An integrated approach to mycotoxin testing from farm-to-fork

Bankok, March 2010

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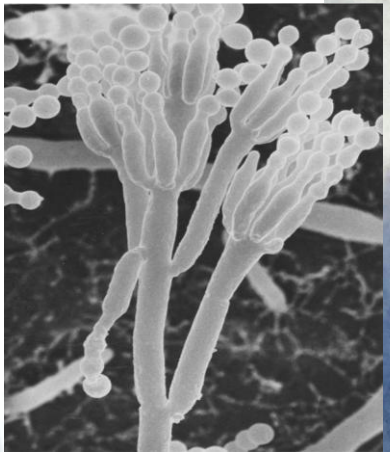
OUTLINE

- Mycotoxins in cattle feed
- Aflatoxins - transfer to milk
- Regulations
- Sampling
- On-site testing
- Laboratory testing

Mycotoxins in animal feed

- Mycotoxins are fungal metabolites
- Formed pre-harvest and post-harvest (during storage) – cereals, seeds, nuts
- Mycotoxins can be present without signs of evident mould – feed looks ‘sound’
- Mycotoxin contamination is heterogeneous (hot spots of contamination)



Mycotoxin contaminated feed materials

- Aflatoxins B_1, B_2, G_1 & G_2 – nuts, seeds, maize
- Fumonisins B_1, B_2, B_3 – maize (corn)
- Deoxynivalenol (DON) – wheat & other small grain
- Ochratoxin A – all cereals

Susceptibility of cattle to mycotoxins

- Aflatoxin B_1 – **clinical signs at 1.5-2.3 mg/kg in feed**

  Transfer as aflatoxin M1 into milk

- Fumonisins – **75 mg/kg decreased milk yield**

  No significant transfer to milk

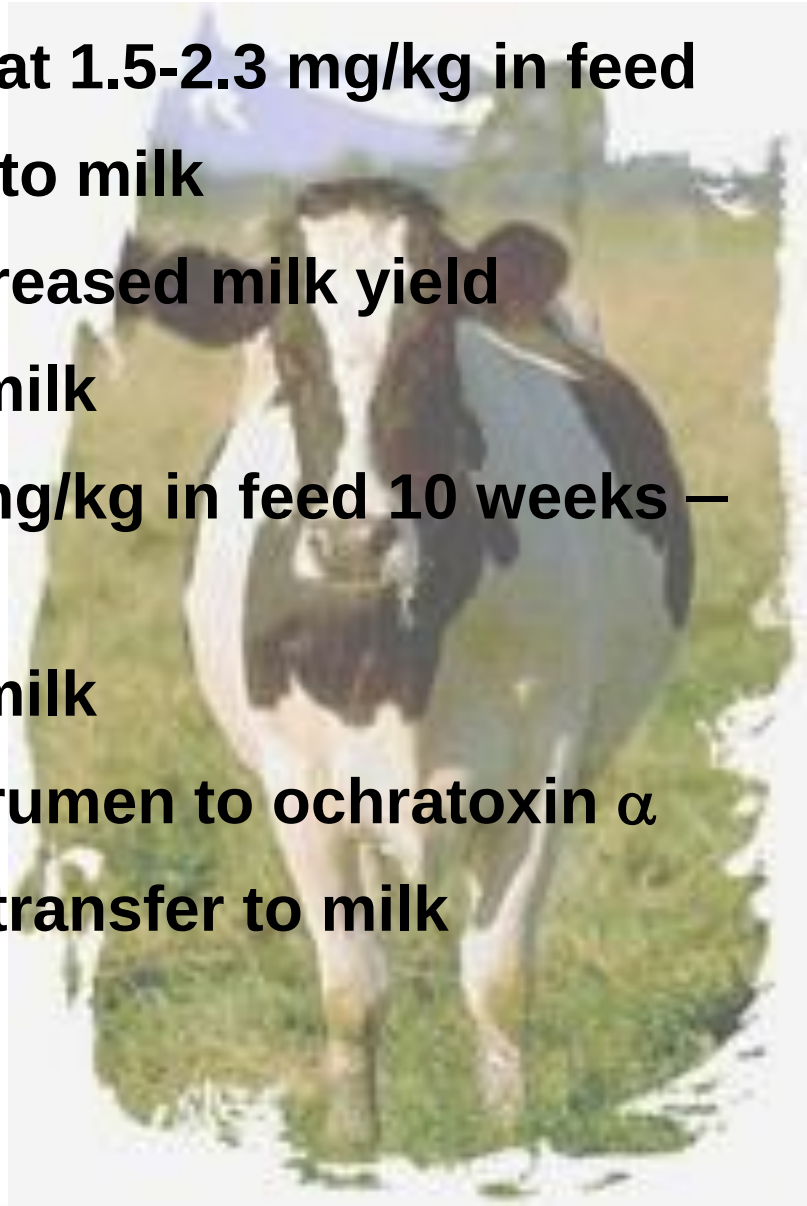
- Deoxynivalenol – **5.0-12.1 mg/kg in feed 10 weeks –**

