Moulds and Mycotoxins

Mould and Mycotoxin, a global issues: New Discoveries, Food and Feed Safeties, Effects, Regulations, Prevention and Treatment
黴和黴菌毒素，世界性的問題：新發現，食品和飼料安全，影響，規範，預防和處理

Maria Angeles Rodriguez, Taiwan, 31st August 2011

Moulds and Mycotoxins

What are moulds? 何謂霉

Moulds are fungi (one of the five Kingdoms of Life):霉菌是真菌生物界五大界

- Fungi are neither plant nor animal, but have some characteristics of each. They cannot move about like an animal Fungi are almost entirely multicellular (with yeast, *Saccharomyces cerviseae*, being a prominent unicellular fungus),
- 真菌既非動物亦非植物，但兼具二者之某些特質，它們不如動物般行動，皆爲多細胞生物(酵母菌爲單細胞真菌)，
- Eukaryotes : They have a true nucleus in their cells and are able to sexually reproduce . (They can also reproduce by spores similar to some of the more primitive plants).

真核生物：有完整的細胞核行有性生殖，(也行孢子生殖類似低等植物)

- Heterotrophic:, have no chlorophyll as do plants, and cannot manufacture their own energy (Symbiotic : Lichens)

異養生物：無葉綠體無法生產能(Lichen是共生)

- Moulds is generic term to describe microscopic fungi
- 霉是形容真菌的總稱

Mycélium

Sporocyste

Hyphae

But moulds are:

- Saprophytes : Must consume organic matter (from dead organisms)

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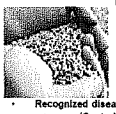
Moulds and Mycotoxins

What are mycotoxins?何謂黴菌毒素

General information一般性的知識



Mycotoxin = Greek word for fungus : « Mykes »希腊文的Mykes
+
Latin word for poison : « toxicum »拉丁文 toxicum

Any potential toxic substance produced by moulds' metabolism任何具有毒性由霉菌產生之代謝物

- Recognized disease in man for centuries : Rye ergot alkaloids produced by Claviceps purpurea (Central Europe)幾世紀來認爲是人類疾病：由Claviceps purpurea產生的黑麥麥角鹼
- First recognition in animals in 1960 in UK (First Aflatoxicosis outbreak in poultry)

1960年英國在動物種發現(家禽其黃毒素第一例)

- Since then more than 200 mycotoxins discovered mostly in the recent years
- 背後200多種黴菌毒素被發現

Mycootxins are a high potential threat to human and animal health through the ingestion of food or feed prepared from infected commodities
含毒素的食品或飼料食入後對人體及動物之健康之危害極大

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Moulds and Mycotoxins

What are mycotoxins?

- As recently defined by Pitt (1996), mycotoxins are 'fungal metabolites which when ingested, inhaled or absorbed through the skin cause lowered performance, sickness or death in man or animals, including birds.'
- 近年來由Pitt氏(1996)對黴菌毒素之定義爲黴菌的代謝產物，當被攝食，吸入，經皮吸收，導致生產性能下降，致人類或禽畜生病或死亡。

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Moulds and Mycotoxins

Moulds and Mycotoxins of world-wide importance 黴及黴菌毒素爲世界性的要務

Table 1 - Moulds and mycotoxins of world-wide importance 表1-重要的世界性黴及黴菌毒素

Mould species	Mycotoxins produced
Aspergillus parasiticus	Aflatoxins B_1, B_2, G_1, G_2
Aspergillus flavus	Aflatoxins B_1, B_2
Fusarium sporotrichioides	T-2 toxin
Fusarium graminearum	Deoxynivalenol (or nivalenol)
	Zearalenone
Fusarium moniliforme (F. verticillioides)	Fumonisin B_1
Penicillium verrucosum	Ochratoxin A
Aspergillus ochraceus	Ochratoxin A

Source: FAO, 2001

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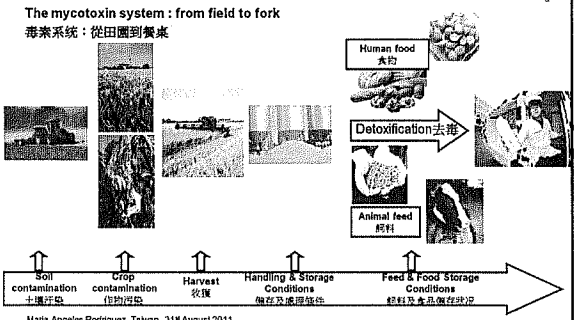
Moulds and Mycotoxins

Occurrence of moulds and mycotoxins, where?在那裡產生霉及黴菌毒素

The mycotoxin system : from field to fork
毒素系統：從田園到餐桌



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Moulds and Mycotoxins

Occurrence of mycotoxins, some data: Europe毒素的生成，歐洲相關資料

Table II: Overview on *Fusarium* toxin occurrence data submitted by the participating countries Fusarium毒素生成資料，由相關國家提供

Fusarium toxin	Countries	Number of samples	Positive samples
Type B trichothecenes 單端孢霉烯			
Deoxynivalenol 脫氧雪腐鐮刀菌烯醇	11	11 022	57 %
Nivalenol	7	4 166	16 %
3-Acetyldeoxynivalenol	5	3 721	8 %
15-Acetyldeoxynivalenol	3	1 054	20 %
Fusarenon X	3	1 872	10 %
Type A trichothecenes			
T-2 Toxin	8	3 490	20 %
HT-2 Toxin	5	3 032	14 %
T-2 Triol	2	1 390	0 %
Neosolaniol	2	1 323	1 %
Diacetoxyscirpenol	3	1 890	4 %
Monoacetoxyscirpenol	1	853	1 %
Verrucarol	1	121	0%
Zearalenone	9	5 018	32 %
Fumonisins			
Fumonisin B_1	9	3 833	43 %
Fumonisin B_2	5	1 010	42 %
Fumonisin B_3	1	239	33 %
	Sum:	44 859	

Source: SCOOP Task 3.2.10

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Moulds and Mycotoxins

Occurrence of mycotoxins, some data: Europe毒素的生成，歐洲相關資料

Table III: Summary of food groups most frequently contaminated with *Fusarium* mycotoxins

Fusarium toxin	Main food items/food groups contaminated (percentage of positive samples)
Type B trichothecenes	
Deoxynivalenol	corn (89 %), wheat* (61 %)
Nivalenol	corn (35 %), oats (21 %), wheat*(14 %)
3-Acetyldeoxynivalenol	corn (27 %), wheat*(8%)
Type A trichothecenes	
T-2 Toxin	corn (28 %), wheat (21 %), oats (21 %)
HT-2 Toxin	oats (41 %), corn (24 %), rye** (17 %)
Zearalenone	corn (79 %), corn milling fractions (61 %), corn based products (50%); wheat (30 %), wheat milling fraction (24 %), wheat based products (11 %), baby food (23 %)
Fumonisins	
Fumonisin B_1	corn (60 %), corn flour (75 %) corn based products (31 %), corn flakes (46 %), wheat (79 %)
Fumonisin B_2	corn (51 %)

