Risks of mycotoxins and plant toxins in food and feed

Monique de Nijs, CON*ff*IDENCE presentation October, 4, 2011

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3. *Mycotoxins and plant toxins (Mw. de Nijs)*
   1. Introduction
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6. *Risk assessment and regulations for mycotoxins (Mr. van Egmond)*

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RIKILT - Institute of Food Safety

www.rikilt.wur.nl

- Part of Wageningen UR since 1998
- About 200 employees

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- Detection, identification, functionality and effects of substances in food and feed;
- RIKILT:
  - Specific research, with CVI and RIVM, for the Netherlands new food safety authority (nVWA);
  - Is laboratory for VWA feed and AID;
- Contract work:
  - According to guidance document;
  - Report duty, as any laboratory, when legal limits are exceeded;

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- Environment & process contaminants:
  - *Dioxins in eggs in Germany;*
  - *Fire in Moerdijk (incl heavy metals);*
- Radioactivity:
  - *Imports from Japan;*
- Pesticides;
- Natural toxins (*mycotoxins, plant toxins, phycotoxins*);
- Animal treatment medicines and residues;
- GMO's;
- Allergens;
- Nutrients / Quality:
  - *Identify organically produced eggs;*
  - *Authenticity identification (is this the fruit juice I selected and ordered several months ago?);*

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Conffidence

- Contaminants in food and feed: inexpensive detection for control of exposure;
- LCP (large collaborative program) Food, Agriculture and Fisheries, and Biotechnology, 2008-2012;
- Simple, fast, multi-analyte, multi-class detection;
- Includes WP Biotoxins:
  - alkaloids (pyrrolizidine, tropane, ergot)
  - *Fusarium* mycotoxins (TCT, ZEA, FUM)
- Intra- and interlaboratory validation studies

www.conffidence.eu

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Introduction – *Natural toxins in food and feed*

Compounds that are poisonous to humans and/or animals that naturally occur in food and feed can be produced by:

1. Microorganisms (fungi (mycotoxins) and bacteria);
2. Plants (plant toxins or phytotoxins);
3. Algae/shellfish

(4. Zootoxins produced by snakes / bees / frogs)

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Introduction – *Zootoxin*

Likken van kop hallucinerende pad kan levensgevaarlijk zijn

In Leeuwarden ontstond vrijdag enige opwinding toen bleek dat drie Zuid-Amerikaanse padden uit een dierenwinkel gestolen waren. De padden zijn populair bij drugsverslaafde paddenlikkers omdat ze hallucinerende stoffen uitscheiden, maar kunnen dodelijk zijn.

Eigenaar Richard Mastenbroek zoekt de daders dan ook bij de naastgelegen dagopvang voor drugsverslaafden.

De gestolen dieren hebben op de kop klieren die een melkachtig slijm kunnen afscheiden, dat het giftige bufotoxine bevat.

De stof veroorzaakt bij mensen hallucinaties die zeven tot acht uur kunnen duren. Bij gebruikers treedt een effect op dat vergelijkbaar is met een lsd-trip. De padslijm simpelweg op te likken. Het slijm wordt echter ook wel gedroogd en vervolgens gerookt.

Gemakkelijk verkrijgbaar

De reuzenpad, Bufo marinus, is afkomstig uit de tropische gebieden van Zuid-Amerika en komt ook voor in het zuiden van Texas, Mexico en Midden-Amerika. De dieren zijn licht- tot donkerbruin en hebben een wrattige huid. De pad werd in veel tropische gebieden ingevoerd om plagen van insecten te bestrijden. De pad komt ook voor op de Nederlandse Antillen en is daarom in Nederland vrij gemakkelijk verkrijgbaar.

Hoewel directielid Peter Brouwer van daklozendagopvang in Leeuwarden niet uitsluit dat de

De Bufo marinus-pad.

Poison, licked from the head of a certain toad, can give strong hallucinations

Can even kill a grown up person

Introduction – *Secondary metabolites*

All natural toxins = secondary metabolites

Secondary metabolite ≠ toxin

Secondary metabolites: metabolites that are not directly related to growth of cells, or to development and reproduction of an organism;

Primary metabolites: amino acids and glucose and substances related to growth of cells, and to development or reproduction

Introduction – *Secondary metabolites*

Secondary metabolites:

- Biological active compounds;
- Important for agriculture: antibiotics, toxins, insecticides or signal compounds;
- Biotechnology uses organisms to intentionally produce certain secondary metabolites such as penicillin production, fermentations.