  No effect on milk yield

  No significant transfer to milk

- Ochratoxin A –**degraded in rumen to ochratoxin α**

  Some reports of low level transfer to milk



Feed risk components for aflatoxin B_1 contamination

- Groundnut
- Copra
- Palm kernel
- Sunflower seeds
- Maize
- Babassu
- Cotton seed
- Rice & rice bran

EU Regulations for aflatoxin B_1 in cattle feed

Council Directive 1999/29/EC of April 29th 1999

- Groundnut, copra etc…. 0.02 mg/kg
- Complete feed for cattle 0.05 mg/kg
- Complete feed for dairy cattle 0.005 mg/kg
- Complementary feed for cattle 0.05 mg/kg
- Complementary feed for dairy 0.005 mg/kg

EU Regulations for aflatoxin M_1 in milk

EC Regulation 1881/2006 of Dec 19th 2006

- Limit for aflatoxin M_1 in raw milk = 0.05 ng/g
- Limit for aflatoxin M_1 in infant formula = 0.025 ng/g

Transfer of aflatoxin B_1 from feed to aflatoxin M_1 in milk

Table 2: Estimated concentrations of aflatoxin M_1 in milk of various animal species considering a carry-over rate of either 6% (reported level for high yielding cows) or 2% (assumed average level).

Species	Case	Milk kg/d	Total feed intake kgDM/d	Compl. feeds in kgDM/d	Feed mat. in kgDM/d	Compl. feeds AFB_1 µg/kg	Feed mat. AFB_1 µg/kg	AFB_1 intake µg/d	Carry over	AFM_1 µg/kg milk
Cattle	A	50	26.0	19.5	6.5	5.0	20.0	227.5	0.06	0.27
	B	25	17.5	7.0	11.5	5.0	20.0	265.0	0.02	0.21
	C	25	17.5	7.0	11.5	5.0	0.0	35.0	0.02	0.03

The EFSA Journal (2004) 39, 1-27

Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Aflatoxin B_1 as undesirable substance in animal feed

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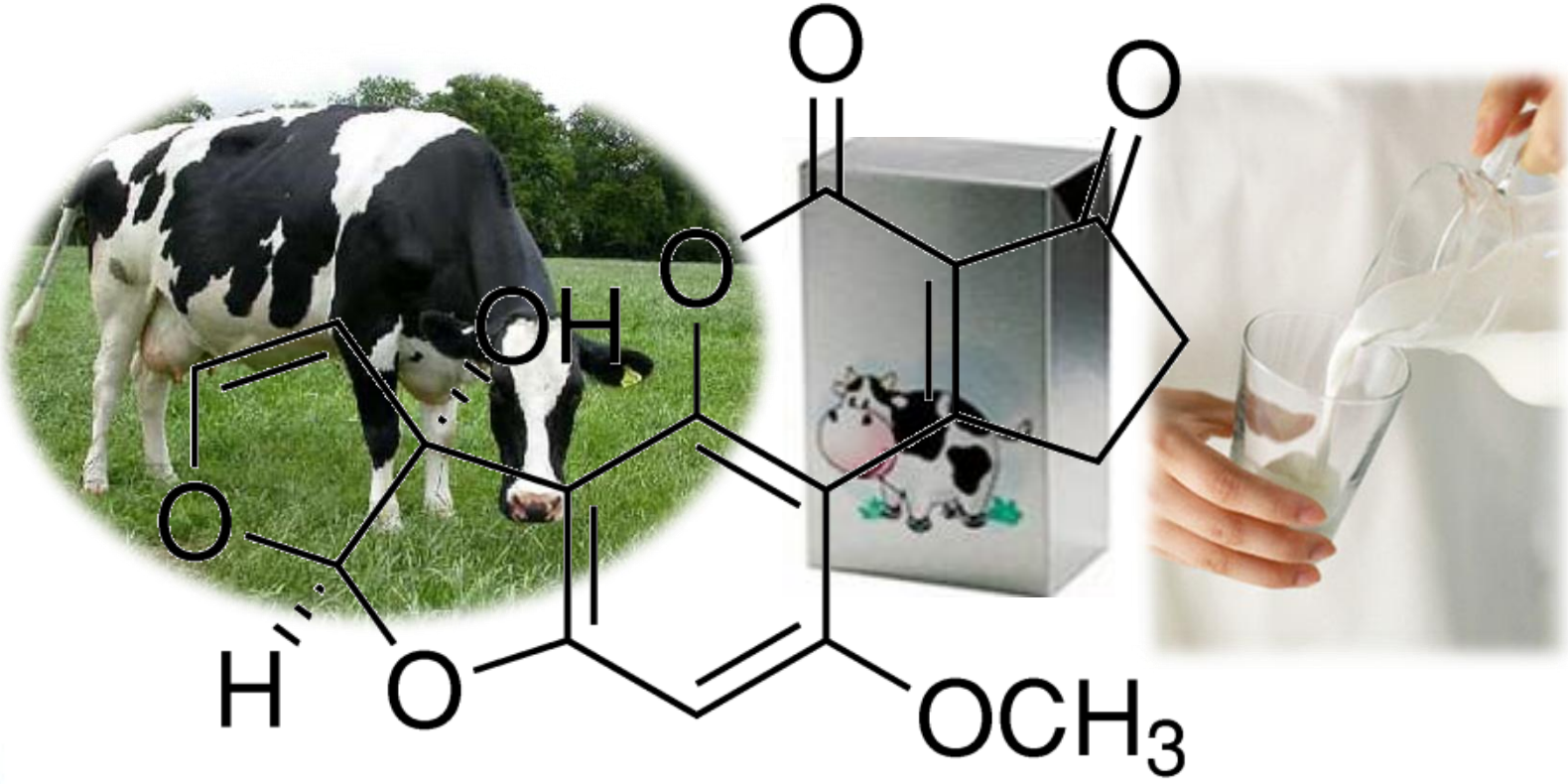
Integrated approach

