fusarium* mycotoxins.

Results and Discussion: The FHBI for the 60 genotypes evaluated ranged from a low of 9.9% to a high of 61.9% and averaged 35.7%. Significant concentrations of DON and ZEA were detected in the grain, chaff, and straw samples, while 15-ADON and zearalenol concentrations were negligible (<0.5 ppm) in the grain but measureable in the chaff and straw. 3-ADON and nivalenol were not detected in any of the samples. The DON and ZEA concentrations in the grain samples averaged 4.7 and 4.4 ppm, respectively, across the 60 genotypes, and were significantly correlated with resistance level (r=0.56 and r=0.51 for DON and ZEA, respectively). In chaff samples, both mycotoxins were present at higher concentrations than in the grain, averaging 16.9 ppm (DON) and 42.9 ppm (ZEA), but the

concentrations of these mycotoxins in chaff were poorly correlated with resistance (r=0.32 and r=0.37; DON and ZEA, respectively). In the straw samples, DON concentrations averaged 3.5 ppm, but, surprisingly, the ZEA concentrations were very high, averaging 55.5 ppm across all 60 entries. The correlation with resistance was much lower (r=0.21), for concentrations of these mycotoxins in the straw than in the grain, indicating that *Fusarium* mycotoxin concentrations in the chaff and straw cannot be reliably predicted by the scab resistance level of the cultivar.

Conclusions: The finding of significant levels of vomitoxin and zearalenone in the wheat straw is potentially clinically relevant to livestock producers.

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P-91 INHIBITION OF OCHRATOXIN A AND HYDROLYTIC ENZYME PRODUCTION BY PROPYLPARABEN ANTIMICROBIAL ON *ASPERGILLUS* SECTION *NIGRI* STRAINS.

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Background: Ochratoxin A (OTA) is one of the most important fungal toxic metabolite of worldwide concern for human and animal health, due to its implication in a diverse range of toxicological effects, including renal toxicity, mutagenicity, teratogenicity and immunotoxicity (1). In previous works in Argentina Magnoli, et al., (2,3) informed the prevalence of OTA producer strains belonging to *Aspergillus* section *Nigri* and variable percentage of storage peanut grain samples contaminated with OTA.
Antimicrobials as propyl paraben (PP) are used safely as traditional fungicidal substitutes for control of fungal species development in several food and agricultural products (4,5). Previous studies demonstrated that this antimicrobial could control fungal growth, fumonisin and aflatoxin production on natural substrates (6,7,8,9). Correlations have been found for fungal contamination of grain with the level of ergosterol and Magan (10) hypothesized that the specfic activity of hydrolytic enzymes was a good early indicator of the activity of spoilage moulds. Thus quantifying enzyme production and its inhibition, is also a good indicator of effectiveness or treatments.

Aim: evaluate the effect of Propylparaben (PP) on ochratoxin A production and his impact on hydrolytic enzyme activity of *A*. section *Nigri* strains.

Materials and Methods: fungal strains: the fungi used in this study were two strains isolated from peanut grains: *Aspergillus carbonarius* (RCP G) and *A. niger* aggregate (RCP 42). Culture medium: peanut meal extract agar was prepared at 2% (w/v). The water activity of the basic medium was adjusted to 0.98, 0.95 and 0.93 with known amounts of glycerol (11). Antimicrobial: the antimicrobial used was propyl 4-hydroxybenzoate (PP); and was added to the culture media to obtain the required concentrations (1, 5, 10 and 20 mmol l^{-1}). Inoculation and incubation conditions: the media for each treatment were centrally needle-inoculated using a sterile loop, with fungal suspended in soft agar. Inoculated Petri dishes of the same a_W were sealed in polyethylene bags and incubated at 25ºC for four weeks. Ochratoxin A detection: three discs of agar from the each treatment plates were removed and extracted with 1 ml of methanol. The mixture was centrifuged and the solutions were filtered, evaporated to dryness, re-dissolved in mobile phase and the extract injected into the HPLC (12). Enzymes activity detection: three discs of agar from the each treatment plates were removed at 0, 24, 48, 72 and 96 h. and placed with potassium phosphate extraction buffer. The bottles were shaken, washing, decanted and centrifuged at 4000 rpm and 4 ºC. The supernatant was removed (13) and the total activity of ∝-D-galactosidase and β-D-glucosidase was assayed using p-nitrophenyl substrates (Sigma Aldrich). The enzyme activity was measured, using a MRX multiscan plate reader (Dynex Technologies Ltd, Billinghurst, UK), by the increase in optical density at 405 nm caused by the liberation of p-nitrophenol by enzymatic hydrolysis of the substrate.

Results and Discussion: Lowest levels of antimicrobial (1 mmol l^{-1}), stimulated the OTA production over all the range of environmental conditions assayed. However, at concentrations higher or equal than 5 mmol l^{-1} of PP, the toxin production was totally inhibited.

In all the treatments PP produced a significant reduction in the total activity of ∝-D-galactosidase and β-D-glucosidase under the different a_W treatments after 96 h in both strains assayed. The inhibition of β-D-glucosidase activity was higher at 20 mmol l^{-1}, 0.95 a_W and 96 h, with 73 y un 80 % for *A. carbonarius* y *A. niger* aggregate respectively. For ∝-D-galactosidase activity, the inhibition percentage was around 75%, at the same conditions ($p < 0.001$).

Conclusion: This study has shown that the inhibition activity of some hydrolytic enzymes may play an important role in enabling these ochratoxin-producing *Aspergillus* section *Nigri* strains to rapidly colonise peanut over a range of water availability conditions.

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P-92 EFFICACY OF ANTIOXIDANT ON OCHRATOXIN A PRODUCTION AND HYDROLYTIC ENZYME ACTIVITY BY *ASPERGILLUS* SECTION *NIGRI* STRAINS.

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Background: *Aspergillus* section *Nigri* species are contaminant of several stored cereal grains and oilseeds (1,2). In last years, these species have acquired interest by their ability to produce ochratoxin A (OTA) (3), a potent nephrotoxin also known to be teratogenic, immunosuppresive and carcinogenic. The use of food grade antioxidants could be an alternative strategy to control of toxigenic fungi in peanut grains; and it is considered as safe (GRAS) chemical by the Food and Drug Administration in the USA. Previous studies demonstrated that these antioxidants as butylated hydroxyanisole (BHA) could control fungal growth, fumonisin, aflatoxin, and OTA production on culture media and on natural substrates (4; 5; 6; 7; 8). We know that on peanuts infected by *Aspergillus* species activity of certain hydrolytic enzymes are indicative of early fungal activity. Thus quantifying enzyme production and its inhibition, is also a good indicator of effectiveness or treatments.

Aim: The objectives of this work were to evaluate the efficacy of Butylated hydroxyanisole (BHA) on control of ochratoxin A production and the impact of these treatments on hydrolytic enzyme activity of *A*. section *Nigri* strains.

Materials and Methods: fungal strains: the fungi used in this study were two strains isolated from peanut grains: *Aspergillus carbonarius* (RCP G) and *A. niger* aggregate (RCP 42). Culture medium: peanut meal extract agar was prepared at 2% (w/v). The water activity of the basic medium was adjusted to 0.98, 0.95 and 0.93 with known amounts of glycerol (9). Antioxidants: the antioxidant used was 2, 3-tert-butyl-4-hidroxianisol (BHA); and was added to the culture media to obtain the required concentrations (1, 5, 10 and 20 mmol l^{-1}). Inoculation and incubation conditions: the media for each treatment were centrally needle-inoculated using a sterile loop, with fungal suspended in soft agar. Inoculated Petri dishes of the same a_W were sealed in polyethylene bags and incubated at 25ºC for four weeks. Ochratoxin A detection: three discs of agar from the each treatment plates were removed and extracted with 1 ml of methanol. The mixture was centrifuged and the solutions were filtered, evaporated to dryness, re-dissolved in mobile phase and the extract injected into the HPLC (10). Enzymes activity detection: three discs of agar from the each treatment plates were removed at 0, 24, 48, 72 and 96 h. and placed with potassium phosphate extraction buffer. The bottles were shaken, washing, decanted and centrifuged at 4000 rpm and 4 ºC. The supernatant was removed (11) and the total activity of ∝-D-galactosidase and β-D-glucosidase was assayed using p-nitrophenyl substrates (Sigma Aldrich). The enzyme activity was measured, using a MRX multiscan plate reader (Dynex Technologies Ltd, Billinghurst, UK), by the increase in optical density at 405 nm caused by the liberation of p-nitrophenol by enzymatic hydrolysis of the substrate.

Results and Discussion: the lowest BHA concentration (1 mmol l^{-1}) stimulated OTA production in RCP 42 strains. With 10 mmol l^{-1} of the antioxidant a significant reduction (33–100%) in the toxin production by *A. carbonarius* strains was detected at 0.980 and 0.930 a_W at 25 ºC, respectively. Also showed a important reduction in OTA production at 10 mmol l^{-1} of antioxidant. However, a_W had an important influence on activity of these dominant enzymes produced by both *A.* section *Nigri* species. In all the treatments BHA produced a significant reduction in the total activity of ∝-D-galactosidase and β-D-glucosidase under the different a_W treatments after 96 h in both strains assayed. The ∝-D-galactosidase activity was least affected by a_W, while β-D-glucosidase activity was higher at 0.98 a_W, but did not show significant difference at 0.95 and 0.93 a_W, ($p < 0.001$).

