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Science & Solutions

Surveying the mycotoxin threat

The where and what of mycotoxin contamination

Inflammation

Improve pig performance by reducing subclinical inflammation

Phytogenic feed additives

Make a difference to the well-being of your sows

Editorial

The art and science of mycotoxin analysis

Mycotoxins continue to pose a challenge to animal health and performance. In the case of pigs, the importance of an effective mycotoxin risk management program cannot be underestimated.

The BIOMIN Mycotoxin Survey provides insights on the distribution as well as levels of occurrence of mycotoxins in selected feedstuffs and regions all over the world. Since the survey began in 2004, more than 25,000 samples have been analysed to date. Today, the BIOMIN Mycotoxin Survey is regarded by scientists and the industry as the standard for global mycotoxin analysis.

Choosing the most suitable method for analysing mycotoxins is essential. The right method of analysis depends also on the purpose of the final data evaluation and interpretations (e.g. HPLC is required to fulfill legal requirements). To quantify the presence of specific mycotoxins in given matrices, enzyme-linked immunosorbent assay (ELISA) is a fast, inexpensive and consistent method in validated raw materials only. For quantitative testing of single toxins at low detection limits, high-performance liquid chromatography (HPLC) provides reliable results but is also more time consuming.

However, due to the natural co-occurrence of mycotoxins, the harmfulness of contaminated feed cannot be determined by the concentrations of one or two toxins only. A novel, sensitive liquid chromatography–mass spectrometry (LC-MS)-based method was specifically developed for the accurate and simultaneous detection of multiple toxins in a wide variety of food and agricultural commodities, all within 45 minutes. Screening for all important mycotoxins provides a macro picture of the trends in the worldwide mycotoxins problem. In close collaboration with experts from the University Department for Agrobiotechnology at IFA Tulln, BIOMIN offers its customers from now on a routine basis for quantitative mycotoxin analysis. More than 380 mycotoxins, including several masked mycotoxins, can be quantified.

A summary of the latest data on the occurrences of the five well-studied, hazardous mycotoxins can be found on page 2.

We hope our readers will find the results informative and useful.

Karin NÄHRER
Product Manager, Mycotoxin Risk Management

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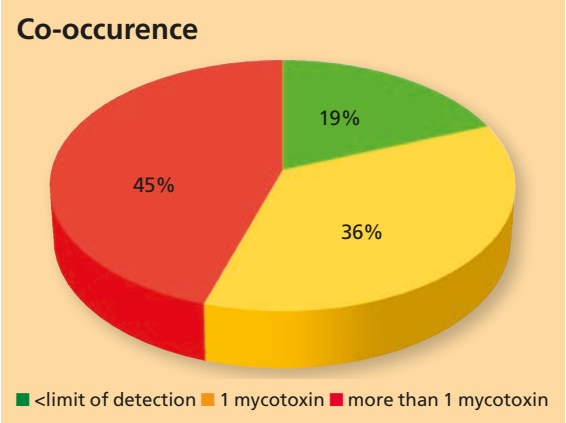
BIOMIN Mycotoxin Survey

A summary of

the major threats

Mycotoxins are a large and growing family produced by different fungi. Analytical tools have so far identified more than 1,000 different mycotoxins. Aflatoxins, zearalenone, deoxynivalenol, fumonisins and ochratoxin A are still among the most researched and frequently occurring mycotoxins worldwide. As part of its approach towards mycotoxin risk management, BIOMIN provides regional insights into the occurrence of the most important mycotoxins in primary feedstuffs.

Figure 1. Global co-occurrence of mycotoxins



From January to December 2013, a total of 4,218 samples were collected worldwide and analysed for the presence of mycotoxins. In total, more than 16,300 single analyses were carried out for the most important mycotoxins in terms of agriculture and animal production. These were aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA). Samples were analysed by high performance liquid chromatography (HPLC) and Enzyme-Linked Immunosorbent Assay (ELISA). Only single commodities were analysed by ELISA.