* Wheat and wheat flour ** Rye and rye flour

Source: SCOOP Task 3.2.10

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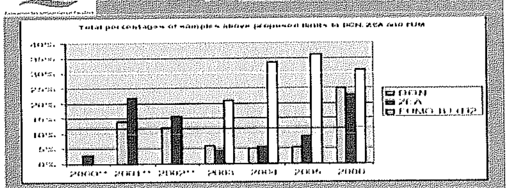
Occurrence of mycotoxins, some data: Europe毒素的生成，歐洲相關資料

aAF Data summary in unprocessed maize (2000-2006)



Total percentages	Number of samples	2000*	2001*	2002	2003	2004	2005	2006
DON	1672	9%	14%	12%	6%	5%	5%	21%
ZEA	1471	3%	22%	13%	7%	6%	9%	21%
FUMO B1+B2*	1214	-	-	-	31%	34%	16%	31%

[illegible]

European Commission Fusarium Toxins Forum (15/16 January 2007)

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Moulds and Mycotoxins

Occurrence of mycotoxins, some data: America毒素的生成，美洲相關資料

Table 1. Main mycotoxins found in animal feed in Brazil.

巴西動物飼料中主要的毒素

Mycotoxin	Samples Analyzed	Positives %	Average ppb
Aflatoxin	82.452	40.8	11.8
Zearalenone	69.417	10.6	43.4
Ochratoxin A	19.739	2.9	0.6
Deoxynivalenol (DON)	15.348	39.4	233.7
Fumonisin	14.162	53.4	1073.2
T-2 Toxin	10.052	1.3	13.9
Diacetoxyscirpenol (DAS)	747	0.0	9.5

Dr. Mallmann et al. LAMIC - UFSM. 2008

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Moulds and Mycotoxins

Occurrence of mycotoxins, some data: America毒素的生成，美洲相關資料

Occurrence of five mycotoxins in corn silage, corn grain and in all feed samples submitted for analysis by producers in North Carolina over a nine-year period

9年間北卡羅來納分析青貯玉米，玉米粒以及所有飼料樣品中5種毒素）

	Aflatoxin > 10ppb			DON > 50 ppb			ZEN > 70 ppb			T2 Toxin > 50 ppb			Fumonisin > 1 ppm	
Sample	n	%	Mean ±s.d.	n	%	Mean ±s.d.	n	%	Mean ±s.d.	n	%	Mean ±s.d.	n	%
Corn silage	461	8	26±19	776	66	1991±2876	487	30	525±799	717	7	569±830	63	37
Corn grain	231	9	170±806	362	70	1504±2550	219	11	208±175	353	6	569±690	37	60
All feeds	1617	7	91±320	2472	56	1739±10880	1780	18	445±669	2243	7	482±898	283	28

n = number of samples樣品數

% = percentage of samples positive above given concentrations 超過標準量的陽性件百分率

Mean of the positive samples plus and minus the standard deviation平均陽性件的量±標準偏差

FEEDSTUFFS, 2008 REFERENCE ISSUE AND BUYERS GUIDE

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Moulds and Mycotoxins

Occurrence of mycotoxins, some data: America毒素的生成，美洲相關資料

Tabla 1. Concentración de micotoxinas en grano de destilería seco (DDGS) y húmedo

	DDGS					WDG				
Toxina	Muestras	Prom.	Normal	Rango	DS	Muestras	Prom.	Normal	Rango	DS
aflatoxina, ppb	30	4.61	2.12	7.09	2.49	28	2.17	0.00	6.79	1.61
vomitoxina, ppm	54	3.62	0.00	7.74	4.12	44	1.91	0.00	4.26	2.35
zearalenona, ppm	16	0.24	0.00	0.51	0.27	14	0.37	0.00	0.87	0.50
T2, ppm	11	0.03	0.00	0.07	0.03	14	0.12	0.00	0.24	0.12
ocratoxina, ppm	4	0.01	0.01	0.01	0.00	3	0.02	0.02	0.02	0.00
fumonisina, ppm	20	0.74	0.00	1.96	1.22	27	0.69	0.00	1.73	1.04

Datos de cosecha acumulados: 05/01/2000 a 04/30/2007; Fuente: www.dairyone.com. 2007

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Moulds and Mycotoxins

Occurrence of mycotoxins, some data: Asia, 2010毒素的生成，亞洲2010料

Table 2a - North Asia (includes China, Taiwan, Korea, Japan)

North Asia	Afla	ZON	DON	FUM	OTA
Number of tests	720	774	778	732	702
Percent Positive (%)	13	56	71	44	22
Average (μg/kg)	9	232	757	780	1
Maximum (μg/kg)	4,687	16,712	19,141	[illegible]	60

Table 2b - South East Asia (includes Malaysia, Philippines, Thailand, Vietnam, Indonesia)

South East Asia	Afla	ZON	DON	FUM	OTA
Number of tests	369	369	369	369	369
Percent Positive (%)	65	49	41	57	32
Average (μg/kg)	22	55	299	493	1
Maximum (μg/kg)	726	2,601	19,096	6,196	53

Table 2c - South Asia (includes India, Pakistan, Bangladesh)

South Asia	Afla	ZON	DON	FUM	OTA
Number of tests	89	89	89	86	89
Percent Positive (%)	66	30	30	58	71
Average (μg/kg)	78	14	47	323	9
Maximum (μg/kg)	593	297	556	1852	174

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Biomin Mycotoxin Survey, 2010

Moulds and Mycotoxins

Occurrence of mycotoxins, some data by commodities 交易商品中毒素的產生

Corn	Afla	ZON	DON	FUM	OTA
Number of tests	600	631	[illegible]	[illegible]	382
Percent Positive (%)	20	46	72	75	9
Average (μg/kg)	20	134	986	1012	1
Maximum (μg/kg)	4,687	2,206	[illegible]	[illegible]	60

Soybean	Afla	ZON	DON	FUM	OTA
Number of tests	93	103	1[illegible]5	87	87
Percent Positive (%)	20	10	32	2	21
Average (μg/kg)	0	5	29	7	1
Maximum (μg/kg)	6	75	[illegible]	315	20

Wheat/bran	Afla	ZON	DON	FUM	OTA
Number of tests	92	172	262	88	63
Percent Positive (%)	4	28	56	11	24
Average (μg/kg)	0	29	1106	28	5
Maximum (μg/kg)	7	465	49,000	634	331