Conclusion: This study has shown that the inhibition activity of some hydrolytic enzymes may play an important role in enabling these ochratoxin-producing *Aspergillus* section *Nigri* strains to rapidly colonise peanut over a range of water availability conditions.

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Poster Section IX: Fungal and Mycotoxin Control.

Wedneday June 30

Group 18 (P-93 to P-97): Each presentation in 10 min.

P-93 STUDY OF PROTEASE INHIBITORS ROLES ON PEANUT SEED AFLATOXIN CONTAMINATION

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Background: Aflatoxin contamination is one of the main factors affecting peanut seed quality. One of the strategies to decrease the risk of peanut aflatoxin contamination is the use of genotypes with resistance to *Aspergillus* infection. In a recent study with the objective to understand the virulence mechanisms of *A. flavus* y *A. parasiticus*, we reported that protease production by these fungi is involved in peanut seed infection and aflatoxin contamination resulting in seed tissue damage, affecting seed viability and facilitating the access of fungi through the testa (Asis, 2009).

Aims: In the present work we proposed to evaluate the function of peanut protease inhibitors (PI) during *Aspergillus* infection and aflatoxin contamination.

Material and Methods: For this study we used a PI337394 and Florman cultivars previously characterized as resistant and susceptible to Aspergillus infection and aflatoxin contamination in “in vitro” infection assay (Asis 2005). Peanut seeds were infected with *A. parasiticus* and then fungal colonization, aflatoxin production (Asis, 2005) and PI activity (Sarath, 1989) were determined. We also measured the PI activity in six infected seed cultivars.
PI extract of resistant cultivar were incubated with *A. parasiticus* spores during 24 hs. to evaluated antifungal activity. Also, the resistant cultivar PI were separated and detected by reverse zymography (Le 2004). In parallel an assay were carried out on the polyacrilamide gel to detect an antifungal activity.
To evaluate the inhibitor activity of PI on *Aspergillus* proteases, a zymography (Asis, 2009) of *Aspergillus* extracellular proteases were incubated with a PI extract of resistant cultivar or a commercial proteases inhibitors mix as a control.

Results and discussion: The results showed a negative correlation between fungal colonization or aflatoxin production and PI activity. Infected seed of resistant cultivar showed an increase of PI activity than uninfected ones, whereas infected seeds of susceptible cultivar showed a decrease of PI activity than the uninfected ones. The PI extract from resistant cultivar exhibited an antifungal activity. The protein separation by electrophoresis showed that PI migrate to the same region where antifungal activity was observed on the gel. Finally, the peanut PI incubated with *Aspergillus* proteases showed a complete inhibition of proteases activity whereas a partial inhibition was observed by IP commercial mix. This results suggest that PI are related to mechanism of peanut seed to diminish the aflatoxin contamination. We proposed that PI in the resistant cultivar are produced as a response to *Aspergillus* infection inhibiting the *Aspergillus* proteases synthesized during seed colonization and probably inhibiting the fungal growth. Actually we are purifying and identifying the proteases inhibitors of peanut seed of resistant cultivar. Thus, protease inhibition during seed infection is shown as an opportunity to reduce aflatoxin contamination

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P-94 INTERACTIONS BETWEEN *SACCHAROMYCES CEREVISIAE* CELL WALL AND AFB_1

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Background. The contamination of animal feed with mycotoxins represents a worldwide problem for farmers. Mycotoxin containing feed can cause serious diseases in farm animals resulting in suffering and even death and thus can cause substantial economic losses (Huwig et al. 2001). Several strategies are available for the mycotoxins detoxification and can be classified as physical, chemical, physicochemical and (micro) biological approaches (Varga and Toth, 2004). One of the most effective methods for controlling mycotoxin hazards in animal husbandry is based on the use of yeast cell wall (YCW) that adsorb mycotoxins, which can be used to reduce the bioavailability of mycotoxins in the digestive tract and alleviate their adverse effects on animals.

Aim. To evaluate the AFB_1 adsorption capacity of a commercial preparation of *S. cerevisiae* YCW using adsorption isotherms.

Materials and methods. Interaction assays between AFB_1 and YCW were performed at pH 2 (50 mL of potassium chloride 0.2 M and 13 mL of hydrochloric acid 0.2 M) and pH 6 (100 mL of potassium phosphate bi acid 0.1 M and 11.2 mL of sodium hydroxide 0.1 M) at 37°C. An aliquot of 500 µL (2 µg/mL) YCW was added to each Eppendorf tube containing 500 µL of 2; 5; 7,5; 10 and 15 µg/mL AFB_1 solution. Each Eppendorf was introduced into a centrifugem Labor 2K15 centrifuge (Sigma) at 37°C with mechanical agitation for 30 min. Eppendorfs were then centrifuged for 10 min at 14.000 rpm, the supernatant was taken and evaporated to dryness under gentle stream of nitrogen gas and analyzed by HPLC using the methodology described by Trucksess *et al*. (1994). Each adsorption test was performed in duplicate and controls were performed. Curves representing the amount of bound AFB_1 as a function of the amount of added AFB_1 were plotted according to the mathematical expressions proposed by three theoretical models (Langmuir, Frumkin-Fowler-Guggenheim and Hill), selected according to the isotherms form.

Results and Discussion. All adsorption isotherms were found to be clearly sigmoid can be adjusted by both, the model of Hill as the FFG. These mathematical models explain situations in which cooperative phenomena occur. The Hill model arises from an extrapolation of the theory that explains enzymatic reactions and therefore assumes that the adsorbent surface there is “n” binding sites. The association of the first adsorbato triggers the immediate association to the remaining sites. The FFG model takes into account the existence of interactions between adsorbed molecules considers that the energy of adsorption depends on the degree of coating of the adsorbent. There were no appreciable changes of isotherms at the studied pH. The YCW adsorption capacity at pH 2 and pH 6 was 1.40 and 2.83 ($g\ g^{-1}$) with an adsorption constant of 0.18×10^{-6} (M^{-1}) and 0.12×10^{-6} (M^{-1}), respectively, using the Hill model (R^2= 0.964 and 0.996). Similar values of adsorption capacity were obtained when the

FFG model was applied. However adsorption constants were lower; at pH 2 and pH 6 constant values of 0.04 x 10^{-6} M^{-1} in both cases (R^2= 0.975 and 0.953) were obtained.

The Hill model has been proposed to explain the shape of adsorption isotherms on yeast cell wall and extracts derived from them (Yianninkouris y col., 2003). This model showed that interaction between the AFB_1 and the YCW is cooperative and supports the hypothesis that the three-dimensional conformation mobility of YCW is probably important in the adsorption event.

Conclusion. The YCW was able to adsorb AFB_1 in appreciable quantities, through a cooperative interaction mechanism. This mode of data representation is more informative of physical and chemical mechanisms involved in the linkage between YCW and AFB_1.

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P-95 REDUCTION OF AFLATOXIN B1 IN PEANUT (*ARACHIS HYPOGAEA* L.) BY *SACCHAROMYCES CEREVISAE*

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Background: Aflatoxins are secondary metabolites produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius* causing various acute and chronic intoxications in human and animals. Aflatoxin B_1 (AFB_1) has been classified as carcinogenic in humans, leading to the appearance of hepatocarcinoma. In tropical developing countries the loss due to post harvest diseases represents a major economical burden and fungal decay is one of the major factors contributing to loss in stored gains. Although various prevention strategies have been implemented in many countries, they were not enough to manage the problem efficiently in the developing world.

Aim: This research was conducted to determine the effect of *S. cerevisiae* in aflatoxin B1 content in peanut kernels, after inoculation with *A. parasiticus*, a strong producer of aflatoxins.

Materials and Methods: *S. cerevisae* YEF 186, isolated from sugarcane fermented; *A. parasiticus* IMI 242695 (International Mycological Institute, UK) isolated from foodstuffs and peanuts seeds cultivar IAC Caiapó and IAC Runner 886 cultivated in Instituto Agronômico de Campinas (SP), Brazil, 2005/2006.

Inoculation: Erlenmeyer flasks 250 mL containing whole peanut seeds of each cultivar (15 g) were divided into the following treatments: (1) Seeds were soaked with 2.5 mL of 10^6 spores/mL of *A. parasiticus* and 2.5 mL of 0.1% Tween 80 as positive control; (2) Seeds were soaked with 2.5 mL of 10^6 spores/mL of *A. parasiticus* and 2.5 mL of 3.2×10^7 cells/mL of *S. cerevisiae*. Simultaneous addition of *A. parasiticus* and *S. cerevisiae*; (3) Seeds were soaked with 2.5 mL of 10^6 spores/mL of *A. parasiticus* and 2.5 mL of 3.2×10^7 cells/mL of *S. cerevisiae*. Addition of *A. parasiticus* was done after 3 h of *S. cerevisiae* addition. Each treatment was replicated five times for each cultivar. All the Erlenmeyer flasks were incubated at 25° C for 7 and 15 days.

Aflatoxins analysis: The aflatoxin B1 concentration was quantified by thin layer chromatography (densitometry/366 nm; Valente Soares and Rodriguez-Amaya, 1989).

Results and Discussion
The table 1 shows the results of the analysis and the percentage of aflatoxin B1 reduction in the peanuts samples after inoculation with *A. parasiticus* and *S. cerevisiae*.