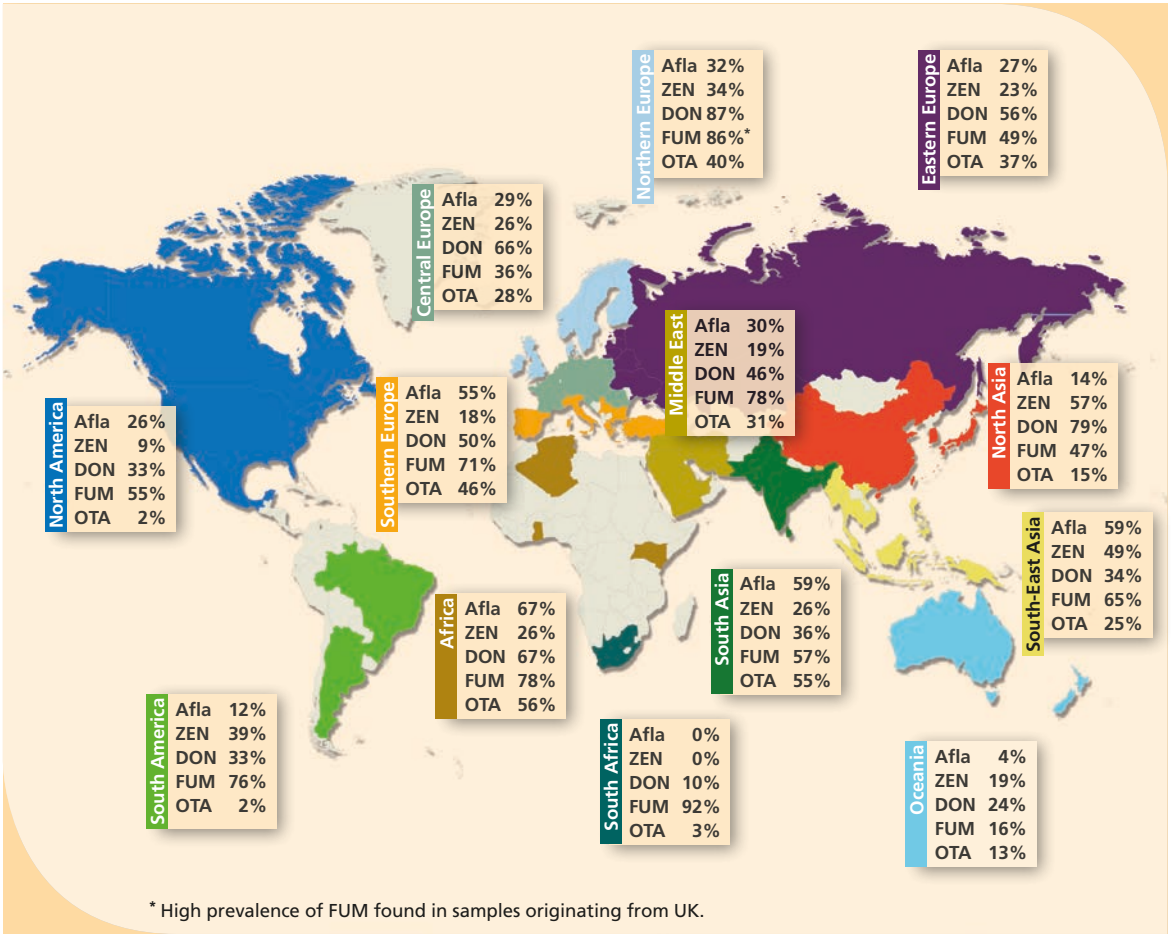
Overall results

In the more than 4,200 samples analysed worldwide, Afla were present in 30%, ZEN in 37%, DON in 59%, FUM in 55% and OTA in 23% of all samples (*Table 1*).