Introduction – *Secondary metabolites*

Metabolites: products of biochemical processes in the cell controlled by enzymes;

Mycotoxins and plant toxins are secondary metabolites produced by resp. fungi and plants with known toxicity to humans or animals.

Synonym of plant toxins: phytotoxins *(analog to mycotoxins; fycotoxins)*, however: phytotoxic is used in the USA for compounds that are toxic to plants. This should be phytotoxic;

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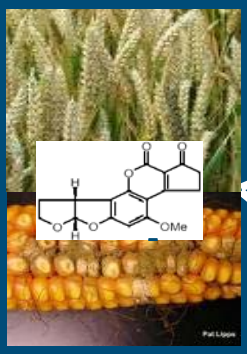
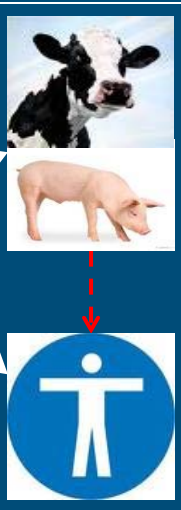
Mycotoxins – *Contamination and exposure route*

Fungi contaminate (harvested) grain



Fungi excete mycotoxins in (harvested) cereals



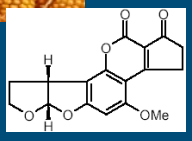
Mycotoxins – *Factors affecting contamination*

- Fungus species: *Aspergillus, Penicillium, Fusarium*
- Substrate: fumonisin mainly detected in maize;
- Environmental circumstances :

  → Changes toxin profile *(Secondary metabolites)*
  - Humidity: fungal infection and growth increase, other fungal species;
  - Dry: grain damaged in field, access for fungi;
  - Temperature: mycotoxin profile changes (enzymes)
  - Insects: access for fungi;
  - Agricultural practices: e.g. use of pesticides influence metabolism;
  - Etc.

Mycotoxins

Hundreds mycotoxins known:



- *Aspergillus; Penicillium*
  - Aflatoxins
  - Ochratoxin A
- *Fusarium*
  - Deoxynivalenol
  - Fumonisins
  - T-2/HT-2 toxins
  - Zearalenone
- *Claviceps*
  - Ergot alkaloids
- *Penicillum; Aspergillus; Byssochlamys; Paecilomyces*
  - Patulin

Mycotoxins – *Incidents and issues*

Year	*Toxicosis*	*Toxin*	*Fungus*
994	Holy fire	lysergic acid deriv.	*C. purpurea*
1890	Cardiac beriberi	citreoviridin	*P. citreo-viride*
1913	Alimentary toxic aleukia	trichothecenes	*F. sporotrichioides*
1952	Balkan endemic nephropathy	ochratoxin	*P. verrucosum*
1960	Turkey X disease	aflatoxins	*A. flavus*
1988	Hole in the head syndr.	fumonisins	*Fusarium*
2004& '05&'10	Hum. aflatoxicosis	aflatoxins	Not determined *317 people ill, 127 fatalities (Kenya)*

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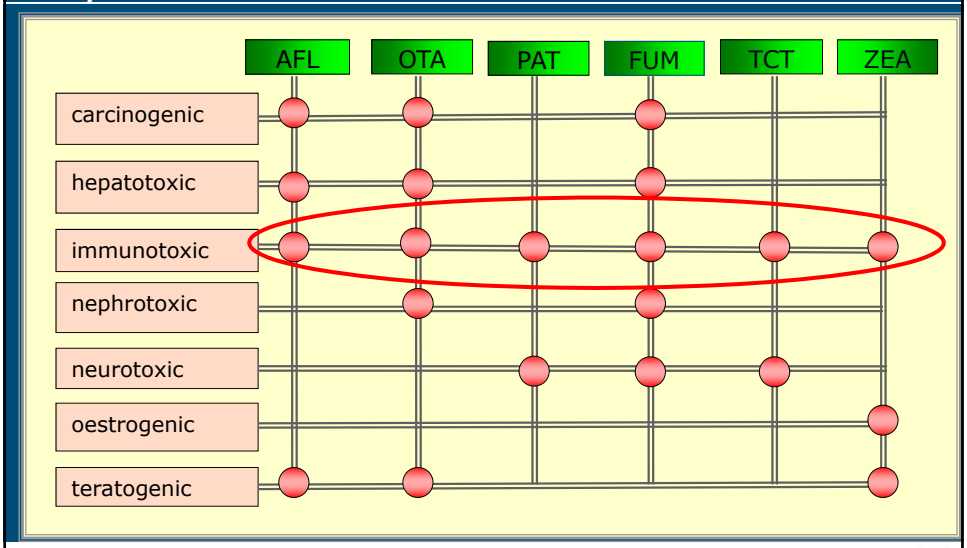
Mycotoxins - *Toxic effects*