Identification of high-risk feed components

- Screening of feed components
- Screening of compound feed
- Monitoring dairy products for aflatoxin M_1



Adoption of adequate sampling strategy

- Mycotoxins in feed are not uniformly distributed
- Heterogeneous contamination – hot spots
- Large sample sizes need to be taken

EU Sampling requirements for aflatoxins in animal feed

Commission Regulation (EC) No 401/2006 of Feb 26th 2006

Lot weight in tonnes	Sub-lot	No of incremental samples	Aggregate sample size
>1500	500 tonnes	100	10 kg
300- 1500	3 sub-lots	100	10 kg
50-300	100 tonnes	100	10 kg
<50	None	3-100	1-10 kg

Testing for aflatoxins in feed and milk

Sampling & Sub-sampling

↓

Grinding and homogenization (10 kg)

↓

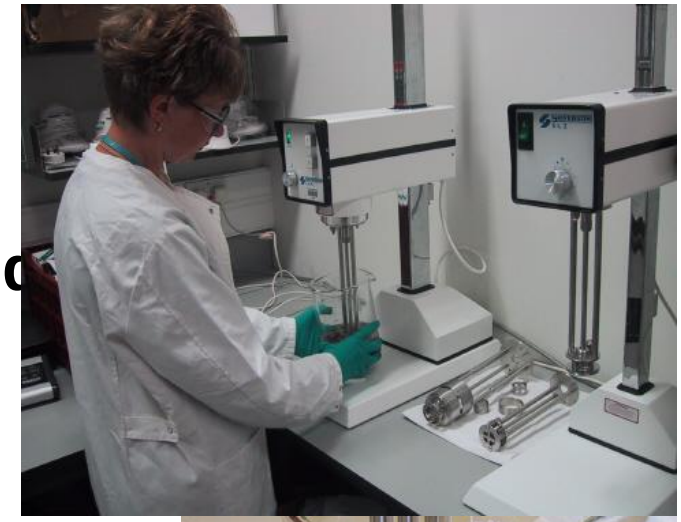
Extraction into solvent

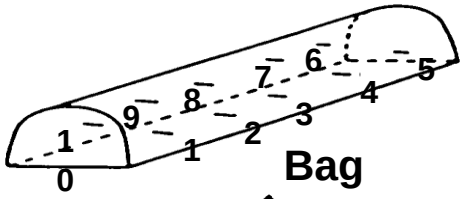
Screening

Instrumental analysis

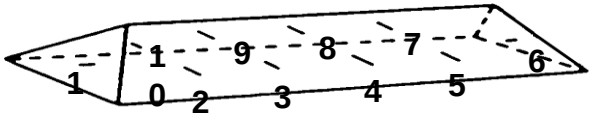
Homogenization of sample prior to analysis

- **Feed samples must be milled**
- **Milled samples thoroughly mixed**
- **Sub-sampled**
- **Slurry-extraction**