Corn Gluten Meal	Afla	ZON	DON	FUM	OTA
Number of tests	19	19	[illegible]	[illegible]	19
Percent Positive (%)	47	95	89	100	74
Average (μg/kg)	34	2248	1544	3807	6
Maximum (μg/kg)	542	16,712	6,965	12,612	[illegible]

Rice/bran	Afla	ZON	DON	FUM	OTA
Number of tests	19	20	20	20	19
Percent Positive (%)	47	55	5	5	26
Average (μg/kg)	3	66	3	38	1
Maximum (μg/kg)	23	297	64	258	14

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Biomin Mycotoxin Survey, 2010

Moulds and Mycotoxins

Occurrence of mycotoxins, some data by commodities

DDGS	Afla	ZON	DON	FUM	OTA
Number of tests	59	86	86	78	57
Percent Positive (%)	6	[illegible]	91	77	35
Average (μg/kg)	2	274	3127	825	1
Maximum (μg/kg)	44	1,690	19,096	8,803	26

Finished Feed	Afla	ZON	DON	FUM	OTA
Number of tests	631	778	791	656	632
Percent Positive (%)	46	57	58	69	39
Average (μg/kg)	14	119	[illegible]	826	2
Maximum (μg/kg)	353	3,576	19,141	10,380	174

Silage	Afla	ZON	DON	FUM	OTA
Number of tests	253	320	359	239	233
Percent Positive (%)	2	36	49	19	14
Average (μg/kg)	0	80	673	168	1
Maximum (μg/kg)	5	2146	14926	2174	96

Straw	Afla	ZON	DON	FUM	OTA
Number of tests	24	21	21	21	21
Percent Positive (%)	0	13	21	0	17
Average (μg/kg)	0	48	87	0	0
Maximum (μg/kg)	0	510	693	0	4

Barley	Afla	ZON	DON	FUM	OTA
Number of tests	7	186	[illegible]	6	16
Percent Positive (%)	0	0	60	0	25
Average (μg/kg)	0	0	658	0	3
Maximum (μg/kg)	0	522	14,137	0	20

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Biomin Mycotoxin Survey, 2010

Moulds and Mycotoxins

Occurrence of mycotoxins, some data: Asia 亞洲黴菌毒素發生狀況

Table 4

Mycotoxin	Sample size	Percent positive	Average of positive (μg/kg)	Highest Level detected (μg/kg)	Commodity found	Country of origin
Aflatoxins total	965	18%	39	341	Peanut Meal	Australia
Zearalenone	963	[illegible]	400	[illegible]	Corn	China
Deoxynivalenol	963	45%	665	18,991	Wheat	China
Fumonisin B1	960	46%	864	10,577	Corn	China
T-2 toxin	748	1%	273	494	Finished Feed	Thailand
Ochratoxin A	128	18%	11.7	143	Corn	Malaysia

Source: Romer Labs Singapore, 2007

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Occurrence of mycotoxins, some data: Asia, 1st quarter 2011 亞洲黴菌毒素發生狀況，2011年第1季

	AFLA	ZON	DON	FUM	OTA
Number of samples tested	267	272	266	267	260
Positive (%)	36	51	59	45	32
Average of positives (μg/Kg)	94	511	1768	1717	12
Maximum (μg/Kg)	677	23278	42423	10979	400

Biomin Mycotoxin Survey, 2011

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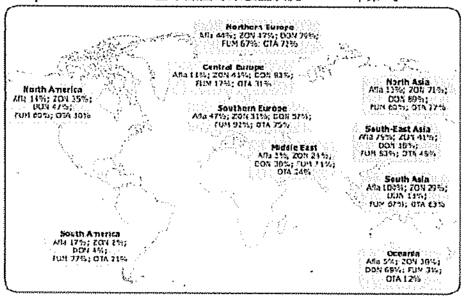
Occurrence of mycotoxins, worldwide data, 1st quarter 2011全球黴菌毒素發生狀況，2011年第1季



Biomin Mycotoxin Survey, 2011

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Moulds and Mycotoxins

What mycotoxins do?黴菌毒素的影響

- occur in a wide variety of foods and feeds廣泛發生於多種食品飼料
- have been implicated in a range of human and animal diseases.與多種人畜疾病相關
- can produce both acute and chronic toxicities, ranging from death to deleterious effects產生急性與慢性之毒素，影响從死亡到生產不佳
- may also be carcinogenic, mutagenic, teratogenic and immunosuppressive.有致癌性致突變性，致畸形和抑制免疫力。
- ability to compromise the immune response and, consequently, to reduce resistance to infectious disease減弱免疫反應故而降低對疾病的抵抗力。
- **This is now widely considered to be the most important effect of mycotoxins, particularly in developing countries. (FAO, 2001)**
- 目前廣泛認爲是開發中國家黴菌毒素重要的影响。

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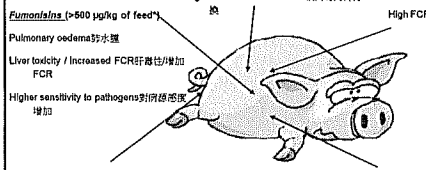
Moulds and Mycotoxins

In swine豬



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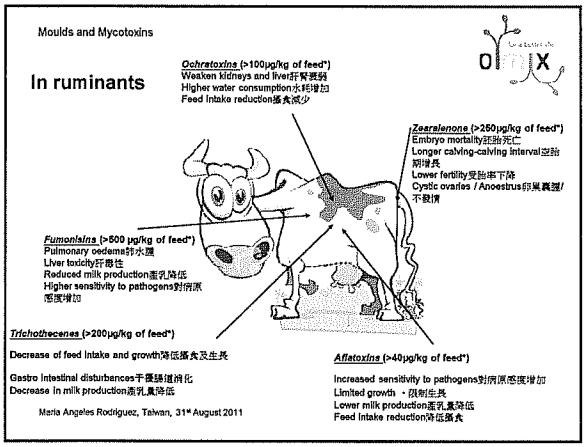
In ruminants



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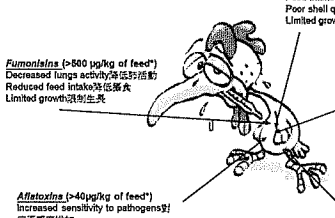
Moulds and Mycotoxins

In poultry



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Moulds and Mycotoxins

New knowledge on DON and FUM effects on digestive system嘔吐毒素與伏馬毒素效應在消化系統的新知

- DON and FUM affect:嘔吐毒素與伏馬毒素效應
  - Nutrient absorption管養分吸卜收
  - Cell proliferation細胞增殖
  - Barrier function障壁功能
  - Immunoglobulin A IgA

Extracted from Isabel Oswald,INRA; 4th International Symposium Mycotoxins, Ghent, May, 2011

