Mycotoxins in Feedstuff and their Effects on Poultry and Swine

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Presentation Outline

- Mycotoxins - Background
- Mycotoxins Involved in Animal Toxicity
- Mechanism of Action
- Occurrence
- Prevention of Contamination
- Prevention of Contamination, Techniques
- Stability during Feed Processing
- Diagnostic Methods
- Absorption Inhibition in GI Tract
- Mycotoxicosis in Poultry and Swine
- Public Health

Mycotoxins - Background

- Secondary metabolites of fungi which are excreted into their host
- Affect plants and their products, that are used for animal/human consumption
- Aflatoxins were the first to be discovered
- Appear throughout history –
  - Dead Sea Scrolls
  - Tenth plague
  - Salem witchcraft trial
  - Chinese medicine
- Two groups: Field and storage fungi

↑

Higher incidence than field fungi
In poultry commercial feed

(Richard, 2007)

Mycotoxins Involved in Animal Toxicity

Mycotoxin	Main fungi producers	Principal toxins	Food supply prone to contamination	Leading factors to mycotoxicosis	Influence
Aflatoxins Toxin incorporated into animal products!	*Aspergillus flavus* and *A. parasiticus*	**B1**, B2, G1, G2	Almonds, cottonseed, peanuts, acorn, chestnuts, corn and grains in general	Inadequate storage conditions (especially >20^{0}C + >14% moisture)	Hepatotoxic ↓ Growth ↓ Milk prod. ↓ Weight gain Teratogenic Immunosupp. Carcinogenic Mutagenic **All species** are affected
DON or vomitoxin (Tricho. Type B)	*Fusarium graminerarum* and *F. culmorum*	---	Corn ("ear rot") and small grains (wheat, barley)	Moist and cool conditions	Vomition syndrome (mainly **swine**) ↓ Growth & reproduction Immunosupp. Inappetance GI tract lesions

The most potent natural carcinogenic agent known

(Richard, 2007; Maciorowski et al, 2007)

Mycotoxins Involved in Animal Toxicity (2)

Mycotoxin	Main fungi producers	Principal toxins	Food supply prone to contamination	Leading factors to mycotoxicosis	Influence
Fumonisins	*Fusarium verticillioides* and *Fusarium proliferatum*	FB1, FB2, FB3	Mostly corn Can be found in rice and sorghum	Drought followed by high humidity and moderate temp. in the next growing season. Inadequate storage conditions (>14% moisture, damaged crop)	Lung edema in **swine** Brain dis. in **equine** Hepatopathy Liver tumors in rats and esoph. Tumors in humans
Zearalenone	*Fusarium graminerarum and F. culmorum*	---	Mostly corn, but also wheat, sorghum and barley	Moist and cool conditions	↑ Estrogen effects, mainly in **swine** ↑ Mortality

(Richard, 2007; Awad et al, 2006)

Mycotoxins Involved in Animal Toxicity (3)

Mycotoxin	Main fungi producers	Principal toxins	Food supply prone to contamination	Leading factors to mycotoxicosis	Influence
T-2 toxin (Tricho. Type A)	*Fusarium sporotrichioides*	---	Wheat, corn, barley, rye, oats, rice	High moisture and temperature range (6-24ºC)	Inhibits: P, DNA and RNA synthesis Inhibits cell division → oral lesions, especially in **poultry** (dose dependent) ↓ BW ↓ Production
Ochratoxin Toxin incorporated into animal products!	*Aspergillus ochraceus* and *Penicillium verrucosum* (potentially more)	---	Corn, grains (including barley), grapes, raising, soy products, coffee	Inadequate storage conditions (especially >20^{0}C + >14% moisture	Carcinogenic Liver and kidney damage (tumors in humans and rats) Swine & human are mainly affected

(Richard, 2007)

Mycotoxins Involved in Animal Toxicity (4)