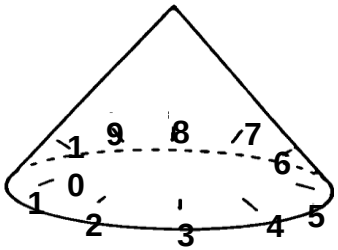




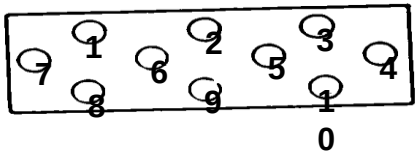
Bag



Wedge Shaped Pile



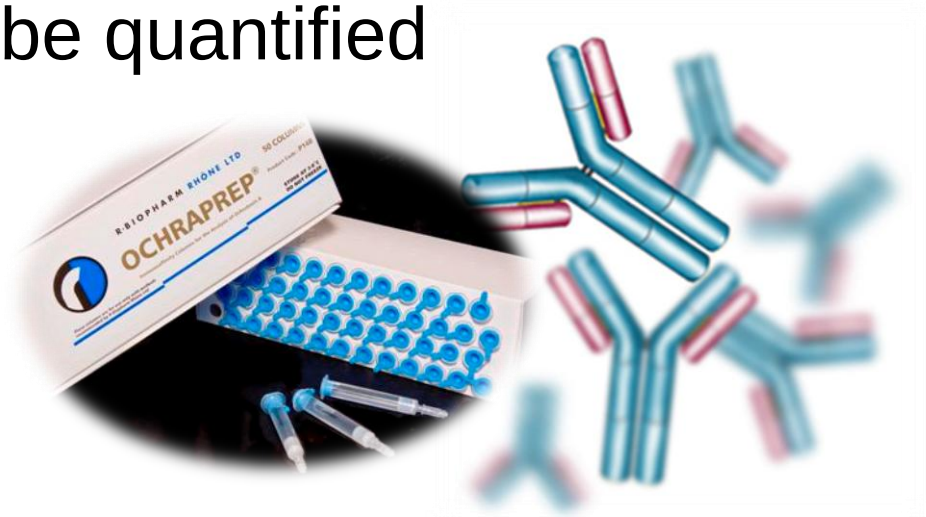
Conical Pile



Truck

Antibody technology

- Antibodies can be obtained specific to aflatoxin B_1
- Antibodies have high specificity
- Antibodies can be used in various formats to provide sensitive and specific test kits
- End-points are usually colour changes
- Cut-off point for test can relate to regulatory limit
- Intensity of colour can be quantified



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Typical rapid screening – R Biopharm AFLACARD B1

Extract 50 g sample in blender

↓ 100 mL methanol/water

Filter minimum of 10 mL

↓ using syringe

Apply 500 uL to card membrane

↓ allow to pass 5 min

Apply 100 uL of conjugate

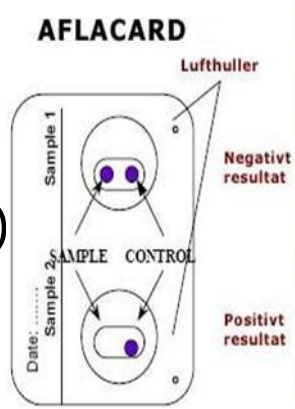
Apply 100 uL buffer

Apply 100 uL substrate wait 5 min

Apply 100uL Stop solution

↓

Read result visually (coloured spot)



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Comparison of screening options for aflatoxin testing in feed

Product	Brand name	No tests	Analysis time	Reading result	LOD ppb
Test card	AFLACARD B1	2/card	5 min	Visual	2
LFD	RIDA®QUICK Aflatoxin	1/LFD	4-16 min	Visual/ reader	4,10,20
ELISA	RIDASCREEN® FAST SC	48 or 6 strips	15 min	reader	2
IAC	AFLASCAN®	1 per column	20 min	Visual UV	1

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Instrumental analysis – using R Biopharm Aflatest® affinity column

- Extract 50 g sample in blender
- Filter or centrifuge
- Apply to immunoaffinity column
- Wash column
- Elute from column
- Carry out HPLC analysis
- Separation of aflatoxins and quantification by fluorescence

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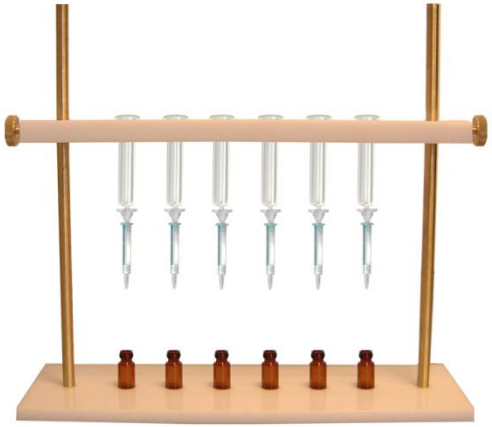

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Conclusions

- Aflatoxin contamination is major concern for dairy cattle
- Controls are focussed on minimising transfer of aflatoxin M_1 to milk
- Some feed components are ‘high risk’ and MUST be controlled
- Simple tests available
- Sampling critical to achieve meaningful result

Teşekkür, Thank you

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