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Moulds and Mycotoxins

New knowledge on DON and FUM effects on digestive system嘔吐毒素與伏馬毒素效應在消化系統的新知

- DON:嘔吐毒素
  - Damages epithelial cells上皮細胞損壞
  - Decreases length of villi纖毛變短
  - Less surface of absorption吸收的表面積減少
  - Less absorption of nutrients吸收少

Extracted from Isabel Oswald,INRA; 4th International Symposium Mycotoxins, Ghent, May, 2011

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•FUM:伏馬毒素

- Impairs epithelial cell proliferation減少上皮細胞增竺

	Animal treatment動物處理	
Villus height (µm) 纖毛高	CONTROL 對照組	FB1
Proximal jejunum 近端空腸	300 ±16[a]	259 ±17[b]
Median jejunum 中段空腸	321 ±13[a]	259 ±21[b]
Distal jejunum 末端空腸	265 ±13[a]	182 ±13[b]

Extracted from IsabelOswald,INRA; 4th International Symposium Mycotoxins, Ghent, May, 2011

Moulds and Mycotoxins

New knowledge on DON and FUM effects on digestive system 嘔吐毒素與伏馬毒素效應在消化系統的新知

•DON: Damages epithelial cells

•嘔吐毒素：破壞上皮細胞

•FUM: Impairs epithelial cell proliferation

•伏馬毒素：減少上皮細胞增生

•Could explain the synergistic effects

•多少解釋了協同作用

Extracted from IsabelOswald,INRA; 4th International Symposium Mycotoxins, Ghent, May, 2011

New knowledge on DON and FUM effects on digestive system嘔吐毒素與伏馬毒素效應在消化系統的新知

•DON:嘔吐毒素

- Decreases trans-epithelial electrical resistance (TEER)降低了通過上皮電阻
- Increases trans-epithelial passage增加了通過上皮通透性。
- Increases the passage of bacteria into the body 細菌通過上皮進入體內增加了
- More severe diarrhea嚴重下

Extracted from IsabelOswald,INRA; 4th International Symposium Mycotoxins, Ghent, May, 2011

Moulds and Mycotoxins

New knowledge on DON and FUM effects on digestive system嘔吐毒素與伏馬毒素效應在消化系統的新知

•DON:嘔吐毒素

- Decreases the intestinal expression of claudin proteins降低小腸中claudin proteins的表施
- Activates MAPKinase that regulates tight junction proteins激活調控緊緻聯結蛋白的MAP激酶
- Decreases the barrier function降低障壁的功能

Extracted from IsabelOswald,INRA; 4th International Symposium Mycotoxins, Ghent, May, 2011

Moulds and Mycotoxins

New knowledge on DON and FUM effects on digestive system嘔吐毒素與伏馬毒素效應在消化系統的新知

•FB1:伏馬毒素B1

- Decreases the intestinal expression IL-8減少腸內IL-8的表現
  - IL-8 is implicated in the recruitment of neutrophils during inflammatory response.在發炎反應中IL-8與聚集嗜中性球的機制相關
  - The decreased IL-8 production could lead to an impaired recruitment of neuthrophils 。 IL-8的生產減少即聚集嗜中性球的機制減弱。
- Decreased intestinal IL-8 is associated with an increase susceptibility to enteric infection 。 IL-8的減產意味著腸內的感染增加。

Extracted from IsabelOswald,INRA; 4th International Symposium Mycotoxins, Ghent, May, 2011

Moulds and Mycotoxins

What mycotoxins do.... toghether?黴菌考素在一起搞什麼

Figure 1. Synergism (S) and Antagonism (A) between mycotoxins.

	AFL	DON	DAS	NIV	T-2	HT-2	ZEA	FUMB1	OTA	CIT
AFL			S		S				S	
DON					S		S	S	A	
DAS										
NIV										
T-2						S				
HT-2										
ZEA										
FUMB1										
OTA										A
CIT										

Depression of immune system 抑制免疫系統 → ⇧Susceptibility to infectious diseases提高疾病易感性

Moulds and Mycotoxins

The risk of mycotoxins黴菌毒素的危害

- world-wide attention because of the significant economic losses associated with their impact on human health, animal productivity and both domestic and international trade.對人類健康和動物生產的危害，造成顯著的經濟損失，已招致全球的關注。
- annual losses in the USA and Canada, arising from the impact of mycotoxins on the feed and livestock industries, are of the order of $5 billion. (FAO, 2001)毒素造成美加在飼料與畜牧的年損失爲5億美元
- In developing countries, where the food staples (e.g. maize and groundnuts) are susceptible to contamination, it is likely that significant additional losses will occur amongst the human population because of morbidity and premature death associated with the consumption of mycotoxins.在開發中國家玉米或花生等主要穀物最易遭受污染而在人類社會中會因爲攝食黴菌毒素造成健康損害或夭折等造成顯著額外的損失

Moulds and Mycotoxins

The REAL risk is higher than that PERCEIVED by consumers真實的危害比感覺上的危害

Classification of food related risk
與食品相關的危害

Risk Perception 感覺上的危害	Real Risk 實際上的危害
GMO 基改	Nutritional errors營養失調
Pesticides 殺蟲劑	Bacteria toxins細菌毒素
Additives 添加制	Mycotoxins黴菌毒素
Nutritional errors營養失調	Pesticides殺蟲劑
Bacteria toxins細菌毒素	Additives添加制
Mycotoxins黴菌毒素	GMO ?基改?

Moulds and Mycotoxins

Transference from feed to food
由飼料向食品的傳遞

Table 1. Deposition of mycotoxins in animal products.

表1. 從動物產品中除去黴菌毒素

Mycotoxin	Deposition (%) in:			
	Chicken meat 雞肉	Chicken fillet 雞胸	Eggs 蛋	Milk 奶
Deoxynivalenol	0	0	0.04	0.0004
Zearalenone	0.005	0.003	0.03	0.06
Ochratoxin A	3.3	0.5	0.2	N/A
Fumonisin B1	0	0	0.0001	0.004

References for table 1: Sypecka, 2006, Prelusky et al., 1994, Doupovec et al., 2008, EFSA Opinion 2005, Gorrish et al., 1993, Hamilton et al. 1995, Hamilton et al., 1994

Moulds and Mycotoxins

Human risk人類危機

Table VI: Range of average dietary intakes* calculated as percentage of the TDI values 平均攝食量以TDI值之百分率計算

Mycotoxin 黴菌毒素	TDI µg/kg bw/day	Population 人口	Adults 成人	Infants 嬰兒
Deoxynivalenol	1	0.8% - 33.8%	14.4% - 46.1%	11.3% - 95.9%
Nivalenol**	0.7	4.2% - 11.1%	0.8% - 8.2%	3.7% - 22.6%
T-2 + HT-2 toxin**	0.06	18.3% - 250%	61.7% - 171.7%	26.7% - 563.3%
Zearalenone**	0.2	13.4%	5.3% -14.5%	3% - 27.5%
Fumonisins $B_1 + B_2$	2	0.8% - 13.2%	0.1% - 14.1%	22.3%