Table 1. Levels of aflatoxin B_1 in peanut seed, cultivars IAC Caiapó and IAC *Runner* 886, inoculated with *A. parasiticus* IMI 242695 (1.0×10^6 spores/mL) and *S. cerevisiae* YEF 186 (3.2×10^7 cells/mL), and incubation for 7 and 15 days at 25°C.

Cultivar	Incubation (days)	Treatment: Control (only fungi) Afla B_1 (µg/kg)	Treatment: Addition of Fungi and Yeast, Simultaneously Afla B_1 (µg/kg)	Treatment: Addition of Fungi and Yeast, After 3 h Afla B_1 (µg/kg)
	7			
IAC *Runner*		515 Aa	231 Ab (55.1%)	248 Aa (51.8%)
IAC *Caiapó*		641 Aa	547 Aa (30.2%)	164 Ba (79.0%)
Coefficient of Variation (%)			10.1	
	15			
IAC *Runner*		6452 Aa	6390 Aa (0.1%)	3056 Aa (52.6%)
IAC *Caiapó*		3033 Ab	1385 Ab (54.3%)	1337 Ab (55.9%)
Coefficient of Variation (%)			7.5	

%: Percentage of aflatoxin B1 reduction is presented in parenthesis.

Means followed by the same letter uppercase, on the line, and lowercase, in column, within the periods the seven and fifteen days, are not significantly ($P>0.05$) different according to the Tukey test.

Conclusion: It was shown for the first time that, aflatoxin B1 contamination in peanut may be reduced by the presence of *S. cerevisiae*. At the same time the reduction of aflatoxin production represents a promising strategy for postharvest contamination control.

Acknowledgments: This research was supported by CNPq (Conselho Nacional Pesquisa e Desenvolvimento) and FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais).

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P-96 USE OF DEATH CONIDIA OF *Aspergillus niger* AGGREGATE AS ZEARALENONE AND AFLATOXIN B_1 ADSORBENT

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Background. Mycotoxins are well-know natural contaminants in foods and feeds. In animal husbandry, the consumption of mycotoxin contaminated diet may induce acute and long term chronic effects resulting in a teratogenic, carcinogenic (mainly for liver and kidney), estrogenic or immunosuppressive impact, in addition, poor feed conversion, diminished body weight gain, increased disease incidence due to immune suppression, and interference with reproductive capacities (Mellor, 2001). One of the most effective methods for controlling mycotoxin hazards in animal husbandry is based on the use of specific materials that adsorb mycotoxins, thus limiting their bioavailability in the body (Miazzo et al. 2005). Conidia of black aspergilli as bioadsorpbents are potentially useful in reducing the toxic effects of mycotoxins in animal production. Physicochemical properties of the surface of conidia make them potential candidates as adsorbents of mycotoxins.

Aims. To evaluate the zearalenone (ZEA) and aflatoxin B_1 (AFB_1) adsorption capacity of death conidia from two non-toxicogenic *A. niger* aggregate strains.

Materials and methods. Conidia were obtained from two strains of *A. niger* aggregate (RC84 and RC104). Strains were grown in Czapek yeast extract agar at 28ºC for 7 days in dark. After the incubation period, a loop of conidia was harvested from the colony surface and placed in a tube. Consecutive 3 washes were done with distilled water to remove any impurities and mycelium. Dead conidia were obtained through boiling living spores in distilled water for 15 min and centrifuged at 5000 rpm, discarding the supernatant. A suspension of dead conidia was inoculated on MEA and incubated at 25°C for 7 days and used as negative control. Concentration of dead conidia ($1x10^7$ conidia mL^{-1}) was performed using a Neubauer chamber. Conidia were resuspended in solution at pH 2 (50 mL of potassium chloride 0.2 M and 13 mL of hydrochloric acid 0.2 M) and pH 6 (100 mL of potassium phosphate bi acid 0.1 M and 11.2 mL of sodium hydroxide 0.1 M) for the subsequent use in the adsorption test. The adsorption test was performed using a concentration of $1x10^7$ conidia mL^{-1} at pH 2 and pH 6, as proposed by Bejaoui et al. (2005). An aliquot of 500 μL of conidia concentration was added to each Eppendorf containing 500 µL of 0.5, 5, 10, 20 and 50 µg/mL of ZEA and 0.1, 0.25, 0.5, 1; 2.5 and 5 µg/mL of AFB_1, respectively. Each Eppendorf was introduced into a centrifugem Labor 2K15 centrifuge (Sigma) at 37°C with mechanical agitation for 30 min. Eppendorfs were then centrifuged for 10 min at 14.000 rpm, the supernatant was taken and evaporated to dryness under gentle stream of nitrogen gas. Each adsorption test was performed in duplicate and controls were performed. Extracts were quantified by high-pressure liquid chromatography (HPLC). Curves representing the amount of bound ZEA or AFB_1 as a function of the amount of added ZEA and AFB_1 were plotted according to the mathematical expressions proposed by three theoretical models (Langmuir, Frumkin-Fowler-Guggenheim and Hill), selected according to the isotherms form.

Results and discussion. Differences among isotherm forms were observed when the pH waschanged. For RC104, the highest maximum adsorption (Γmax) was observed at pH 2 (2.8 x 10^{-6} g conidia^{-1}), meanwhile, the situation was reversed when RC84 was used as adsorbent (2.23 x 10^{-6} g conidia^{-1}) at pH 6. The efficiency of ZEA adsorption depended on the strain from which the conidia were obtained and could be attributed to differences in their chemical composition. Considering that conidia are hydrophobic structures, since they have deposits of fat and a hydrophobic proteins, their interaction with ZEA might be due to the lipophylic nature of this toxin. The conidia isolated from the black aspergilos strain RC104, were able to adsorbed AFB_1 at pH 2, but did not show any considerable adsorption at pH 6. The maximum adsorption of AFB_1 by conidia was 0.175 x10^{-6} g conidia^{-1}. Conidia of black aspergilos strain RC84 were unable to adsorb the AFB_1 in all conditions studied.
Since there are not published works on the uptake of mycotoxins by conidia analyzed by mathematical models, this report interprets the results using three different mathematical models. The Hill model is applied to sigmoid isotherms as type L. Its mathematical expression includes the dissociation of the constant (KD), the maximum adsorption (Γmáx) and the minimum number (n) of binding sites required for cooperative adsorption. The inverse of KD is the constant of adsorption called (β). Besides comparable adjustments are obtained with the different models, for purposes of comparison, Hill's model was chosen to explain the results of this report study. The values of Γmáx and β obtained using the Hill model could be used together with the affinity to define and describe the entire phenomenon. The value n was considered more as an indication of the occurrence of a cooperative interaction than as a number of active sites.

Conclusion. This study shows a novel tool to be applied in the adsorption of ZEA with importance in livestock production.

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Miazzo R, Peralta M F, Magnoli C, Salvano M, Ferrero S, Chiacchiera S M, Carvalho E C Q, Rosa C A R and Dalcero A (2005). Efficacy of Sodium Bentonite as a Detoxifier of Broiler Feed Contaminated with Aflatoxin and Fumonisin. *Poultry Sci.* 84:1-8.

P-97 Aflatoxin B_1 adsorption by yeast cell wall from commercial origin®

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Background. Among more than 300 mycotoxins described as yet, aflatoxin B_1 (AFB_1) and the group of fumonisins (FBs) are the toxins of major concern in tropical and sub-tropical regions. Aflatoxins are toxic secondary metabolites produced by species of *Aspergillus* genus, mainly *A. flavus* and *A. parasiticus* and its effects are carcinogenic, mutagenic, teratogenic and hepatoxic. Aflatoxin B_1 is one of the most potent known hepatocarcinogens. Mycotoxin contamination of feed is a serious problem because they reduce feed consumption, decrease growth rate and reduce immunity. Besides the health aspects, which involves the presence of toxins or toxic metabolic products in food and / or by-products such as meat intended for human consumption, its presence in animal feed has also important economic connotations causing losses by increasing mortality and production (Rosa et al., 2001, 2006). One of the most effective methods to control risks of mycotoxins in animal husbandry is based on the use of specific materials that adsorb mycotoxins. These substances adsorb the toxins in the gastrointestinal tract to form insoluble complexes that are eliminated in the feces. Thus, the toxic effects are diminished by reducing the bioavailability of mycotoxins. In particular, the use yeast cell β-glucans and mannans- walls (PL), mainly composed of oligosaccharides. They are usually introduced as a food additive in the animal production industry since the 90s.

Objective. To evaluate the efficacy of a commercial yeast cell wall to adsorb AFB_1.

Materials and methods. Adsorbent: a yeast wall of commercial origin (Safmannan ® - Saf do Brasil - Agricultural Division) was resuspended in buffer at pH 2. **pH 2 buffer solution:** 50 mL of a solution of potassium chloride (KCl) 0.2 M was added 13 mL of a solution of hydrochloric acid (HCl) 0.2 M. **Aflatoxin B_1 Solution:** 10 mg of AFB_1 (Sigma) were resuspended in methanol (MeOH) to obtain a solution of 2 mg/mL. From this, the toxin was diluted to obtain the required concentrations for each test. **Adsorption test:** to determine the potential adsorption, saturation isotherms were previously made with different concentrations of the mycotoxin (250, 200, 100, 50, 10, 5, 2, 1 and 0, 1 mg/ml). Then, adsorption isotherms were made between the PL (2 mg/ml) with AFB_1 (15,346, 10:33, 7:34, 2:08 and 4.83 mg/ml). Tests were assayed at pH 2 and 6, in triplicate. All mycotoxin concentrations were evaluated using high performance liquid chromatography (HPLC).