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Mycotoxins – *Challenges - Products*

994: Holy fire (lysergic acid deriv. *C. purpurea)*

1568: Pieter Brueghel *The Cripples*
'Holy fire' by ergot alkaloids in **rye**
(gangreen followed by *necrosis / hallucinations*)

2011: Ergots in **cereals**; EFSA opinion expected in 2012
Regulatory limits on groups of toxins (as opposed to parts)

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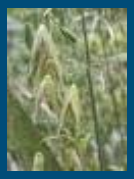
Mycotoxins – *Challenges - Products*

- (Re-)introduction of crops:
  - Introduction of lupin as GMO-soy replacer: risk of phomopsin contamination (Australia & NZ limit of 5 µg/kg)

- Increased area of oats as healthy grain: no recent reports on occurrence of mycotoxins in oats in the Netherlands.



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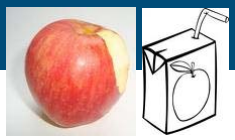
Mycotoxins – *Challenges - Handling*

- Up to now: patulin contamination of apple juice seems to be controlled:
  - bulk production of apples;
  - controlled storage and sorting;
  - quality control on juice;
- Currently: handicraft / local production:
  - lack of knowledge on contamination routes (uncontrolled storage, no sorting prior to processing);
  - use of new or old races (no experience);

  → patulin in artisanal produced apple juice (2009):

  (Gillard et al (2009) WMJ (1) 95-104)



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Mycotoxins - *Challenges– Masked mycotoxins*

- Masked mycotoxin = mycotoxin that is metabolised by the plant into a, for the plant, harmless compound;
- Eg: deoxynivalenol-3-*β*-D-glucoside (D3G);
- First report 1985 by Miller JD & Young JC

  (Deoxynivalenol in an experimental Fusarium graminearum *infection of wheat, Canadian Journal Plant Pathology, (1985), 7, 132-134);*
- Relevance:
  - Occurrence: D3G analyzed by LC MS/MS;
  - Toxicity:
    - Increased exposure by release in intestinal tract?
    - Absorbed by intestinal cells?

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Mycotoxins – *Challenges - Climate*

- Expected changes in range of latitudes, where certain fungi are able to compete;
  - Example: *F. graminearum* growth: NIV increase;
- Drought, flooding and other consequences of climate change may result in more mycotoxins and changed toxin profiles;
  - Example: aflatoxins found in Italy since 2003, and in other parts of Central Europe;
- Response of insects and plant diseases to climate change poorly understood, but increases expected

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Mycotoxins - *Regulations - EU Food (1/2)*

- Commission *Regulation* (EC) No 1881/2006 (and its amendments): Setting maximum levels for certain contaminants in food
- Commission *Regulation* (EC) No 401/2006 (and its amandments): Laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in food

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Mycotoxins - *Regulations - MS Food (2/2)*

- The Netherlands: Warenwetbesluit bereiding en behandeling van levensmiddelen Artikel 12

  Fungal and bacterial toxins in quantities that can be harmful to the public health must be absent in food, drinks and raw materials

Mycotoxins - *Regulations - EU Feed (1/2)*

- Commission *Directive* (EC) No 2002/32 (and its amendments): On undesirable substances in animal feed
- Commission *Reccomendation* 2006/576/EC: On the presence of deoxynivalenol, zearalenon, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding

Mycotoxins - *Regulations - EU Feed (1/2)*

- Commission *Regulation* (EC) No 152/2009: Laying down the methods of sampling and analysis for the official control of feed

Regulations - *EU import controls*

- Commission *Regulation* (EC) No 669/2009 (and its amendments): Implementing Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards the increased level of official controls on imports of certain feed and food of non-animal origin and amending Decision 2006/504/EC
- Commission *Regulation* (EC) No 1152/2009: imposing special conditions governing the import of certain foodstuffs from certain third countries due to contamination risk by aflatoxins and repealing Decision 2006/504/EC

Mycotoxins - *Decontamination*

EU regulatory limit for mycotoxins in feed: aflatoxin

EU recommended limit for mycotoxins in feed: deoxynivalenol, zearalenon, ochratoxin A, T-2 and HT-2 and fumonisins

- Decontamination is not allowed in EU;
- Dilution to lower contamination is not allowed in EU;

Mycotoxins - *Decontamination*

- Feed: allowed are 'technological additives' according to EU 1831/2003: substances for reduction of the contamination of feed by mycotoxins;
  - that can suppress or reduce the absorption;
  - promote the excretion of mycotoxins;
  - modify their mode of action.