* Mean food consumption and mean 1 occurrence data. For details see parts A-C.
** Temporary TDI

Moulds and Mycotoxins

Legal status of mycotoxins: EU
歐盟黴菌毒素法規狀況

- The only mycotoxin legislated with a maximum level in feed is AFLATOXIN B1 (COMISSION DIRECTIVE of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed (2003/100/EC))

只有黃麴毒素B1在飼料中有最大限制量(2003年10月31日委員會修訂附錄I 指導2002/32/EC 歐洲議會會議動物飼料中令人無法接受的物質 (2003/100/EC))

- No legislation on maximum levels of other mycotoxins in feed, only an official recommendation (COMMISSION RECOMMENDATION of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (2006/576/EC))

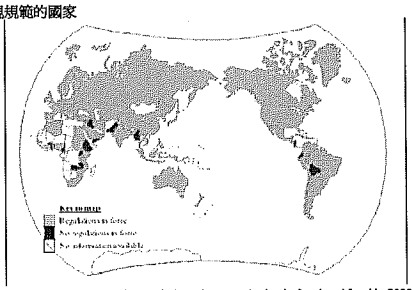
其他飼料中黴菌毒素沒有最高限量之規定只有建議量(2006年8月17日在動物飼料中存在deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins等之建言義量

- It's very likely that this recommendation will become a Legislation in the near future.這些建議量很可能最近完成立法

Moulds and Mycotoxins

Legal status of mycotoxins: Worldwide
全球黴菌毒素法規狀況

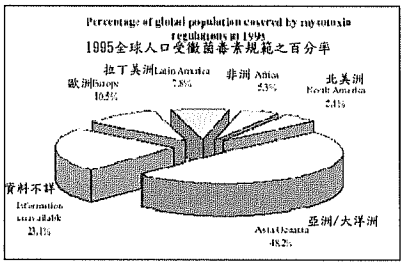
Countries with and without regulations for mycotoxins
有無法規規範的國家



Worldwide regulations for mycotoxins in food and feed in 2003, FAO, 2004

Moulds and Mycotoxins

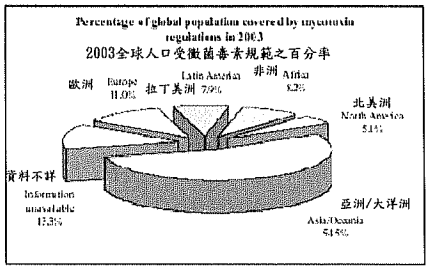
Legal status of mycotoxins: Worldwide
全球黴菌毒素法規狀況



Worldwide regulations for mycotoxins in food and feed in 2003, FAO, 2004

Moulds and Mycotoxins

Legal status of mycotoxins: Worldwide
全球黴菌毒素法規狀況



Worldwide regulations for mycotoxins in food and feed in 2003, FAO, 2004

Moulds and Mycotoxins

Legal status of mycotoxins: Asia
亞洲黴菌毒素法規狀況

-Asia and Oceania cover a very large part of the globe, with most countries in the tropics and subtropics, so it is expected that most mycotoxin problems be caused by fungi.亞洲與大洋洲占地頗廣，多數國家位於熱帶及亞熱帶因此理所當然的會有黴菌造成的黴菌毒素的問題。

-Twenty-six countries in Asia/Oceania were known to have specific mycotoxin regulations (88 percent of the inhabitants of the region).亞洲與大洋洲中的26國有特別的規範。(占地區居民百分之88)

-Regulations for total aflatoxins dominate in food, whereas regulations for aflatoxin B1 dominate in feed. 食品相關規範中涵蓋的所有的黃麴毒素，飼料相關規範中針對黃麴毒素B1 。

-Australia and New Zealand have harmonized their mycotoxin regulations, which include limits for the "exotic" mycotoxins agaric acid and phomopsins .澳紐使用共同的蕈菌毒素規範包括特別的mycotoxins agaric acid and phomopsins

-By far the most extensive and detailed regulations can be found in China and the Islamic Republic of Iran.目前規範最完整詳細的爲中國和伊朗

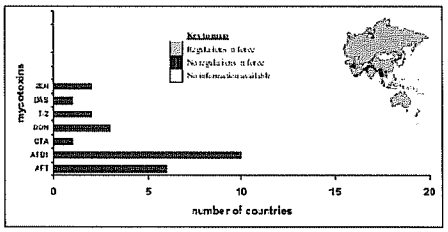
Worldwide regulations for mycotoxins in food and feed in 2003, FAO, 2004

Moulds and Mycotoxins

Legal status of mycotoxins: Asia
亞洲黴菌毒素法規狀況

Mycotoxins regulated in feed in Asia/Oceania

亞洲與大洋洲飼料中黴菌毒狀態



Worldwide regulations for mycotoxins in food and feed in 2003, FAO, 2004

Moulds and Mycotoxins

Is there any solution? 有解決的方案嗎？

Prevention: 預防:

- During plant growth: planting of more resistant grains, balanced fertilization...
  在作物的生長時期: 種植抵抗力強的穀物, 均衡的施肥...
- During storage: humidity, temperature and insect control...
  穀物貯存的期間: 控制溼度、溫度與蟲害...
- During distribution: good shipping conditions...
  在運輸的過程: 良好的運輸條件...

Moulds and Mycotoxins

Is there any solution? 有解決的方案嗎？

•Physical methods: 物理方法:

- Washing, polishing, mechanical sorting and separation, density segregation, flotation, autoclaving, roasting and microwave heating, UV irradiation, ultrasound treatment and solvent extraction. 洗滌、磨光、機械化的分類與隔離、密度離析、漂浮、高壓蒸煮、烘培和微波加熱、紫外光照射、超音波處理以及溶劑萃取.
- Efficiency highly depends on the grade of contamination and the distribution of mycotoxins throughout the grain. 成效視汙染的程度與黴菌毒素在穀物間分佈的情況而定.
- Subsequently the results obtained are uncertain and often connected with high production losses. 之後得到的結果無法肯定, 且往往與大幅生產損失相關.
- Moreover, some of these physical treatments are relatively costly and may remove or destroy essential nutrients in feed. 此外, 部份物理方法相當昂貴, 並且可能損毀飼料中的必需營養成份.

Moulds and Mycotoxins

Is there any solution? 有解決的方案嗎？

- **Chemical methods: 化學方法:**
  - Oxidizing and reducing agents, acids, bases, salts and chlorinating substances. 氧化還原劑, 酸、鹼、鹽類以及氧化反應的物質.
  - Only a limited number turned out to be effective without diminishing nutritional value or palatability. 結果只有少數證實具有效果又不會降低營養價值或適口性.
  - Treatment of contaminated feed with ammonia was the most attractive method in the past. 以氨處理受汙染的飼料是過去最吸引人的方法.
  - Although this technique was apparently safe and effective in earlier studies, it has not been approved by the US Food and Drug Administration due to the potential toxicity and carcinogenicity of reaction products. 雖然這樣的技術在早期研究中是相對較安全及有效的, 它卻沒有被美國 FDA 核准使用, 因爲可能會產生毒性和致癌性.