Results and Discussion. Figures 1 and 2 show the adsorption isotherms at pH 2 and 6, respectively. Visual inspection shows a S-type isotherm, following a Hill model that explains the cooperative adsorption isotherm between the toxin and the adsorbent. The mathematical expression of the adjustment equation is $\theta = \theta_{max} [ZEA]^n / k_D^n + [ZEA]n$ where θ is the mass of adsorbed ZEA by g of PL, n the number of sites and k_D constant adsorption site.

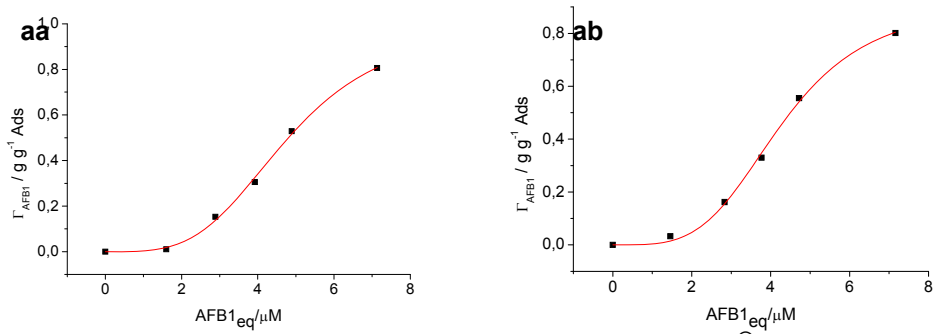

Figure 1. Adsorption isotherm of AFB_1 by Safmannan®, (a) pH 2, (b) pH 6.

Among the most important parameters we consider a saturation value of 0.918 ± 0.056 mg of adsorbed AFB_1/g PL at pH 2 and 1.002 ± 0.089 mg of adsorbed AFB_1/g PL at pH 6. The association constant per site was 0.233 ± 0.044 and 0.208 ± 0.063 ng/mL^{-}1 (Table 1).

Table 1. Setting parameters obtained by Hill model to evaluate the adsorption isotherms from AFB_1 and Safmannan® at different pH.

Adsorbent	ppH	k_d (μM)	$10^{-6}\beta$ (M^{-1})	Γ_{max} (g/g)	*n*	N	RR^2
Safmannan®	D2,0	4,291±0,189	0,233±0,044	0,918±0,056	3,829±0,453	5	00,996
	S6,0	4,806±0,303	0,208±0,063	1,002±0,089	3,619±0,459	5	00,996

k_d = dissociation constant, β = association constant, Γ_{max} = masimum capacity of adsorption, *n* = number of sites for coorperativism, N = number of curve points. Each point is the mean of triplicates.

Conclusion. Aflatoxin B_1 was efficiently bound by PL through a cooperative attraction mechanism. This result shows the potential of this PL to prevent the toxic effects caused by the intake of ZEA.

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Poster Section IX: Fungal and Mycotoxin Control.

Wednesday June 30

Group 19 (P-98 to P-102): Each presentation in 10 min.

P-98 PHOTOCATALYTIC DEGRADATION OF FUMONISINS (B1 and B2) IN MAIZE

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Abstract

Maize has been generally contaminated by mycotoxins during the cultivation, storage, or in distribution process, produced by fungal pathogens (*Fusarium verticillioides and F. proliferatum*. The different methods such as dilution, molturation, sifts, and ultraviolet (UV) radiation have been used to decontaminate mycotoxins, which present in cereal. It has been demonstrated that although the mycotoxins can be hydrolyzed, it is unable to eliminate completely by even several washes. Photocatalytic degradation is an efficient method to oxidize several organic contaminants in soil and foods; thus, the photocatalytic degradation is considered to eliminate fumonisin B_1 and B_2 (FB_1 and FB_2) in maize. The objective of this work is to determinate the effect of the photo-degradation over FB_1 and FB_2 in presence and absence of photocatalyst (TiO_2) to consider as a new chemical control technique and also to determine the role of: a) the matrix complexity, b) fat content, c) humidity of maize samples, and d) titanium dioxide (TiO_2) in fumonisins degradation. The fumonisins degradation results indicate that UV or gamma radiations are favored the oxidation when the temperature of fumonisins treatment increases; furthermore, in the presence TiO_2, the decomposition of fumonisins is increased. During the treatments, the fumonisins liberation from the maize was occurred that is the reason why the inconsistent degradation curve was obtained. However, the effect of UV/TiO_2 or gamma radiation depends with the fat content present in the sample, meaning that the high fat content in the sample facilitates higher fumonisins degradation, suggesting that the UV/TiO_2 or gamma radiation can be used as alternatives techniques to eliminate the fumonisin levels in maize.

P-99 SÍNTESIS *IN VITRO* DE OCRATOXINA-A POR AISLADOS VENEZOLANOS DE *ASPERGILLUS NIGER* DE DIFERENTES SUSTRATOS

In vitro synthesis of ocratoxin-A by venezuelan strains of *Aspergillus niger* isolated from different subtrates

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Antecedentes. *Aspergillus niger* van Tieghem tiene un amplio uso en la agroindustria por la capacidad amilolítica, celulolítica y lipolítica que posee, entre otras. Además, es utilizado como suplemento en la dieta de algunos animales por el contenido de proteínas y vitaminas de la biomasa que se desecha. Es un moho considerado como GRAS (*generally recognized as safe*) por la FDA (Food Deparment of Agriculture USA). Sin embargo, se ha encontrado síntesis de ocratoxina-A (OTA) en algunos aislados. Esta micotoxina tiene efectos nefrotóxicos, inmunosupresivos, neurotóxicos y teratogénicos, es clasificada como cancerígeno tipo 2 según el Centro Internacional de Investigaciones contra el Cáncer (IARC) y su producción se asocia principalmente con *Aspergillus ochraceus* Wilhelm. Sin embargo, otras especies de la sección *Nigri* como *Aspergillus carbonarius* Bainier Thom y algunos aislamientos de *A. niger* suponen un riesgo a la salud humana y animal como productores de esta toxina (Astoreca *et al.*, 2007). La producción de OTA por aislados con potencial agroindustrial limitaría su uso en derivados alimenticios.

Objetivo. Esta investigación se realizó con el fin de evaluar *in vitro* la capacidad ocratoxigénica de trece aislados venezolanos de *A. niger* obtenidos de diferentes sustratos naturales.

Materiales y Métodos. Los aislados de *A. niger* fueron obtenidos de granos de algodón, arroz, frijol, girasol, lenteja, maíz (02), maní y sorgo, así como de cebolla, lodo cervecero, pan y suelo, empleando el método de siembra directa de granos previamente desinfectados y el método de aislamiento por dilución en placas en el resto de los sustratos. En todos los casos se utilizó malta-sal-agar como medio de cultivo y se incubó durante 7 días a 24$\pm$2ºC. La producción *in vitro* de OTA se evaluó cultivando los aislados en el medio arroz estéril durante 14 días de incubación a 15ºC. OTA fue cuantificada usando columnas de inmunoafinidad (Ochratest®, Vicam Sci. Tech.) y fluorometría. Los resultados fueron analizados con el programa estadístico SAS® mediante ANOVA y comparación de medias por la prueba de rangos múltiples de Duncan, bajo un diseño totalmente aleatorizado con tres observaciones por tratamiento.

Resultados y Discusión. Se obtuvo producción de OTA en todos los aislados de *A. niger* (Samson *et al.*, 2004). El análisis de varianza arrojó diferencias al 1% en la producción de OTA entre aislados, con un coeficiente de variación de 19.8%. Los contenidos promedio de OTA en el medio arroz variaron desde 0.5 en el aislado de lenteja hasta 12.5 ng/g (ppb) en el de maní, este último diferente de todos los demás en la prueba de comparación de medias, excepto del aislado de sorgo. El segundo grupo de mayor producción de OTA lo conformaron los aislados provenientes de sorgo (10.7 ng/g), maíz II, suelo, frijol y lodo cervecero (8.3 ng/g). En el tercer grupo solo se ubicó el aislado maíz I (5.2 ng/g). Finalmente los aislados de pan (3.2 ng/g), arroz, girasol, algodón, cebolla y lentejas (0.5 ng/g) fueron los de menor producción de OTA.

Conclusión. Aun cuando todos los aislados de *A. niger* probados en este estudio *in vitro* produjeron OTA, podrían ser utilizados en procesos de bio-conversión de desechos agroindustriales y en la posterior formulación de alimentos para animales sin que represente riesgos a su salud, toda vez que la biomasa del moho sería solo una fracción de las raciones.

