

Other aspects of the use of humic substances in livestock

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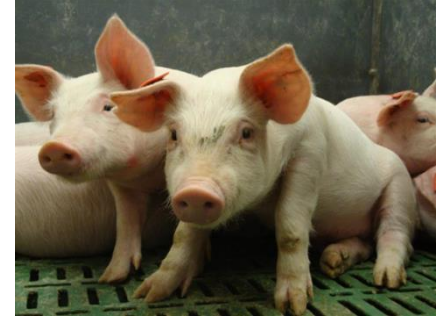


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The group of Animal Nutrition

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Expertise

- impact of **different types of feed additives** on animal health and performance
- prevention and treatment of **diarrhoea in weaned piglets** caused by enterotoxigenic *Escherichia coli*

Services and consulting services

- **serum biochemical profiles** (Mindray BS200 biochemical analyser)
- determination of essential **nutrients in feed** (AOAC method)
- **experiments using experimental animals** and collection of samples (blood, organs, tissue, feces)

Research project

The use of humic substances as a feed additive for the prevention of diarrhoeal diseases of piglets and improvement of pig performance 2012 – 2016

The aim of the project

- effects and safety of **humates with trace elements** in **prophylaxis of diarrhoea** and growth efficiency improvement in pigs
- **technological parameters** suitable for their production from natural raw materials



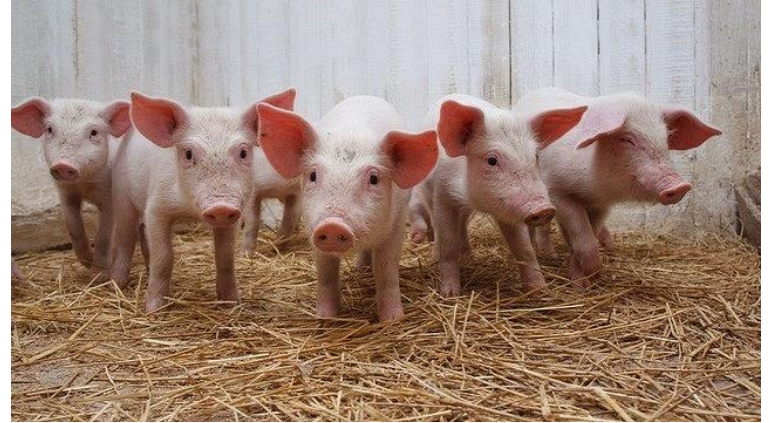
Effect of humic substances on lipid and fatty acid profile

HS in diet of animals:

- decrease serum **cholesterol** (Samudovska and Demeterova 2010; Mista et al. 2012; Ozturk et al. 2012)
- affect unsaturated (U), saturated (S) **FA** and **UFA:SFA ratio** in meat (Wang et al. 2008)
- redistribution of lipids:
reduce backfat thickness (Wang et al. 2008)
improve marbling of meat (Wang et al. 2008)



Effect of HS on lipid and FA profile in weaned piglets



C.....basal diet

ZnO2.5..... basal diet + 2.5 g ZnO/kg

ZnO1.7+HNa(f)...basal diet + 1.7 g ZnO + **20.0 g HNa/kg**

ZnO1.7+HNa(w)..basal diet + 1.7 g ZnO/kg and drinking
water with 0.2% HNa

Conclusions

- **Cholesterol, HDL and LDL were not affected** by HNa supplementation
- Partial replacement of ZnO by HNa in feed provided significantly **lower serum TGC, lower total amount of FA** in serum in comparison with ZnO2.5
- Partial replacements of ZnO by HNa - **positive effect on PUFA n6/n3** ratio in serum in comparison with ZnO2.5



Mycotoxins (MT)



structurally diverse secondary fungal metabolites

> **300: deoxynivalenol, zearalenon, fumonisins, T-2 toxin, ochratoxin A, aflatoxin (B1)**

2015: 84% from 8271 of agricultural commodities contaminated with MT

	Afla	ZEN	DON	T-2	FUM	OTA
EUROPE	11%	64%	77%	42%	54%	26%

corn, dried distillers grain



Mould and MT hazard in the feed chain

Aspergillus, Penicillium, Fusarium...