Moulds and Mycotoxins

Is there any solution? Detoxifying agents 有解決的方案嗎？吸附劑

Some material have a natural ability to adsorb mycotoxins on their surface
有些物質天生就具有能將黴菌毒素吸附於其表面上的能力

Mineral materials: 礦物質:
- Clays (some Montmorillonites) 黏土 (某些蒙脫土)
- Diatomites 矽藻土
- Activated carbon 活性碳

Organic materials: 有機物質:
- Cholestyramine (quaternary ammonium chloride anion-exchange resin) 膽酸結合樹脂 (四級胺的氯化鹽陰離子交換樹脂)
- Synthetic polymers: PVPP (Polyvinyl Polypyrolidone) 合成聚合物: PVPP (聚乙烯聚吡咯啶酮)
- Yeast cell walls 酵母細胞壁

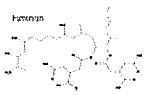
WHY？ 爲什麼？

- **SURFACE 表面**
- **POROSITY 多孔性**
- **IONIC EXCHANGE CAPACITY 離子交換能力**
- **ELECTRONICAL REACTIVITY 電荷反應**

Moulds and Mycotoxins

Detoxifying agents, are they effective? 吸附劑有效嗎？

- Aflatoxins are small and polar mycotoxins, and are easily adsorbed by some kinds of clays, specially montmorillonite type. 黃麴毒素是小而帶極性的黴菌毒素, 並且容易被一些黏土類吸附, 尤其是蒙脫土類.
- Zearaleone is less polar and a bigger molecule so it's more difficult of adsorb. 玉米赤黴烯酮毒素較不帶極性且分子較大, 所以比較不容易被吸附.
- Tricothecenes and fumonisins are even less polar and bigger molecules so are even more difficult to adsorb. In fact, these are the most difficult mycotoxins to be adsorbed. 新月毒素和伏馬鐮孢毒素的極性更低且分子更大, 所以更加不易被吸附. 事實上, 這些都是最不容易被吸附的黴菌毒素.



Moulds and Mycotoxins

Detoxifying agents, are they effective? 吸附劑有效嗎？

TABLE II Reduction of the concentrations of ZON and DON [% as compared to the blank] in the supernatant buffer solution by various detoxifying in the in vitro detoxification test (mean and SD of 4 independent replications). 在含多種吸附劑的懸浮緩衝液的體外吸附試驗(4個獨立重複組的平均值和標準差)中觀察玉米赤黴烯酮毒素(ZON)和嘔吐毒素(DON)降低的濃度(與對照組比較後的%)

	ZON 玉米赤黴烯酮毒素		*DON* 嘔吐毒素	
Product	***Mean*** 平均值	***SD*** 標準差	***Mean*** 平均值	***SD*** 標準差
Activated carbon 活性碳	100[a]	0	67[a]	6
Cholestyramine 膽酸結合樹脂	94[b]	1	10[b]	15
Modified aluminosilicate 改良矽酸鹽	81[c]	6	17[b]	16
Toxisorb®	55[d]	1	1[b]	2
Mykosorb® Extra	24[e]	1	24[b]	18
Klinosan®	20[e]	4	0[b]	5
Mycofix® Plus	17[e]	2	1[b]	4
Bentonite	13[f]	12	1[b]	1
Fix A Tox®	5[g]	1	21[b]	31
Likratox®	5[g]	2	2[b]	3

Values in one column with different superscripts are significantly different ($P<0.05$) 同一個管柱中不同標示的數值明顯不同 ($P<0.05$)

Döll et al, 2004

Moulds and Mycotoxins

In vitro trials 體外試驗

- **Demonstration of the effectiveness of a potential mycotoxin detoxifying agent in contaminated feed is often primarily conducted in *in vitro* conditions. 要試驗某種黴菌毒素吸附劑在受到汙染的飼料中的效果, 通常都是從體外試驗著手.**
- **Classical in vitro systems used for that purpose are simple but very far from the natural in vivo conditions. 爲此目的所使用的體外試驗很簡單, 但是跟自然的體內環境有很大的差異.**
- **Important factors in relation to the digestion and the fate of feed compounds during passage through the gastrointestinal tract are: 消化作用和飼料混合物在通過胃腸道時的相關重要因子有:**
  - **the composition and pH of gastric and intestinal contents. 胃及腸道內容物的組成和酸鹼值.**
  - **the gastrointestinal transit conditions. 胃腸道輸送的狀況.**
  - **the activity of bio-chemicals (enzymes) and of the intestinal micro flora in the gastrointestinal tract. 酵素和胃腸道內的微生物活性.**

Moulds and Mycotoxins

In vitro trials 體外試驗

Percent of adsorbed mycotoxin (mean ±S.D., n=3) 所吸附的黴菌毒素的 % (平均值±標準差, n=3)

	pH	DON (2 µg/ml) 去氧雪腐鐮刀菌烯醇; 嘔吐毒素	DON (10 µg/ml) 去氧雪腐鐮刀菌烯醇; 嘔吐毒素	NIV (2 µg/ml) 雪腐鐮刀菌烯醇	NIV (10 µg/ml) 雪腐鐮刀菌烯醇	Source 來源
Myco AD®	3	11±1	3±2	10±0	1±1	*Special Nutrients Inc., Miami, Fl. USA*
	6	1±0	10±2	4±1	11±1	
Mycosorb®	3	18±5	0±0	6±4	1±0	*Alltech Ltd. Lincs. UK*
	6	3±3	9±5	10±3	7±5	
Glucomann	3	1±3	0±1	2±2	1±0	*Dan Shen s.a.s. Milan, Italy*
	6	1±6	12±1	3±1	14±5	
Tirolex 28®	3	9±0	16±2	11±1	13±4	*Filazoo Aventis, Modena, Italy*
	6	1±2	10±0	3±0	10±1	
Mycofix Plus®	3	9±0	9±1	9±1	10±1	*Biomin GmbH, Herzogenburg, Austria*
	6	1±2	13±1	7±1	13±1	
Cholestyramine 膽酸結合樹脂	3	4±3	7±5	5±3	7±4	*Sigma-Aldrich, Milan, Italy*
	6	10±1	4±7	12±2	5±7	
Zeolite 沸石	3	5±4	2±1	3±1	1±0	*Sigma-Aldrich, Milan, Italy*
	6	2±1	3±1	2±1	0±0	
Bentonite 斯賓土	3	2±2	9±5	4±1	9±0	*Sigma-Aldrich, Milan, Italy*
	6	3±3	13±2	3±2	10±1	
Activated carbon 活性碳	3	84±2	59±5	62±3	33±7	*Sigma-Aldrich, Milan, Italy*
	7	84±0	52±1	60±0	23±1	
	6	95±9	57±5	63±1	30±6	

Extracted from Avantaggiato et al, 2004

Moulds and Mycotoxins

In vitro dynamic trials 體外動力學試驗

- The activities of those factors through the gastrointestinal tract are dynamic processes. 這些吸附反應在通過胃腸道時的活動是動態的過程.