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P-100 ADSORPTION OF FUMONISIN B1 WITH ALUMINOSILICATES IN "*IN VITRO*" ASSAYS

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Background: Fumonisins are a distinct group of mycotoxins produced by several field fungi, including *Fusarium verticilloides* and *Fusarium proliferaturatum*. Fumonisin B1 is considered to be the most prevalent and most toxic derivative within the group of fumonisins (EFSA, 2005). The presence of fumonisins, especially fumonisin B1 (FB1) in corn, is a big concern in the poultry industry. Specially in Brazil, where it has been reported that levels as low as 5 mg/kg (ppm) of FB1 cause statistically significant differences in the weight of the birds that are given feeds contaminated with this toxin, compared with the control group that is free of contamination. In Mexico it has been reported that this situation is not present with the inclusion of 100 ppm of this toxin (Fierro at al., 2009). The scientific literature specifies that minimum levels of 250 ppm are required to cause the problem of weight reduction in animals (EFSA, 2005). It has been published that aluminosilicates are being incorporated into diets for human consumption to reduce the effects of aflatoxins. Likewise, aluminosilicates have been incorporated into diets for birds to reduce the effects of aflatoxins, for more than 20 years. Recently it has been reported that aluminosilicates have been incorporated as non nutritious additives in diets for human consumption aimed at reducing the effect of FB1 (Phillips, et al., 2009).

Aim: The purpose of this work is to evaluate the capacity of 5 commercial products to adsorb fumonisin B1 at levels of 200mg/kg, in "*in vitro*" assays.

Material and Methods: 5 products were selected for evaluation; these are commercialized in the most important animal feed markets of Latin America (Brazil and Mexico).
The process of "in vitro" adsorption was performed following the method developed in NUTEK: It establishes the contact between mycotoxin FB1 and the adsorbent in a solution of pepsin to pH of 2.0 (the solution is the specified by the AOAC for the analysis of digestibility of protein in flours of animal origin). 3 hours of contact with agitation to 37 °C, this mixture is centrifuged and an aliquot is taken and submitted to HPLC analysis, previous derivatization.

Results and discussion: The capacity of adsorption of the evaluated aluminosilicates on a concentration of 200 ppm of FB1 were: Product A: 98 %, Product B: 73 %, Product C: 71%, Product D: 55 % and product E: 43%. The essays were performed by triplicate and an average of the obtained values is reported. Based on these results an essay will be done later "*in vivo*" to demonstrate the efficiency of adsorbent A.

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Conclusion: Though there is not always a correlation between the "*in vitro*" and "*in vivo*" tests, it is possible that product A has the possibility of being an adapted adsorbent that diminishes the effects of FB1, but it is necessary to perform "*in vivo*" experiments but against more than 200 ppm. Brazil performs these assays with levels below 100 ppm.

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P-101 BIOCONTROL AGENTS AGAINST FUSARIUM HEAD BLIGHT IN WHEAT ISOLATED IN ARGENTINA: VIABILITY AND FUNGICIDE TOLERANCE

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Background: *Fusarium* head blight (FHB) caused by *Gibberella zeae* (anamorph = *Fusarium graminearum*) is a devastating disease that causes extensive yield and quality losses to wheat in humid and semi-humid regions of the world. Different strategies have been used to reduce the impact of FHB, but none of these strategies by themselves are able to substantially reduce the impact of the disease. Biological control offers an additional strategy and can be used as part of an integrated management of FHB. In previous studies 3 bacteria, *Bacillus subtilis* RC 218, *Brevibacillus sp.* RC 263 and *Streptomyces sp.* RC 87B were selected by their potential to control FHB and DON production in greenhouse trials (Palazzini *et al.*, 2007).

Aim: The goal of this work was: - to test the tolerance *Bacillus subtilis* RC 218, *Brevibacillus sp.* RC 263 and *Streptomyces sp.* RC 87B to common fungicides, utilized to control FHB and, - to evaluate the viability and stability of spray dried cell formulations.

Materials and Methods: Three fungicides were used in the bioassay (prothioconazole, tebuconazole and metconazole) at concentrations ranging from 0.5 to 80 µg/ml. Bacterial cells were adjusted to 2 x 10^3 cells ml^{-1} in a haemocytometer chamber. An aliquot of 0.1 ml of each bacterial strain was inoculated in Petri dishes using the spread plating technique containing the different fungicides. Inoculated plates were incubated at 28 ºC for 48 h. Colony counting were done at 24 and 48h. Bacteria were cultured for 48 h under culture media with different a_w and solutes and then spray dried. Spray drying conditions were: 140/55 ºC Inlet/Outlet temperatures, 10 ml/h flow, 40 mbar atomizing pressure and $MgSO_4$ 10 % as protectant. Dried cells were stored at room temperature and 4 ºC and viability was evaluated up to 6 months.

Results and Discussion: *Bacillus subtilis* RC 218 and *Streptomyces sp.* RC 87B showed better tolerance to fungicides than *Brevibacillus sp.* RC 263. Complete inhibition of growth was observed at concentrations of 20 µg/ml for metconazole, 40 µg/ml for tebuconazole and 80 µg/ml for prothioconazole. Dried cells of *B. subtilis* RC 218 retained a viability of 80% (4 x 10^6 cfu/gr) of the initial concentration after 6 months. Viability of *Brevibacillus sp.* RC 263 decreased up to 10^4 cfu/gr.

Conclusion: The results obtained indicate the possibility to use the biocontrol agents in combination with fungicides as part of an integrated management to control FHB of wheat. Spray drying technique was adequate for *B. subtilis* RC 218 formulation.

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P- 102 The work is in Poster 100.

Poster Section IX: Fungal and Mycotoxin Control.

Wednesday June 30

Group 20 (P-103 to P-107): Each presentation in 10 min.

P-103 DESTRUCTION OF SPIKED AFLATOXIN B1 IN DOUGH DURING THE TORTILLA-MAKING PROCESS

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Several studies have shown that different recipes to make tortillas, based on the *nixtamalización* process, are effective in destroying aflatoxin B_1 (AFB_1). The so-called ecological *nixtamalización* reduced AFB_1 levels by 61%, while the traditional recipe led to the elimination of up to 96% AFB_1. Usually, when corn used for human consumption has low levels of AFB_1 (~20 µg Kg^{-1}), the resulting dough utilized to make tortillas will not be contaminated on the other hand, high AFB_1 levels in corn (~252 µg Kg^{-1}) will result in a contaminated dough (~26 µg Kg^{-1} AFB_1). Although the remaining contamination of AFB_1 in the dough can be considered low, the risk for toxicity in humans is still high. Therefore, the objective of this work was to investigate the effects of dough pH, dough resting time before cooking, and cooking temperature for tortillas, on residual aflatoxin B_1 content. In this work, dough was obtained by the traditional *nixtamalización* process of white corn. Ten replicate samples of dough (50g) were spiked with high concentration standard solutions of aflatoxin B_1.Two different dough pH values, four different resting times, and two cooking temperatures were tested. The experiments were repeated three times. All treatments effectively destroyed the spiked aflatoxin. Interestingly, a 98% reduction in aflatoxin content was achieved after a 30 minutes dough resting time at pH 10 followed by cooking at 78° C. The data show that the tortilla making process can destroy the remnants of aflatoxin after the *nixtamalización* process, even when highly contaminated corn is used. At the moment, other Mexican recipes used to prepare corn-based foods are being analyzed.

P-104 PHENOLIC COMPOUNDS AS POTENTIAL ANTIFUMONISIN AGENTS: A STRUCTURE–PROPERTY–ACTIVITY RELATIONSHIP STUDY

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Background: Fungi of the genus *Fusarium* are widely found in plant debris and crop plants worldwide. Several species from this genus are economically relevant by their ability to cause tissue destruction and produce mycotoxins on important crops such as corn, wheat and other small grains on the field, and in storage. *Fusarium verticillioides* and *F. proliferatum* are probably the most important producers of fumonisin B1 (FB1). It has been found to be associated with several animal diseases. With respect to humans, their occurrence in corn has been associated with high incidences of esophageal cancer and liver cancer. In recent years, particular interest has been focused on the potential application of natural products such as monoterpenes and phenolic compounds, to prevent fungal growth and mycotoxins production in grain and cereal. However, little is knowledge about the molecular properties related to the antitoxigenic activity of them.

Aim:

1) To study the effects of phenolic compounds on FB_1 by *Fusarium verticillioides* MRC 4316.

2) To determine the links between the structure and molecular properties and the antitoxigenic activity of the phenolic compounds

Materials and Methods: The phenolic compounds used were: thymol, carvacrol, eugenol, methyl-eugenol, isoeugenol, vanillin, orto-cresol, meta-cresol, para-cresol, creosol, estragol y guaiacol. The MYRO liquid culture was prepared as described Blackwell et al. (1994). It was inoculated with 500 µL of a conidia suspension *of F. verticillioides* MRC 4316. Cultures were incubated with agitation in darkness at 28 ºC for 21 days. The phenolic compounds were inserted into the flask on the 5th day post inoculation, in a final concentration of 1.0 mM. Quantification of FB1 was done as described Shephard, et al. (1990). Multivariate methods of numerical analysis were applied here to determine the links between the structure and molecular properties and the antitoxigenic activity of the phenolic compounds. These are principal components analysis and Canonical correlations analysis. All statistical analyses were calculated by using InfoStat software.

Results and Discussion: All the phenolic compounds evaluated showed some inhibitory effects on FB1 production by *F. verticillioides*. The most active inhibitors were carvacrol, thymol and isoeugenol, followed by eugenol and methyl-eugenol, respectively. The rest of the phenolic compouds evaluated had little effect on FB1 production.

Structural studies have revealed that the 75% of the antifumonisin activity of the phenolic compounds can be explained by the radical scavenger activity (DPPH), the acidity (pka), the Van der Waals surface (vdWSA) and the lipophilicity (Log P) of them.

Conclusions: The results obtained in this work reveal the molecular properties of phenolic compounds, necessary to exercise inhibitory effects on FB1 production. Such findings would allow a large contribution to guide the search for new compounds with antitoxigenic activity.