- **mould growth-alterations in the nutritional content**
- **production of MT**
 - ↓ feed intake, ↓ weight gain, carcinogenic, mutagenic, teratogenic, oestrogenic, immunotoxic, hepatotoxic, nephrotoxic, neurotoxic, reprotoxic...
 - presence in tissues - OTA (kidney, liver), milk-AFM1
 - synergistic effect, subclinical effect
 - **masked/bound mycotoxins!**



Legislative regulations

- DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on undesirable substances in animal feed (**aflatoxin B1**)

maximum content in mg/kg

- COMMISSION RECOMMENDATION on the presence of **deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins** in products intended for animal feeding (2006/576/EC)

guidance value in mg/kg



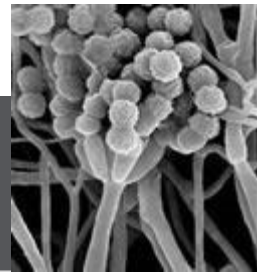
Strategies for detoxification/inactivation of MT

1. prevention of fungal infection
2. nutritional feed additives
3. physical, chemical, biological methods for **reduction of the contamination by MT**



➤ **BIOTRANSFORMATION, BIODEGRADATION** of MT
microorganisms and their enzymes (*Eubacterium* spp.,
Trichosporon spp., *Nocardia* spp., *Flavobacterium aurantiacum*)

➤ **ADSORPTION** - high affinity to MT, ↓ dissociation, high
binding capacity
multi-binding capacity, mix of adsorbents +++



NON-NUTRITIVE ADSORBITIVE MATERIALS

INORGANIC: bentonites, montmorillonites, zeolites, modified clays...

BUT

- *adsorption of trace elements*
- *aflatoxins +++, other MT – limited*
- *contaminants*

ORGANIC: activated charcoal, synthetic polymers, **humic substances**, esterified glucomannan (cell wall of yeast), yeast, lactic acid bacteria, dietary fibres

- *efficient against a larger range of MT*
- *biodegradable, (immunostimulants)*

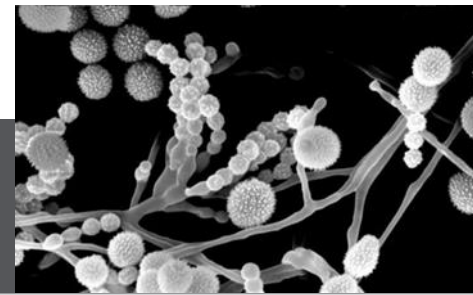
PROMISING – FURTHER RESEARCH IS NEEDED!

Regulation (EC) No 1831/2003 on additives for use in animal nutrition

Technological additives

Substances for the reduction of the contamination of feed by

- **deoxynivalenol** - strain DSM 11798 of the *Coriobacteriaceae* family (pigs)
- **fumonisin** - fumonisin esterase produced by *Komagataella pastoris* (pigs)
- **aflatoxin B1, fumonisin** - bentonite
(ruminants, poultry, pigs)



Humic substances-natural complexing compounds

- **reactive groups**-hydroxyl, phenol, carboxyl, methoxy...
- large specific **surface**, hydrophilic, flexible
- strong **adsorbtion** (microbial toxins, haevy metals, mutagens) ion exchange, chelation, **complexation** activity
- high **mycotoxin adsorbtion** capacity-reduction of its bioavailability (zearalenon, aflatoxins)



FULVIC ACID



HUMIC ACID

Humic substances in prevention of mycotoxicosis in animals

GOOD ADSORPTION

- **oxihumate, humate - AF B1 - broilers** (van Rensburg et al., 2006; Ghari et al., 2010)
- **sodium humate - AF B1 - *in vitro*** (Ye et al., 2009)
- **natural humic acid polymers - ZEN *in vitro*** (Sabater-Vilar et al., 2007; de Mil et al., 2015)
- **humic acid polymers - ZEN, OTA - adsorption at pH 3, desorption at pH 8.4** (Santos et al., 2011)

INEFFICIENT ADSORPTION

- **DON** (Sabater-Vilar et al., 2007; Dänicke et al., 2012)



Humic substances as MT binders in animal diet



- **↑ albumin and total protein levels**
- **↓ enzyme activities (AST, LDH, GGT)**
in serum
- reduction of the immunotoxic effect of MT
- protective effect on the **liver** and **bursa of Fabricius**
- **not adsorb other nutrients**
- **antioxidants, growth promoter, antimicrobial effect, improvement of gut health**

Study of the effects of MT binders

- *IN VITRO studies* - pre-screening

Adsorbent binding capacity - adsorption isotherms

Effect of

- ✓ pH (3-8), time
- ✓ amount of adsorbent, MT
- ✓ feed (adsorption of nutrient and MT)

toxic effect of binders

gastrointestinal models

Desorption at different pH

- *IN VIVO studies* - effectiveness in animals

NEW RESEARCH PROJECT COLLABORATION...

???

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