Table 1. Overview of worldwide survey results (2012 and 2013)

Global results	Afla 2012	Afla 2013	ZEN 2012	ZEN 2013	DON 2012	DON 2013	FUM 2012	FUM 2013	OTA 2012	OTA 2013
Number of tests	2,636	2,839	3,320	3,470	3,712	3,931	2,570	2,699	2,230	2,459
Percent positive (%)	25	30	46	37	64	59	56	55	31	23
Average of positives (µg/kg)	34	33	251	133	1,088	770	1,350	1,421	5	10
Maximum (µg/kg)	6,323	1,563	9,854	5,324	30,200	29,267	42,120	26,828	170	595
Commodity tested	Ground-nut cake	Maize	Corn Gluten Meal	Maize	Maize	Barley	Maize	Dried Distiller's Grains	Maize	Finished Feed
Source country	Myanmar	China	China	China	USA	China	Malaysia	US	India	Spain

Figure 2. Prevalence of mycotoxins in different geographic regions according to percentage of positive samples (>limits of quantification).



* High prevalence of FUM found in samples originating from UK.

Distribution of mycotoxin contamination by risk levels

Field mycotoxins such as DON, FUM and ZEN were the most frequently occurring ones. The risk levels of these mycotoxins were evaluated according to the percentage of samples in the different contamination ranges.

Important results were gained in this survey especially in the case of the type B-trichothecene DON. Of all the surveyed samples, 42.5% showed a DON contamination above 200 µg/kg which represents a medium risk level for pigs. Of all the feed samples, 12.5% were above the EU guidance values for DON (900 µg/kg) in complementary and complete feedstuffs for pigs (EC, 2006).

General findings

- More than half of all worldwide samples contain DON and FUM (*Table 1*).
- Corn was found to contain the highest concentrations of singly occurring Afla and ZEN in addition to FUM in corn DDGS.
- In half of all samples, more than one mycotoxin was detected. Multi-mycotoxin occurrence is a challenge due to its potential synergistic effects in animals (*Figure 1*).

A clear concern

From the mycotoxin survey results in which more than 4,200 samples worldwide were investigated, it is clear that mycotoxins are a topic of concern in animal feed and multi-mycotoxin occurrence continues to be a threat. Constant monitoring and continued research on the prevention and mitigation of mycotoxin contamination are therefore necessary.

A first step towards preventing the negative effects of these harmful substances is the application of good agricultural practices and storage conditions. An effective mycotoxin risk management program is also important in order to protect animals from the negative effects of mycotoxins on health and performance.

Christine Hunger
Product Manager, Phytogenics

INFLAMMATION

An underestimated cause of poor performance in pigs

The key to profitability in animal production lies in optimal animal performance. To attain the genetically achievable level of performance, inflammation and its impact on performance should be controlled.

*The aim is to **reduce** subclinical **inflammatory** processes in order to **increase** the availability of **energy** and nutrients for performance.*

Inflammation is an innate process that occurs in response to physical or chemical damage in the body or invasion by an infectious agent or feed toxin. A prolonged subclinical inflammatory response in the animal leads to continually reduced performance.

Inflammatory processes reduce feed intake (anorexia) and energy is directed towards cellular defense mechanisms instead of being used for production, e.g., the accretion of muscle and production of milk.

The effects of inflammation

An independent study was conducted to show the effect of a stimulated inflammation on piglet performance (*Table 1*). The piglets were challenged with LPS (Lipopolysaccharides) by intraperitoneal injection.

Table 1. The effect of induced inflammation on performance parameters and blood markers of post-weaning piglets

Concentrations in blood	No LPS	With LPS
IL-1ß (pg/mL)	32	114
PGE2 (pg/mL)	490	1285
Cortisol (ng/mL)	55	206
IGF-1 (ng/mL)	182	101
Performance (day 14 – 28)		
Average daily gain (g/d)	604	525
Average daily feed intake (g/d)	962	838
Feed conversion	1.59	1.59

Source: Adapted from Liu et al.*, 2003*

Blood parameters were analysed and it was found that cortisol levels were elevated when fighting the inflammatory stimulus of LPS administration. This signified that a catabolic metabolism was taking place in order to provide the body with energy to fight the inflammation. Levels of IGF-1 (insulin-like growth factor 1), a factor which enhances the hypertrophy of muscle cells, were reduced. Decreased levels mean a reduced potential for muscle growth.

The detrimental effect on performance was reflected in reduced feed intake as well as reduced weight gain of over 10%. The correlation between inflammatory processes and poor performance is frequently observed in practice. Particularly during times of weaning or feed change, a reduction in feed consumption is indicative of inflammatory reactions in the gut.