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P-105 INFLUENCE OF BUFFERS ON AFLATOXIN B1 ADSORPTION ON SODIUM BENTONITE. COMPETITIVE ADSORPTION OF IONS OR pH EFFECT?

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Background: Aflatoxins (AFs), fungi secondary metabolites produced by *Aspergillus Penicillium* and *Fusarium,* are highly toxic and produce both healthy and economic problems. In animal production supplementation of the diet with non-nutritive adsorbent is a useful strategy to reduce problems caused by ingestion of these toxins, especially the most toxic of all (AFB_1). Sequestration of toxins by the adsorbent reduces the bioavailability in the gastrointestinal tract of animals. Studies in vivo and in vitro have demonstrated the ability of sodium bentonite to adsorb AFS from their aqueous solutions through the formation of an inert and insoluble complex that is excreted in the feces.
Aim: To determine the reason on the observed buffer influence on adsorption isotherms of AFB_1 on NaB.
Materials and Methods. Aflatoxin was produced via fermentation of rice by *Aspergillus parasiticus* NRRL 2999, and was purified according to the methodology proposed by AOAC. For the adsorption isotherms 15 dilutions from the AFB_1 stock solution were made in: water brought to pH 6, buffers at pH 6, pH 4 and pH 2 and 0.15M NaCl in buffer pH 2 solutions. A previously characterized NaB (Magnoli y col 2008) was used, 40µL of the sorbent suspension were added to 4 mL of each working AFB_1 solution, taking care that the total incubation period was equal to 1 h. Two replicates were performed for each analytical concentration. Controls of AFB_1 without adsorbent and blanks with adsorbent were also included for comparison. After incubation, the solutions were centrifuged for 5 min at 16,060*g*, and the supernatant was decanted carefully into a clean tube. The AFB_1 concentration in each supernatant was determined by HPLC (Trucksses et al., 1994). Aflatoxin B_1 adsorption was estimated from the amount of unbound (free) toxin remaining in the supernatant after incubation. The data were adjusted according to the theoretical model according to criteria proposed by Hinz (2001).
Results and Discussion: The adsorption isotherms of AFB_1 on NaB are rather sensitive to the influence of buffer. The capacity of the adsorbent to uptake AFB_1 is maxima a pH = 6, followed at pH 2 and minimum a pH 4. In order to find if the reason of such tendency is the pH or the specific influence of the buffer ions, the present study was carried down. A comparison between the curves obtained both in phosphate buffer (pH = 6) and in water brought to pH 6 (conditions of pH-stato) was performed. Both the NaB affinity for the toxin and the capacity (mol of AFB_1/Kg adsorbent) were affected. The shapes of the isotherms were all sigmoid. The experimental data were adjusted according to the Frumkin-Fowler-Gugenheim (FFG) adsorption model (Masel, 1996). This model takes into account the heterogeneity of sites on the NaB surface. From the positive sign of the FFG adjusting parameter “a”, a cooperative adsorption mechanism seems to be operating. In such mechanism some kind of attractive interactions occur between the molecules at the surface with those in solution assisting their adsorption. Phosphates ions ($HPO_4^{=}$, H_2PO_4 y $P_2O_7^{-4}$) strongly interacts with montmorillonite, one of the main components of NaB, increasing the charge density of borders and therefore

also the coagulation of the particles (Lagaly, 2004). The buffer at pH 6 contain $HPO_4^=$ y H_2PO_4 ions which can be adsorbed changing the affinity and/or capacity to sequester AFB_1. Probably, changes in aggregation might favor the adsorption at pH 6. On the other side, the main constituents of the buffer at pH 4 are phthalic acid and potassium hydrogen phthalate. Both compounds have the same characteristics of AFB_1, i. e., plane chemical structures, donor acceptor and chelating properties. Therefore, these adsorbates can interact with the surface in the same way and be competitive adsorbed. An excess of NaCl not only decrease the adsorption uptake capacity but also modify the surface nature of the adsorbent. The shape of the isotherm changes from S to L-type when the concentration of NaCl increases. This change reflects an alteration in the nature of the adsorbent surface. The affinity of NaB for AFB_1 significantly increases meanwhile the capacity of the adsorbent is severe decreased due to the lesser number of availables sites in the surface. Furthermore, NaCl-occupation of sites on the adsorbent surface may inhibit lateral interaction that favors the adsorption of AFB_1 with the increase in the occupancy of sites. The results highlight the sensitivity of the isotherms to the presence of ions that may compete for sites on the adsorbent and therefore block and/or facilitate the uptake of adsorbates

Conclusion: The affinity and uptake capacity AFB_1 is affected by the presence of competing ions on the adsorbent sites blocking and/or altering the interaction of toxin with surface .

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P-106 EFFECT OF BILE AND LOW pH ON OCHRATOXIN A BINDING BY *Saccharomyces cerevisiae* STRAINS.

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Background. Ochratoxin A (OTA) is a secondary metabolite produced by two genera of fungi *Aspergillus* and *Penicillium*. A big concern is about its occurrence in many commodities (feeds, foods and beverages), because it is suspected to be nephrotoxic, teratogenic, hepatotoxic and carcinogenic (Marquardt and Frohlich 1992). Several technologies in food processing may play an important role for reducing the content of OTA in foods and beverages like physical, chemical, and microbiological methods. However, few of them have practical applications (Bata *et al*., 1999; Heilmann *et al*., 1999, Belajová *et al*., 2007; Ringot *et al*., 2007). *Saccharomyces cerevisiae* strains, due to their GRAS status and their use as probiotics, are of particular interest on reducing the bioavailability of mycotoxins (Shetty *et al*, 2006; Var *et al*., 2009). The use of *Saccharomyces cerevisiae* strains to reduce the animal exposure to OTA has a great promise; however additional studies are required as well as GI relevant conditions that effect OTA binding.

Aim. To examine four strains of *Saccharomyces cerevisiae* from animal ecological niches for the ability to bind OTA using adsorption isotherms and the effect of bile and low pH on OTA binding.

Materials and Methods. To determine the effect of exposure to bile salts and low pH on OTA binding of *Saccharomyces cerevisiae* strains, 1 ml of each strain (RC008; RC009; RC012 and RC016) were incubated for 4 h at 37 ºC in YPD broth (30 mL) containing 0.5 % (w/v) bile salts and YPD pH 3 modified with HCl (1M). After incubation, cells were harvested, used in the AFB1-binding assay. .The OTA binding assay was performed according to Bueno *et al*. (2007) with modifications. Solution stock of OTA was suspended in PBS to obtain concentrations of 1; 5; 10; 40 and 100 µg ml^{-1}. Yeasts (10^7cells ml^{-1}) were washed twice with PBS and incubated for 1 h at 37ºC in a shaking bath with 1 ml of PBS OTA. Then, cells were pelleted by centrifugation at 5,000 rpm at room temperature for 15 min, and the supernatant containing unbound mycotoxin was collected and stored at -20ºC for high-performance liquid chromatography (HPLC) analysis using the methodology described by Ringot *et al*. (2007). Each adsorption test was performed in duplicate and controls were performed. Curves representing the amount of bound OTA as a function of the amount of added OTA were plotted according to the mathematical expressions proposed by Hill's theoretical model, selected according to the isotherms form.

Results and Discussion. The amount of OTA bound was strain specific with a percentage ranging from 56.7% to 74.24%. The amount of OTA bound by *Saccharomyces cerevisiae* RC0016 was higher than the other examined strains. Growth in the presence of 0.5% bile salts (except RC016) increased OTA binding (71.33% to 78.66%) by *Saccharomyces cerevisiae* strains. Moreover, an increase in the OTA binding when all strains were subjected to pH 3 was observed (75.88% to 83.32%). Therefore, to screen strains of *Saccharomyces cerevisiae* for the ability to bind OTA during passage through the gastrointestinal tract, it is important to evaluate the influence of bile and low pH on

OTA binding. The results presented in this study demonstrate that exposure of yeast strains to gastrointestinal conditions have a significant impact on OTA binding. In general, all strains bound more amount of OTA when they were exposed to bile and low pH. This finding could have an important biological significance, since probiotic yeast are normally exposed to bile secreted in the small intestine, which suggest that probiotics may be more capable of binding OTA in the intestine, and thus reduce OTA bioavailability in the gut.

Conclusion. *Saccharomyces cerevisiae* strains were able to adsorb OTA in appreciable quantities. Exposure of the yeast cells to bile and low pH significant increased OTA binding.

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P-107 INTERACTION OF AFLATOXIN B1 WITH MODEL LIPID BIOMEMBRANES.

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Background:
Most of the mycotoxicoses in human beings and in animals arise after consumption of diets contaminated with any or several fungal metabolites. Aflatoxin B1 (AFB1) absorption from the gastrointestinal system results in its immediate transport to the liver, which may contribute to aflatoxin hepatotoxicity. The lipophilicity of this mycotoxin is crucial in the determination of its uptake into living cells, including hepathocytes, to then exert its toxic effects. Non-ionic diffusion (Muller & Petzinger, 1988) and membrane transport (Tachampa *et al.*, 2008) appears to be the main entry pathway of AFB1 into cells. However the initial toxin-membrane interactions, and the changes that such process may induce in the latter, are scarcely known.

Aim:
To characterize the interaction of AFB1 with model lipid biomembranes, in order to evaluate the probable modulation of the cell plasma membrane functionality by the toxin.