- Therefore, these processes cannot be simulated in static *in vitro* models. 然而, 這些動態的過程無法用*靜態的體外試驗模型*來模仿.

- To demonstrate in the most reproducible and reliable conditions the efficacy in vitro of a sequestrant/chelator material, the TNO TIM-1 in vitro gastrointestinal model can be used. 爲證明在大多數可複製和可信賴環境下的某種螯合吸附物質的功效, 可以使用 TNO TIM-1 體外胃腸道試驗模型儀器.

Maria Angeles Rodriguez, Taiwan, 31st August 2011

Moulds and Mycotoxins

In vitro dynamic trials 體外動力學試驗

The TNO *in vitro* gastrointestinal models simulate in high degree the successive dynamic processes in the stomach and small intestine (TIM-1) and in the large intestine (TIM-2). These models are unique tools to study the fate of compounds during passage through the gastrointestinal tract.
TNO 體外胃腸道試驗模型高度模仿胃和小腸中(TIM-1)與大腸中(TIM-2)之連續動態的過程. 這些模型是研究混合物在通過胃腸道的過程之獨一無二的工具.

The studies for testing mycotoxins detoxifying agents are performed in the TIM-1 system, the TNO dynamic, multi-compartmental system of the stomach and small intestine (Figure 2). This computer-controlled model simulates the successive dynamic conditions in the gastric compartment and in the three successive compartments of the small intestine. In this system the gastrointestinal conditions were simulated digestive conditions of the pig after the intake of a pig feed. 測試黴菌毒素吸附劑作用的研究以使用 TIM-1 系統爲佳, 也就是 TNO 動力模型, 分成多個部份模仿胃和小腸的系統(圖 2). 這個由電腦控制的模型仿照胃部以及三個部份的小腸的連續動態狀態. 在這個系統的胃腸道環境仿照豬隻食入飼料後的真實消化狀態.

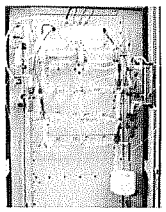


Figure 2. TNO dynamic model of the stomach and small intestine (TIM-1)
胃和小腸的 TNO 動態模型 (TIM-1)

Maria Angeles Rodriguez, Taiwan, 31st August 2011

Moulds and Mycotoxins

In vitro dynamic trials 體外動力學試驗

Performance of active carbon in TIM-1: 活性碳在 TIM-1 系統的吸附表現:

Dr. Giussepina Avantaggiatto, from CNR Institute of Sciences of Food Production (ISPA) in Italy, has run out several trials using this system to evaluate the efficacy of several commercial binding agents and substances potentially useful as chelating agents (Avantaggiato et al., 2003; 2004 y 2007). 義大利食物製造科學協會國家研究會議 (ISPA-CNR) 的 Dr. Giussepina Avantaggiatto 已經使用這套系統進行幾項試驗, 來評估一些市售吸附劑以及像螯合劑等可能有用的物質的效果 (Avantaggiato et al., 2003; 2004 y 2007).

In those studies in the TIM system with activated carbon, she demonstrated a reduction of the bioaccessibility of **DON of 29-45%** in comparison to the control experiment (Avantaggio et al., 2004). However, the level of activated carbon in the feed ranged from **0.5% to 2%.** 在使用 TIM 系統進行的活性碳試驗中, 她證明了嘔吐毒素 (DON) 比對照組減少了 29~45% (Avantaggio et al., 2004). 然而, 飼料中添加的活性碳含量範圍從 0.5% 到 2% 都有.

Maria Angeles Rodriguez, Taiwan, 31st August 2011

Moulds and Mycotoxins

Olmix innovation *Olmix 的新發明*

Amadéite® is a new natural Pillared Interlayered Clay (P.I.L.C), patented by Olmix.
奈米態 (Amadéite®) 是一種新的天然柱狀夾層的黏土 (P.I.L.C), Olmix 公司並取得專利.

It's produced through a new patented technology process in which the layers of a phyllosilicate clay (Montmorillonite) are separated by seaweeds polysaccharides (ulvans).
它是使用一種新的專利技術製造, 利用海藻多醣 (ulvans) 將頁狀矽酸鹽類黏土 (蒙脫土) 的層狀構造分開.

That modification of the structure of the clay increases up to ten times the space between the layers thus opening access to big size molecules and increasing dramatically the surface area available for adsorption of mycotoxins.
這樣改變黏土的構造讓層與層之間的空間增加了 10 倍, 如此得以允許大分子的吸附並且大大增加了可以吸附黴菌毒素的表面積.

The production process is 100% environmental friendly.
整個製造過程 100% 綠色環保.

Maria Angeles Rodriguez, Taiwan, 31st August 2011

Moulds and Mycotoxins

In vitro dynamic trials 體外動力學試驗

Performance of Amadéite® in TIM-1: 奈米態在 TIM-1 系統的吸附表現:

The availability for absorption (bioaccessibility) of mycotoxins from the jejunum and ileum was measured during gastrointestinal transit of pig feed contaminated with both: 當受到下列兩種黴菌毒素污染的豬隻飼料通過胃腸道時, 測量空腸段到迴腸段間吸收黴菌毒素的能力:

- **DON (1 ppm), 嘔吐毒素 (1 ppm)**
- **and Fumonisin B1 (2 ppm), 和 伏馬鐮孢毒素 (2 ppm)**

simulating the gastrointestinal conditions of pigs in the TIM-1 system®. 在 TIM-1 系統中模仿豬隻的胃腸道環境.

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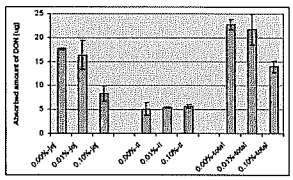
In vitro dynamic trials 體外動力學試驗

Performance of Amadéite® in TIM-1: 奈米態在 TIM-1 系統的吸附表現:

Deoxynivalenol (DON) 嘔吐毒素

The bioaccessibility of DON from contaminated pig feed was not significativly inhibited by the addition of Amadéite® at the levels of 0.01%. 在含 0.01% 濃度的奈米態飼料, 受到汙染的豬飼料中嘔吐毒素並沒有明顯的受到抑制.