All in all, underlying subclinical inflammation prevents the animal from reaching its full growth potential, therefore leading to lower profitability.

The importance of paying attention to inflammatory reactions was confirmed by Niewold (2007), who stated that effective growth promoters should focus on inhibiting the intestinal inflammatory response.

Reducing inflammatory processes

One of the major mediators of inflammatory processes is the transcription factor NF-κB (nuclear factor κB), which is present in nearly all cells of the body. The activated form of NF-κB results in an increase in the pro-inflammatory gene-expression.

The Nrf2 (nuclear factor erythroid 2–related factor 2) is an antioxidant transcription factor involved in cell protection mechanisms in two different ways. First, the Nrf2 antioxidant pathway acts as a defence against reactive oxygen species, and secondly, it reduces the susceptibility of cells to the harmful properties of pro-inflammatory cytokines.

To increase the efficiency of animal production, these two systems should be kept in close balance. The aim is to reduce subclinical inflammatory processes in order to increase the availability of energy and nutrients for performance.

By measuring the target genes of the Nf-κB and the Nrf2 systems, the effect of a treatment on the health status of the animal can be measured at the cell level.

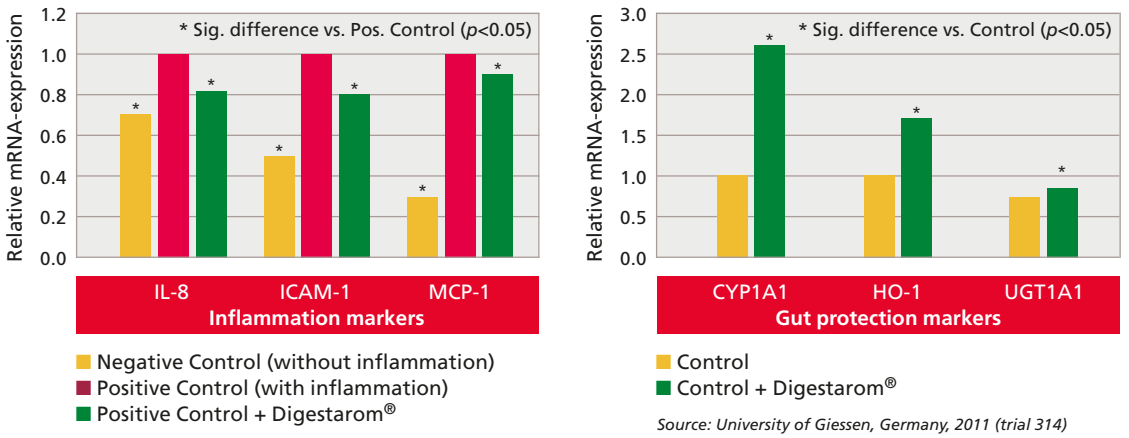
The phytogenics effect

In an *in vitro* cell test with Caco-2 intestinal epithelial cells, the effect of the phytogenic (plant-derived) feed

Why fight subclinical inflammation in the GIT?

Reducing inflammation and subclinical inflammatory processes improves the performance of pigs through higher feed intakes and feed efficiency. Furthermore, dietary energy and nutrients are used for growth rather than defense mechanisms.

Figure 1. *The effect of Digestarom® on inflammation markers and gut protection markers*



Source: University of Giessen, Germany, 2011 (trial 314)

additive Digestarom® on an inflammatory stimulus was measured (*Figure 1*). The NF-κB target genes (IL-8) (Interleukin 8), ICAM-1 (Intercellular Adhesion Molecule 1) and MCP-1 (Monocyte chemoattractant protein 1) were measured to assess the inflammatory status.

The phytogenic feed additive Digestarom® reduced the mRNA expression of the NF-κB target genes significantly compared to the positive control (1.0), and therefore indicated a significant reduction in the pro-inflammatory mediator NF-κB.

The cytoprotective effect of Digestarom® on intestinal epithelial cells was also assessed by measuring the Nrf2 target genes CYP1A1, HO-1 and UGT1A1. The mRNA-expression of the NRf2 marker genes showed a significant increase compared to the control (1.0).