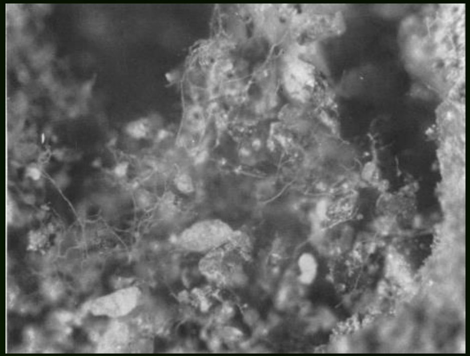
Mycotoxin	Main fungi producers	Principal toxins	Food supply prone to contamination	Leading factors to mycotoxicosis	Influence
Ergot (alkaloids)	*Claviceps* , as well as *Neotyphodium* or *Epichloe*	---	Mostly grass species (millet, fescue, etc)	Strong wind and rain (field fungi)	GI and CNS signs Gangrene Agalactia in **swine** Loss of appendages (e.g. ears) Other affected species – **cattle, equine, human**

(Richard, 2007)

Mechanism of Action

Infection occurs through silks and kernel **wounds**, or systemically through the root

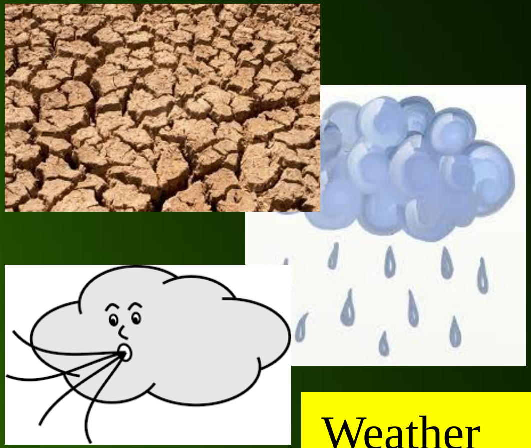
Infected corn



Birds or insects

Fungi survives in soil debris or surface

Fungi are soil/ airborne



Weather conditions

(Richard, 2007)

Occurence

- Differs between geographical regions and environmental conditions, e.g.:
  - T-2 toxin – occurrence throughout the world
  - Zeralenone – occurrence differs throughout the years due to difference in conditions
  - Aflatoxins – in the US, occur mainly in southern area
- Worldwide survey conducted between 2004-2011:
  - 56,672 individual analyses
  - Five mycotoxins prevalence was assessed:
    - DON – 55%
    - Fumonisin – 54%
    - Zearalenone – 36%
    - Aflatoxin – 27%
    - Ochratoxin – 25%

(Streit et al, 2013)

→ Although high levels of mycotoxins were found in the samples > 80% were within the normal limits of the European Union regulations

Prevention of Contamination

- Codes of practice were developed by the Codex Alimentarius
  - Good Agricultural Practices (GAP)
  - Good Manufacturing Practices (GMP)
  - HACCP is considered as complementary quality control system

(Kabak et al, 2006)

Prevention of Contamination, Techniques

- Pre-harvest techniques (**zearalenone, DON, T-2 toxin**):
  - Resistant varieties – some cereal species might be more genetically resistant → difficult to obtain
  - Field management – appropriate management, including irrigation (↓ during anthesis and ripening of crops), soil cultivation, crop rotation and fertilization influence on mycotoxin formation in the field
  - Environmental conditions – temperature, humidity and wind
  - Biological and chemical agents - biocompetitive micro-oraganisms

(Kabak et al, 2006; Jouany et al, 2007)

Prevention of Contamination, Techniques (2)

- Harvest-related techniques:
  - Moisture – most important
  - Avoiding mechanical damage
  - PREVENT contact with soil

- Post-harvest techniques:
  - Mainly techniques to improve drying (↓ moisture)
  - Better storage conditions

(Kabak et al, 2006; Jouany et al, 2007)

Stability during Feed Processing

- **Cleaning, trimming, and sorting**
  - Reduce prevalence of infected grains or kernels
  - Cleaning process efficiency is influenced by the severity of the contamination and the type of fungi involved (aflatoxin by up to 80%)
- **Mycotoxin inactivation**
  - Thermal processing – frying, cooking, baking and canning.
    - Stability depends on process, temperature, time and mycotoxin itself

(Jouany et al, 2007; Bullerman et al, 2007)

Stability during Feed Processing (2)

- **Mycotoxin inactivation (cont.)**
  - Extrusion processing
    - Heat is added in the form of steam→ temp > 150^{o}C
    - Pressure is generated → shortened cooking period
    - Stability of mycotoxins depends on: screw speed, moisture content, *temperature generated, time in extruder*
    - Other factors – ammonia, Maillard reaction
  - Ionization radiation
    - Gamma radiation → success depends on moisture: Higher moisture → water radiolysis → free radicals
    - UV radiation → removal of aflatoxin M1 from milk; Solar energy with UV rays can be used