However, a strong inhibition of absorption was found by the addition of Amadéite® at the level of 0.1%. The reduction was approximately 40% in comparison to the control. 然而, 在含 0.1% 濃度的奈米態飼料, 可以發到明顯的吸附作用. 與對照組相比, 降低將近 40%.



Absorption of DON (ng) from the jejunum (jej) and ileum (il) compartments and from both compartments together (total) in the control experiments (0.00%) and in the experiments with the addition of Amadéite® at the level of 0.01% and 0.10% during gastrointestinal transit in the TIM-1 system of pig feed contaminated with DON (0.8 ppm) and fumonisin B1 (2 ppm) 受到嘔吐毒素 (0.8 ppm) 和伏馬鐮孢毒素 B1 (2 ppm) 污染的豬飼料通過 TIM-1 系統之胃腸道轉運速率時, 對照組(0.00%) 、奈米態 0.01% 組以及奈米態 0.10% 組在空腸段 (jej) 、迴腸段 (il) 以及兩段合併 (total) 之嘔吐毒素的吸收 (ng).

Maria Angeles Rodriguez, Taiwan, 31st August 2011

Moulds and Mycotoxins

In vitro dynamic trials 體外動力學試驗

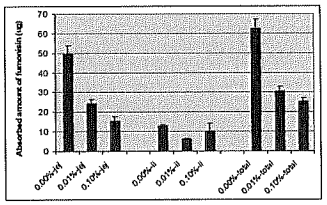
Performance of Amadéite® in TIM-1: 奈米態在 TIM-1 系統的吸附表現:

Fumonisin 伏馬鐮孢毒素

The bioaccessibility of fumonisin from contaminated pig feed was strongly inhibited by the addition of Amadéite® at the levels of 0.01% and 0.1%. 在含 0.01% 和 0.1% 濃度的奈米態下, 受到汙染的豬飼料中伏馬鐮孢毒素受到明顯的抑制.

The total absorption of fumonisin was reduced from 62 µg (control) to 30 µg and 25 µg in the experiments with Amadéite® in the feed at the levels of 0.01% and 0.1%. 伏馬鐮孢毒素的吸附從 62 µg (對照組) 降低至含 0.01% 奈米態飼料的 30 µg 和含 0.1% 奈米態飼料的 25 µg.

This means a reduction of 50% to 60% of the bioaccessibility of fumonisin. 這表示伏馬鐮孢毒素的生物可接觸性減少 50~60 %.



Absorption of Fumonisin (µg) from the jejunum (jej) and ileum (il) compartments and from both compartments together (total) in the control experiments (0.00%) and in the experiments with the addition of Amadéite® at the level of 0.01% and 0.10% during gastrointestinal transit in the TIM-1 system of pig feed contaminated with DON (0.8 ppm) and fumonisin B1 (2 ppm). 受到嘔吐毒素 (0.8 ppm) 和伏馬鐮孢毒素 B1 (2 ppm) 汙染的豬飼料通過 TIM-1 系統之胃腸道輸送過程時, 對照組(0.00%)、奈米態 0.01% 組以及奈米態 0.10% 組在空腸段 (jej)、迴腸段 (il) 以及兩段合併 (total) 之伏馬鐮孢毒素的吸收 (µg).

Moulds and Mycotoxins

Remarks 評論

- There are big differences between performance of a toxin binder in vitro and in in vitro dynamic system: 毒素吸附劑在一般體外試驗和體外動力學試驗的結果表現有很大的差異:
  - Active Carbon: 84-95% of DON in vitro, 29-45% of DON in TIM-1 活性碳: 在一般體外試驗吸附嘔吐毒素 84~95%, 而在 TIM-1 系統吸附嘔吐毒素 29~45%.
- Active Carbon is a good mycotoxin binder, much better than the rest of commercial toxin binders, in vitro. 在體外試驗中, 活性碳是很好的黴菌毒素吸附劑, 比其他市售的毒素吸附劑要好得多.
  - Active Carbon 84-95% of DON vs others, maximum Mycosorb, 18% of DON at pH 3. 在 pH 3 下, 活性碳可吸附 84~95% 的嘔吐毒素, 而其他品牌, 像是 Mycosorb, 則只能吸附18% 的嘔吐毒素.
- However, in practice, the use of activated carbon in animal production has some limitations. High concentration of activated carbons (> 0.5%, w/w) should be avoided in order to minimize the risk of nutrient adsorption as well as an impairment of the caloric/nutritional value of the feed (NOSB, 2002; Ramos et al., 1996). 然而, 實際上, 活性碳在畜產動物的使用上有一些限制. 爲了讓活性碳吸附營養成份以及飼料中卡路里和營養價值喪失的風險降至最低, 應避免高劑量的使用活性碳 (> 0.5%, w/w)(NOSB, 2002; Ramos et al., 1996).

Moulds and Mycotoxins

Remarks 評論

Amadéite® has a better capacity of adsorption of DON than active carbon, cause to reduce adsorption of Don in TIM-1 it needs 1Kg/Tm instead of 5 to 20 Kg/Tm for the active carbon.
奈米態吸附嘔吐毒素的能力優於活性碳, 因爲奈米態只需要每噸添加 1 公斤就能夠減少嘔吐毒素的吸收, 而活性碳卻需要每噸添加 5~20 公斤.

- Moreover, Amadéite® has no besides effects on nutrient adsorption. (Demais and Havenaar, 2006)
此外, 奈米態對於營養的吸收沒有任何不好的作用. (Demais and Havenaar, 2006)

Moulds and Mycotoxins

Conclusions 結論

- Mycotoxins are widely spread all over the world in different feedstuffs and foodstuffs. 黴菌毒素廣泛存在於所有世界上的飼料及食物中.
- They posses a risk to both animal and human health. 黴菌毒素對於動物和人類的健康都會造成傷害.
- They are difficult to eliminate from feedstuffs. 黴菌毒素很難自飼料中被清除.
- Not all of them can be adsorbed or detoxified by conventional detoxifying agents. 不是所有的黴菌毒素都可以被普通的吸附劑吸附或去毒.
- Activated carbon is able to detoxify difficult mycotoxins such as DON, but also impairs nutritional value of feed. 活性碳能夠處理比較難吸附的黴菌毒素, 像是嘔吐毒素, 但是也會導致飼料喪失部份的營養價值.
- Still, there's an effective solution, based in the latest technology patented by Olmix: **Amadéite®**. 儘管如此, 還是有一種有效的解決之道, 就是利用 Olmix 取得專利的最新科技所製造的: 奈米態.
- You can only find **Amadéite ®** in our top toxin binder. 您只能在我們頂尖的毒素吸附劑產品中找到奈米態.

Moulds and Mycotoxins

for a better life

Thanks for your attention

謝謝您的聆聽!

敬請指教!