Materials and methods:
Penetration of AFB1 in lipidic interfaces: 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (dpPC, Avanti Polar Lipids, USA) monomolecular layers (MML) at the air-water interface were prepared and monitored as described (Theumer *et al.*, 2008). The lateral surface pressure (π) was measured by the Wilhelmy plate method. The penetration of AFB1 (Sigma-Aldrich, USA) dissolved in EtOH was evaluated varying: a) the toxin concentration (0-8.4 μM) injected in the subphase of MMLs at similar initial molecular packing ($\pi_i \approx 10$ mN/m), and b) the MML molecular packing (5-35 mN/m) and injecting the AFB1 (3 μM) in the subphase.
Steady state fluorescence: Multilamellar vesicles (MLV) were prepared by hydration of a dpPC film with bidistilled water, intensive vortexing and heating at 50 °C. The fluorescent probes DPH (2 μM) and TMA-DPH (6 μM) were added to the dpPC MLV suspension and incubated for 1 h at room temperature. The effects of AFB1 (0-8.9 μM) on the DPH and TMA–DPH steady-state fluorescence anisotropy were studied. Anisotropy values were calculated from the emission fluorescence intensities at λ_{em} = 430 nm (λ_{ex} = 356 nm) (Theumer *et al.*, 2008).

Results and discussion:
When injected in the subphase of dpPC Langmuir films at similar molecular packing ($\pi_i \approx$ 10 mN/m), the mycotoxin (0-8.4 μM, corresponding to 0-42 μL of EtOH used as vehicle) induced a linear increase in the lateral surface pressure (π) of the MML. Although the addition of EtOH alone, in amounts equivalent to those applied in the AFB1 containing samples, also increased π, its effect was significantly lower than that observed with AFB1 (slope 0.0374 mN/m μL^{-1} EtOH). Moreover, in dpPC Langmuir films the maximal π allowing the AFB1 penetration ($\pi_{cut\text{-}off}$) was $\pi \approx 30$ mN/m, which was determined by

monitoring the increments in the π values seven minutes at the plateau in π value reached after the injection of AFB1 (3 μM) in the subphase of the dpPC MML at different initial molecular packing (π_i 5-35 mN/m). Surface pressure changes may be interpreted either as a toxin penetration in the monolayer and/or an interfacial deformation accompanying the toxin adsorption.

The possible disturbances in the membrane environment due to the incorporation of AFB1 to dpPC MLVs were studied using DPH and TMA-DPH as probes. AFB1 (0-8.9 μM) decreased in a dose-dependent manner, the fluorescence anisotropy of TMA-DPH (from 0.399±0.020 to 0.044±0.002) and of DPH (from 0.341±0.002 to 0.090±0.003). Steady-state fluorescence anisotropy provides information about the organization of the membrane environment around the fluorescent probe. DPH is known to be located within the hydrocarbon chain region of the membrane core and its parent compound, TMA-DPH, stabilizes its DPH moiety at the polar head group region of bilayers. The changes in the fluorescence anisotropy of both probes indicate that AFB1 localize deeply in the bilayers membrane structure with a consequent decrease in the molecular order and an increase in the molecular mobility at the surface, and also affecting the membrane dynamics at the hydrocarbon chain region.

Conclusion:

AFB1 modified the molecular order and mobility of the model lipid biomembranes studied. Then a modulation of the cell membrane functionality should be expected by its sole interaction with the AFB1.

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PHYCOTOXIN SECTION
YUCATAN I
POSTER PRESENTATIONS
(Afternoons 13:30-17:00)

Poster Phycotoxin Section X: Cyanobacterial and Phytoplankton Toxins. Group 21.
Monday June 28
Group 21 (P-108 to P-110): Each presentation in 20 min.

P-108 IN VITRO CITOTOXICITY OF CYANOBACTERIAL CRUDE EXTRACT FROM ANTIOQUIA (COLOMBIA)

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INTRODUCTION: The occurrence of cyanobacterial blooms are a potential dangerous related to water quality, since some species of cyanobacteria produces several toxins (Hudnell 2008). Human and animal illness and in some cases death have occurred from exposure to cyanotoxins, toxic secondary metabolites produced by cyanobacteria, through skin contact and consumption of potable and recreational waters (Oehrle 2010).

Toxicity assays are important to ensure good water quality but only few studies have determined the potential exposition to these toxins (Anjos 2006). The main research has focused on the predictions of influence of environmental factors in the formation of harmful cyanobacterial. (Sarnelle 2010). In recent years, in vitro-toxicity-tests involving the use of cultured cells have been developed to provide a substitute for the mouse bioassay (Masango 2008). In the current study, toxicity of algal samples collected during October of 2009 were measured using Vero and U-937cells assays. Also, liquid chromatography coupled with mass spectrometry (LC-MS) analyses evidenced some cyanotoxins.

MATERIALS AND METHODS

Collection of cyanobacterial samples

From a cyanobacterial bloom occurred at October 2009 at Antioquia /(Colombia), 500 g of material was collected and stored at -4°C until lyophilization.

Extracts preparation

Lyophilized cyanobacterial samples (160 g) were extracted with 1000 mL of 80% methanol (5x200), with ultrasonication for 15 min; after that, solvent was evaporated until dryness in a rotaevaporator. Then extract was separated using silica gel and sephadex colums and finally until obtain 20 fractions, according to tlc.

***In vitro* assay of citotoxicity**

The evaluations were performed in vitro cytotoxicity of 5 cell lines which were: U-937 (human promonocytic cells), Vero (kidney cells from African green monkey), using the enzymatic MTT micromethod. The tests were performed twice with 2 replicates for each concentration tested and the results are expressed as the lethal concentration 50 (LC_{50}) which was calculated by the Probit method. The amphotericin B ® was used as the control of cytotoxicity.

Mass spectrometry

The LC–MS experiments were carried out on an Agilent 1200 binary pump. Injection volumes were 10 µl. The mobile phase consisted of 0.2% formic acid (solvent A) and

acetonitrile (solvent B) with the following linear gradient programme: star with 10%B at 20 min 55% B. Flow rate of 0.8 ml/min.

RESULTS AND DISCUSSION: A high-biomass of cyanobacterial blooms was detected being *Microcystis* sp. was the main microalga; columns chromatography on silica gel and then sephadex brought a good separation of chlorophylls and fatty acids; high concentrations of carotenes were also separated, according to nmr spectroscopy. The last fractions contained several compounds were monitored through HPLC/MS, using mass scan mode to detect the peaks at *m/z* 1045, 1024, 953, 934 corresponding to several compounds reported before by others authors (Prakash 2009). Additionally Microcystins AR, WR, LR were detected in other peaks at m/z 953, 925, 728, 484, 440, 375, 213, 135; 1068, 1040, 934, 599, 626, 375, 213, 135; 728, 682, 599, 375, 213, 135, corresponding to specific fragmentations of each compound.

Although all compounds displayed high toxicity (Table 1), Vero cells were less sensitive than U-937 and main toxicity was detected in fraction 9, which contain nitrogen compounds, according to tlc using Dragendorf's reactive. These cells could be a more accurate and fast method to detect cyanotoxins instead of mice.

Table 1. Results in vitro assay of citotoxicity from cyanobacterial fractions.

	CL_{50} (µg/ml) X ± SD	
Name	U-937	Vero
A4	41.2 ± 6.2	39.7 ± 8.0
A5	> 200.0	> 200.0
A7	84.8 ± 2.8	51.3 ± 6.3
A8	29.7 ± 0.34	> 200.0
A9	16.8 ± 2.0	> 200.0
Amphotericine B	35.9 ± 5.5	61.5 ± 4.0

CONCLUSIONS: Analysis of the microcystins in algae has been successfully accomplished by using both HPLC- MS and bioassays, Both offer good potential for the analysis of microcystins in algae.

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P-109 SEASONAL VARIATION OF TOXICITY IN MARINE MACROALGAE OF VERACRUZ, GULF OF MEXICO

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Background. Algae are consumed by a lot of herbivorous, chemical defense is a powerful barrier against these herbivorous, these substances can be a risk for human consumers and they are an ecological element for the survivability of algae (Lozano, 1991). The chemical defense of algae is a collection of a wide variety of substances (Magallanes *et al.,* 2003). The toxicity of some algae has been an evolutionary factor that have been important in relation to distribution and abundance of these organisms (Lubchenco and Cubit,1980; Hay, 1996).

Aim. The setting up of biological tests using guppy *Poecillia reticulata* and plecostomus *Hypostomus plecostomus* in order to get a toxicity degree comparison related to the algae collecting season.

Materials and methods. Algae were collected in several localities in Veracruz State during February 2006; April 2007; September 2007. The total number of collected algae species was 17. Algae were frozen to avoid decomposition and were transported to laboratory. After, epibiontic organisms were eliminated from algae. The algae were extracted using distilled water, ethanol or acetone. The extracts were used for toxicity essays using guppys, *Poecillia reticulata* and plecostomus *Hypostomus plecostomus.*

Resuts and discussion. Macroalgae present a dissimilar toxicity activity depending on collecting season. *Acantophora* is a good example of this behavior. However *Cymopolia barbata* produces toxic metabolites during any collecting season. *Cymopolia* was toxic with any solvent used. It is possible that cimopol, a sesquiterpene isolated from *Cymopolia* be the responsible substance. Some Rhodophyta produce very toxic brominated phenols, however this kind of substance has not been isolated from *Gracilaria* genus. Two species of Gracilaria were toxic and could be an agar source.