(Kabak et al, 2006; Jouany et al 2007; Bullerman et al, 2007)

Diagnostic Methods

- Thin Layer Chromatography (TLC)
- High Performance Liquid Chromatography (HPLC)
- Mass Spectrometry – GC/MS, HPLC/MS – more efficient
- Immunological methods, e.g. ELISA → shortcomings
  - Used only as semi-quantitative methods

(Mallmann and Dilkin, 2011)

Absorption Inhibition in GI Tract

- Mycotoxin binders (non-nutritional adsorbents) → highly preferable approach, applied worldwide
- Adsorbents available: hydrated sodium calcium aluminosilicate (HSCAS, most preferable), activated carbons, zeolites, bentonies and certain clays
- Adsorption efficiency:
  - Physico-chemical structure of agent
    - Total charge, charge distribution (polarity), surface area, dose and size of pores
  - Mycotoxin itself
- Alternative rout of inhibition – GI microbiota
  - Dairy strains of lactobacillus and bifidobacteria → efficient in mycotoxin binding → mechanism unclear (cell structure?)

(Kabak et al, 2006)

Mycotoxicosis in Poultry and Swine - Aflatoxin

- Poultry are highly susceptible to aflatoxin B1
  - Metabolism includes cytochrome P450 and glutathione S-transferase (GST)
  - glutathione conjugation can be the rate-limiting factor to aflatoxicosis
  - Poultry are highly deficient of GST (non-existent in some cases) → ↑susceptibility
- Clinical manifestation – acute and chronic
  - Acute illness – hours to a week after initial consumption (moderate-high doses)
  - Chronic illness – discrete CS, hard to diagnose (may resemble nutritional deficiency), can extend weeks to months after consumption (low-moderate doses)
  - Lack of uniformity in the same infected herd/flock

(Hossain et al, 2011)

Mycotoxicosis in Poultry and Swine - Aflatoxin (2)

Species	**DL_{50} (mg/kg BW)**
Swine	0.62
Chicken	6.5-16.5
Sheep	2.0
Duckling	0.34-0.56
Dog	1.0
Cat	0.55
Rabbit	0.3-0.5

(Patterson, 1973; Christensen et al, 1976)

Mycotoxicosis in Poultry and Swine - DON

- No detrimental effects on poultry performance
- DON < 15 mg/kg → no negative effects on weight gain, feed consumption and its efficiency in broilers
- DON consumption → correlated to decreased titre to NCD in hens and IB in broilers
- DON >2 mg/kg → not recommended in swine
- DON > 1 mg/kg → decrease in feed consumption → economicaly significant; Complete refusal when DON > 15 mg/kg
- DON > 20 mg/kg → vomition syndrome
- Organ weight in poultry does not alter significantly with DON intoxication (aside to liver, pancreas and heart in ducks) – initial swelling, and then reduction in size
- Liver size in swine increases

(Danicke et al, 2001; Awad et al, 2012; Girish et al, 2007)

Mycotoxicosis in Poultry and Swine - T-2 Toxin and Fumonisins

- **T-2 Toxin**
  - Young swine more resistant than mature swine
  - Safe concentration is considered < 0.5 mg/kg feed
  - T-2 toxin > 3 mg/kg feed → decrease in feed consumption
  - T-2 toxin > 16 mg/kg feed → complete refusal of feed
  - High levels of T-2 toxin cause diarrhea and lesions throughout the GI system, including perineal lesions → no need of metabolic activation
- **Fumonisins**
  - Toxemia in swine is aggravated by the synergistic effect with other mycotoxins, especially trichothecenes
  - No known treatment for this mycotoxin

(D'Mello et al, 1999)

Mycotoxicosis in Poultry and Swine - Zearalenone

- Poultry resistance > swine
- Levels of zearalenone for swine should not surpass 10ug/kg feed → basically feed free of zearalenone
- Feed refusal → often associated with zearalenone intoxication due to its bad taste
- Clinical symptoms are similar to estrus → vulvoganitis is the principal clinical symptom of zearalenone
- Clinical signs appear 1-4 weeks after ingestion of contaminated feed → continue long after the contaminated feed was replaced

(Mallmann and Dilkin, 2011)