  Need to be evaluated according to EU 429/2008
  - Toxicity of the substance to animals;
  - Efficay;
- <u>Only when EU regulations are met !</u>

Mycotoxins - *Decontamination*

'Technological additives' according to EU 1831/2003:

- Biotransformation
  - E.g.: enzymes; enzyme producing microorganisms;
- Organic binders
  - E.g.: yeast cell wall components; synthetic polymers; humic substances; dietary fibres;
  - Nutritional feed additives:
  - E.g.: antioxidants; immunostimulatory agents;
- Inorganic compounds (absorbents):
  - E.g.: clays; activated carbons

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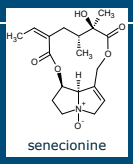
Plant toxins – *Contamination and exposure route*

Plant toxins – *factors affecting contamination*

- Role: protect plant against being eaten (insects and animals) and infections;
- Plant species (star anise);
- Environmental circumstances:
  - Temperature;
  - Growing season;
  - Insects;
  - Etc. – more research.

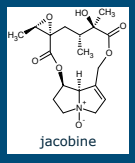
Plant toxins

Hundreds plant toxins known



Mostly mentioned:

- PA's: pyrrolizidinalkaloids;
- TA's: tropane-alkaloids;
- Aromatic compounds: essential oils; alkenylbenzenes (estragol; methyleugenol; safrool) (herbs);
- Lectins (legumes);
- Furanocoumarins (cruciferous plants);



Plant toxins – *Incidents and issues*

1990-1991 Belgium: >100 women kidney damage (transplants, cancer development)
Cause: aristolochic acids from *Aristolochia spp* ingredient in herbal preparations/TCM Slimming agents

Aristolochia Clematis (birthwort; pijpbloem)

2001 Netherlands: >60 poisoning cases (epileptic seizures)
Cause: anisatin from ingredient in herbal tea
(Japanese instead of Chinese star anise)

Plant toxins – *Incidents and issues*

NACHRICHTEN-ARCHIV

Supermarkt: Giftiges Greiskraut im Rucola

In einer Packung Rucola-Salat hat ein Kunde der Discounter-Kette Plus in Hannover nach Medienberichten einzelne Stängel einer giftigen Pflanze entdeckt. Das Gemeine Greiskraut (Senecio vulgaris) könne in größeren Mengen mitunter gefährliche Leberschädigungen hervorrufen, schrieb «Spiegel Online». Nach Angaben von Plus-Sprecherin Christina Stylianou sei die betroffene Charge komplett aus dem Sortiment genommen worden, um jedes Risiko auszuschließen. Der Lieferant des Salats sei kontaktiert und um eine stärkere Kontrolle der Lebensmittel gebeten worden.

2006 en 2009 Germany:
Weed (*Senecio vulgaris*) in rocket <2.5 mg/kg PAs
Issue: pyrrolizidine alkaloids, liver toxicity
⇒ feed: roughage
⇒ food: honey, herbs preparations/TCM

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Plant toxins – *Incidents and issues*

Cor de Vries (links) en Merijn Vreugdenhil oogsten hun in de kas gekweekte rucola. Foto Joost van den Broek / de Volkskrant

Rucola van de gestoomde grond

De pittig smakende slasoort is definitief doorgebroken in Nederland. Ook telers ontdekken de jonge markt. 'Rond de 70 procent van de productie is al voor de oogst verkocht.'

Door **Tjerk Gualthérie van Weezel**

VK 110804

Slasoorten

Alle rucola is wild, ook die tussen de tegels

De grootste angst van de telers is klein kruiskruid. 'Als je daar een bak van eet, kun je het niet meer naver-

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Plant toxins – *Incidents and issues*

2008 Afganistan: wheat flour contaminated and carry-over to goat milk; 270 persons ill, 50 fatalities due to acute liver lailure;
Cause: *Heliotropium popovii* H. Riedl subsp. *gillianum* in wheat flour.

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Plant toxins – *Incidents and issues*

2010 Netherlands: Alfalfa contaminated with *Senecio vulgaris*:
- Alfalfa grown in coastal area's;
- Alfalafa used as animal feed for cattle and horses (pellets);

2010 Toxicity in cattle: little known on dose-response;
Carry-over several PA's to milk suspected (Jacoline)

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Plant toxins – *Challenges – Carry-over*

Carry-over from feed to animal products:

- Jacoline from *Senecio jacobaea* (jacobskruiskruid) to milk
  - *Hoogenboom et al. (2011) FAC Part A (28) 359-372;*
- Incident in Afghanistan: possible PA's from other weeds carried over to goat milk/cheese;
- No reports on occurrence of plant toxins in meat.