Conclusion. Information about toxic macroalgae in Mexico is scarce. Knowledge of toxic macroalgae biology can give us useful information about how to use them as biological control against overpopulation of exotic introduced fish species in some water bodies, these algae could be a good alternative to more aggressive biocides.

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P-110 BIOASSAYS TO DETECT TOXICITY IN CULTURES OF MARINE MICROALGAE

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Background. As it happen in many places around the globe, dinoflagellates are economically important in Mexico. These organisms produce many molecules that present a diverse toxicity levels. The dinoflagellate growth population is known as Harmful Algae Bloom (HAB) and these events are ecologically important because toxic molecules can be concentrated along food chain producing severe health troubles in a variety of organisms, humans included (Cortés-Altamirano *et al*. 2006; Tiffany *et al*. 2001). Isolation and controlled conditions culture of dinoflagellates will let to know life cycle and metabolite production in a better way. Although it exists several research centers that make investigation on dinoflagellate culture is a very evident reality that is impossible that an investigation center could to get a complete collection of these organisms. Therefore is better to obtain organisms from the places where the HAB is produced in each country and cultivated them in order to make experiments for understanding physiological issues and, dynamic of HAB's and the imminent toxicity (Band-Schmidt *et al.,*2006; Rodriguez-Palacio *et al.,* 2009).

Aim. Test for toxicity in marine dinoflagellate cultures by bioassays using brine shrimp *Artemia salina* and guppy *Poecillia reticulata.*

Material and method. Dinoflagellate cultures were harvested during exponential phase. After, cultures were lyophilized in order to get cellular disruption; metabolites were obtained using a buthanol plus water solution. Extracts were dried up; at this point they were added with 9% salt-water solution. Each strain was tested three times with a control experiment. When brine shrimps were hatched they were nourished with *Chlorella vulgaris* for the three initial days; after three days are placed in a flask and algal extract is added. Organisms in control experiment were nourished with *Chorella*. Experiments with guppys were carried out in triplicate and just salt-water solution was added.

Results and discussion. Extracts from *Amphidinium* sp., *Chattonella marina, Heterocapsa pymaea, Gyrodinium uncatenum, Gyrodinium instriatum, Prorocentrum rathymum, Protoceratium reticulatum* were tested during exponential phase. Extracts were classified considering the behavior of experimental organisms when they were nourished with *Chlorella*. Key: (T) toxic; (MT) moderately toxic; (NT) not toxic.

Conclusion. Toxic molecules in nature represent a wide research topic in several science fields. Bioassays using *Artemia salina* and some ornamental fishes could be a powerful toxicity detecting test as good as mouse bioassays. An opportune specific detection test for harmful algae could help us to take ecological and fishery actions in order to avoid damage in human populations.

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Poster Phycotoxin Section XI:
Phycotoxins in the Atlantic area. Group 22.

Tuesday June 29

Group 22 (P-111 to P-112): Each presentation in 20 min.

P-111 KNOWLEDGE TRANSFER NETWORK FOR PREVENTION OF MENTAL DISEASES AND CANCER IN THE ATLANTIC AREA (PHARMATLANTIC)

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Climate change on the Atlantic Area is responsible for the settlement of favourable ecological conditions for marine compounds reproduction because marine organisms develop unique metabolic and physiological capabilities to be able to survive in such new habitats. These sometimes negative effects can be taken in a positive way because marine compounds can be studied by research centres of the Atlantic Area as new chemicals that should be used by enterprises to be benefited from such research in the future (chemistry, pharmacology, environmental, maritime, fishing, etc…).

PHARMATLANTIC is a 36 months EU project promoted by the Pharmacology Department of University of Santiago de Compostela (Veterinary Faculty). It was created on the necessity to provide involved industries situated in the Atlantic seaboard with research and innovation advances in prevention of mental diseases and cancer already developed by the most specialized centres in this field at EU level. A partnership of 10 EU institutions from Spain (University of Santiago de Compostela and ANFACO-CECOPESCA), Portugal (CIIMAR and University of Algarve), France (CNRS), United Kingdom (Queens's University Belfast and Agri-Food & Biosciences Institute) and Ireland (Cork Institute of Technology and Dublin City University) and the main research centres, universities and industries are represented in this project.

The main objective of PHARMATLANTIC is to build a stable network of knowledge transfer between researchers and industries by developing a joint method for the use and exploitation of marine resources through previous research in order to further transfer to the enterprise from involved sectors.

The specific objectives are:

- Data exchanging among research centres to create a joint valid methodology and industrial applications at transnational level.
- Establishment of a joint method aiming to transfer knowledge from research centres to companies.
- Exchanging and transfer of knowledge and innovation addressed to the industries of the sectors, particularly the pharmaceutical industry; raising awareness on the importance of innovation to companies of involved sectors.

Short term outputs:

- Establishment of a microalgae collection.
- Scaled specifications for microalgal culture in bioreactors.
- Comparative analysis of developed methods with current state of art.
- Development and introduction of new efficient (simplified, inexpensive, reliable) alternative biotoxin detection, refinement and control methods.
- Creation of an Internet Accessible Database.

Expected mid and long term outputs:

- Constitution of at least one transfer network in each one of involved Regions of participant partners.
- Study about state of knowledge transfer in the involved territories and at EU level.
- Achievement of a valid methodology of knowledge transfer from research centres to enterprises at EU level.
- Transnational Working Group of Knowledge Transfer and Valorisation.
- Creation of Innovation and Knowledge Transfer Network PHARMATLANTIC.

PHARMATLANTIC has an innovative character highlighted under all its aspects: thematic, implementation and results expected. Even its background is based on the innovative idea of using marine harmful organisms, which have resulted as a consequence of climate change and global temperature rising, in order to be used as a new source of chemicals for research in the fields of pharmacology.

The project theme is also based on a new and innovative approach which consists in establishing a reliable protocol in order to obtain bioactive compounds from marine harmful organisms with effect on neurodegenerative diseases and cancer.

P-112 ADVANCED TESTS ABOUT NEW TOXINS APPEARED IN THE EUROPEAN ATLANTIC AREA (ATLANTOX)

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Global warming is causing negative impacts in marine ecosystems all over the world. One of the effects derived from the rise of ocean temperature is the increasing frequency of harmful algal blooms, and the subsequent risks for seafood consumers and economical losses for coastal communities that depend on the exploitation of marine fish and shellfish resources and on tourism. This is an issue of transnational importance that requires joint actions in order to find adequate solutions. Besides the increasing number of toxic episodes, global ocean warming causes changes in the distribution of phytoplanktonic communities; thus, toxic microalgal species typical from warm or temperate waters may appear in higher latitudes or new locations. Toxic episodes referring to new toxins appeared for first time in the Gulf of Mexico, Gulf of California and Indo-Pacific ocean regions, but intoxications due to toxins so far unknown in European waters have been recently reported.

EU authorities consider therefore a priority the development of quick and reliable methods to detect new toxins, as well as the enhancement of knowledge about their effects on human health, since EU currently lacks legislation regulating maximum permitted limits for these compounds. The ATLANTOX project, launched in April 2009, is fully coincident with this aim, establishing tools and methods for the detection of newly appeared marine biotoxins which will contribute to the safe and sustainable management of coastal environments in the EU Atlantic Area.

ATLANTOX follows the priorities and objectives described in the EU Atlantic Area Operational Programme: protect, secure and enhance the marine and coastal environmental sustainability. The general objective of this project is to support and accelerate the development and introduction of a proper and efficient method of fast alternative controlling, based on antibodies and functional assays for biotoxins that causes problems of food safety with touristic and economic consequences.

Specific objectives are:

- Developing a reliable method of detection and control of marine toxins, which applies to the whole maritime zone of Atlantic Area, specially tetrodotoxin, palytoxin and cyclic imines such as spirolids.
- Establishment of a collaborative network for the collection and analysis of samples of marine toxins in the Atlantic Area.
- Promoting the exchange of knowledge on the topic addressed by promoting, among others, the mobility of researchers in the Atlantic Area.
- Enhancing the sustainable management of marine resources in the Atlantic coastal area.
- Encouraging the adoption of transnational tools to protect marine ecosystems.
- Avoiding negative impacts that the emergence of marine toxins exerts on the economies of Atlantic coastal areas linked to concepts of food safety and tourism promotion.

Expected short term outputs:

- Biotoxin and reference material production.
- Toxicological studies about new toxins appeared in the Atlantic Area coastline and identification of risks for humans.

- New and reliable detection methods.
- ITC outputs: webpage, Internet accessible database about toxins.
- Technical outputs: evaluation plan, evaluation report and communication and dissemination plan.

Expected long term outputs:

- Enhancing cooperation through stable research networks
- Contributing to the objectives of the EU research policy
- Replacement and/or reduction of in vivo toxin detection

ATLANTOX project is leaded by University of Santiago de Compostela (Spain). Other members of the partnership are: Institute of Agri-Food and Land Use of Queen's University Belfast (United Kingdom), Agri-Food & Biosciences Institute (United Kingdom), Cork Institute of Technology (Ireland), CIIMAR (Portugal), CNRS (France) and ANFACO-CECOPESCA (Spain). IFRÉMER (France) and IPIMAR (Portugal) participate as supporting partners.

More information: www.atlantox.com.