Mycotoxicosis in Poultry and Swine - Ochratoxin

- Intoxications usually happen in spring and summer
- Polydipsia and polyuria are characteristics of ochratoxin A intoxication in swine, especially when ochratoxin > 1 mg/kg feed
- Dose of 200 ug/kg in swine → nephropathy and negative effects on feed consumption and its conversion
- Death rates can reach up to 90% in affected herds
- Promotes degenerative changes in liver, kidneys and brain of chicks → target organs for carcinogenic effects

Adenocarcinoma in chick’s liver

(Mallmann and Dilkin, 2011; Stoev, 2010)

Mycotoxicosis in Poultry and Swine - Ergot

- Ergotamine has considerable biological activity
- Swine metabolism → very efficient in bio-transforming ergot alkaloids → eliminated quickly through urine
- Polydipsia in swine due to unpleasant flavor of the alkaloid
- Effect on feed digestion → insignificant in swine
- Gangrene and lesions on extremities → characteristic of ergot intoxication

Ergot sclerotia and barley grain

(Mallmann and Dilkin, 2011)

Public Health

- In laying hens, aflatoxin B1 accumulates in high concentrations in the muscles.
- Ochratoxin and T-2 toxin also accumulate in poultry products → implications on public health
- "A food shall be deemed adulterated—(a)(1) If it bears or contains any poisonous of deleterious substance which may render it injurious to health; but in case the substance
- is not an added substance such food shall not be considered adulterated under this clause if the quantity of such substance does not ordinarily render it injurious to health."
- **Federal Food, Drug, and Cosmetic Act** §402 [21 U.S.C. 342]
- Only 5 mycotoxins are monitored under FDA surveillance programs: aflatoxins, DON, zearalenone, fumonisins, ochratoxin A

(Kahn et al , 2014; Rawal et al, 2010)

Public Health (2)

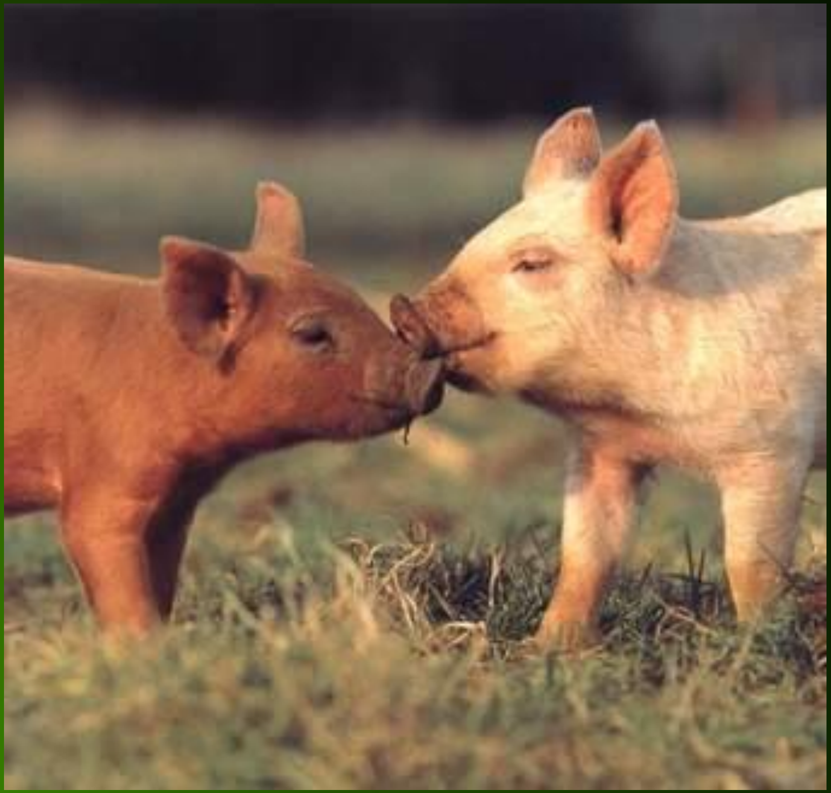
- The FDA has established
  - Action levels for aflatoxin
  - Advisory levels for DON
  - Guidance levels for fumonisins
- European Union established much stricter regulations, directives and commission’s recommendations:

https://ec.europa.eu/jrc/en/eurl/mycotoxins/legislation

- Climate and weather monitoring can be used worldwide as tools to predict mycotoxicosis risk, and perform appropriate testing

Thank You…

Questions?



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