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Plant toxins – *Challenges - Climate*

- Growing area of weeds can change;
- Drought, flooding and other causes;
- (Re-)introduction of crops;
- New plants /weeds due to import / export.

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Plant toxins – *Legislation*

Food: 37/2010 Aristolochia spp. (table 2 forbidden substances, vet.drugs. AO)
396/2005 nicotine, pyrethrins, rotenone, azachdirachtin (MRL pesticides)
Feed: 2002/32 hydrocyanic acid, gossypol, theobromine, allyl-ITC (glucosinolates)
Botanical impurities: weed seeds/fruits with alkaloids, glucosides, other tox subst.

2001 act on herbal preparations (≡ food)
No aristolochic acids
Pyrrolizidine alkaloids < 1 µg/kg
List of plants not to be used in herbal preparations (monk's hood, fox glove,..)

Pyrrolizidine alkaloids, tropane alkaloids, glucosinolates, opium alkaloids, …
General: safety of botanicals
EFSA compendium botanicals

Other

Increased interest/concern:
Climate changes => changes in habitat/abundance weeds
Quality control herbal preparations (gap)
Herbal preparations to replace antimicrobial growth promoters in feed

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Challenges – *Analytical methods*

Single compound chemical analysis

Multiple compound chemical analysis

Single / multiple compound effect analysis

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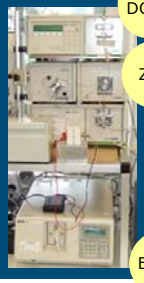
Challenges – *Chemical analysis*

Single compound methods:

- Optimized extraction;
- SPE and/or IAC clean-up (derivatisation);
- LC-UV, LC-Flu, GC-ECD



Multi-compound methods:

- Generic extraction;
- (limited) no clean up;
- LC-MS/MS

(RIKILT 37 mycotoxins + 10 plant toxins)

Aflatoxins
OTA DON
Fumonisins
ZON T-2/HT-2
Ergot alkaloids
Many other mycotoxins

Spanjer et al, *Food Additives and Contaminants (2008), 25, 472;*
Mol et al, *Anal. Chem. (2008), 80, 9450;*
Mol et al. *Food Additives and Contaminants (2011) accepted*

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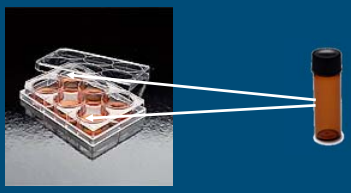
Challenges – *Effect analysis (1/2)*

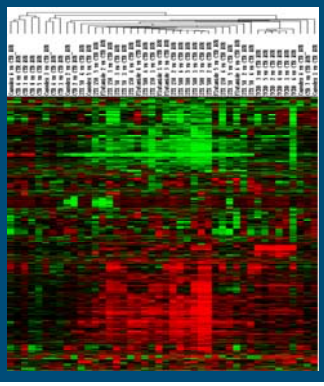
In vitro testing: effects of mycotoxins on gene expression



1. Culture human Caco-2 cells
2. Seed cells; expose to compounds or sample extracts of interest
3. Isolate RNA from Caco-2 cells;
4. Conversion in cDNA, amplify cDNA, labelling;
5. Hybridisation, detection and data acquisition;
6. Data analysis.

Challenges – *Effect analysis (2/2)*

Affected genes whole array
(44000 spots from 25000 genes)
- Red: up-regulated
- Green: down-regulated



Further method development, e.g.:
- Caco-2 cell line clones with luciferase expression when exposed to mycotoxins;
- multiplex qRT-PCR on several specific up- and down-regulated genes;

Conclusions

- Similarities in occurrence and toxic effects of mycotoxins and plant toxins:
  - Route of exposure: mainly through plant materials;
  - Carry-over through animals (meat, milk) is known;
  - Toxin profiles occur and toxicity differs between toxins;
  - Animal species vary in sensitivity;
  - Climate will affect occurring profiles and concentration;
  - Gaps in knowledge about and toxicity of many myco- and plant toxins;
- Plant toxin intoxication often resulting from mistake. The results can be devastating.

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Challenges

Challenge: perform risk assessment on mycotoxins and plant toxins with any change in agrichain (new supplier, new recipe, new harvest);

This demands exchange of information through the whole production chain and close cooperation between authorities, science, plant breeders and produceres of feed and feed.

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Natural toxins


Past performance is no guarantee of future results!

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Thank you for your attention

Questions ?

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