The anti-inflammatory properties of Digestarom® were also shown in an *in vivo* study with piglets. The gene expression (mRNA) of NF-κB in the gastrointestinal tract (GIT) of piglets was measured, comparing a negative control to a positive control (Avilamycin) and a Digestarom® group (*Table 2*).

Table 2. Gene expression of NF-κB in the GIT of piglets[1]

Tissue	Phytogenic[2]	Avilamycin
Ileum	-1.12*	-1.53*
Colon	-0.59(*)	-0.53(*)
Mesenteric lymph nodes	-1.06*	-1.83*[ł]
Liver	-0.57*	-0.37

[1] Values are expressed as 2^x-folds compared to the negative control group
[2] Digestarom® P.E.P.
* linear contrast of means compared to the negative control group ($p<0.05$)
(*) linear contrast of means compared to the negative control group ($p<0.1$)
[ł] linear contrast of means between the phytogenic and avilamycin group ($p<0.05$)

Source: Kroismayr et al., *2008*

Compared to the negative control, the pro-inflammatory transcriptional factor NF-κB was down-regulated in the colonic tissue and significantly reduced by the phytogenic in the ileum, mesenteric lymph nodes and the liver. Altogether, the results of the Digestarom® application *in vitro* and *in vivo* indicate a down-regulation of pro-inflammatory cytokines and a stimulation of the anti-oxidative status and cytoprotective marker genes.

References are available on request.

Thomas Weiland
Phytogenics Technical Manager

Phytogenic feed additives

for sow well-being

Photo: temmuz can arsiray

After some initial confusion about the exact mode of action of phytogenic feed additives, on-going research has proven their positive effects beyond doubt. So which are the benefits for sows, both during gestation and lactation?

In practice, feed producers or farmers find themselves confronted with an unprecedented number of products of widely varying composition and efficacy claims. Randolf Nott, a German pioneer of complex phytogenic feed additives (PFAs) who launched the first generation of the Digestarom® product line way back in 1989, once said, "The fine art of formulating phytogenics lies in finding a suitable combination of the right plant materials.

"There is a very thin line between a successful formula and a mere mixture of different components. A combination of different plant materials, maximises the synergistic effects of the active constituents."

These principles provide the only basis for developing complex PFAs that are consistent in their effects under a wide range of production conditions.

Navigating PFAs

It is difficult to conclusively assess the effectiveness of phytogenic compounds on intestinal functions. Another problem is that most properties were studied *in vitro* because of the natural variability in the composition of plant secondary metabolites, depending on botanical origin, processing and composition of the plant.

Too many product claims lack proof in terms of scientific and field trials, and are imprecise in their statements. One example is that antimicrobial properties are stated for a wide range of PFAs. But no differentiation is made between the bactericidal (bacteria killing) and bacteriostatic (inhibition of growth and reproduction) modes of action. Bactericidal activity, which is generally recognised in synthetic antibiotics, bears the risk of developing resistance. Bacteriostatic effects, however, do not bear this risk.

A multitude of essential live-processes can be influenced by PFAs.

- Stimulating endogenous secretions, one of the most important effects of PFA, improves digestibility of nutrients, especially protein and most amino acids
- Modulating the gut microbiota which stabilises gut health and functionality, thus supporting the immune system
- Exerting positive effects on the liver and stomach function, gut motility and a multitude of other bodily functions
- Reducing stressor-effects by downgrading inflammatory protein release and/ or enhanced cyto-protective protein production, thus positively affecting metabolic processes and animal well-being
- Complex PFAs exert flavouring properties which are equivalent to conventional flavors.

PFAs – What for?

When PFAs were first used, there was some confusion on whether they could work as antibiotic replacements. Scientists were divided over the issue. The dispute was simply because the term 'antibiotics' was generally used instead of the correct term 'antibiotic growth promoters' (AGPs).

Today it is commonly accepted that PFAs can replace AGPs and have a much wider range of positive effects on the animal than AGPs ever had.

Complex PFAs can help the animal protect itself (prophylactic and overall stabilising properties) and better exploit available nutrients (feed intake and digestibility enhancement).The use of complex PFAs also improves animal performance (fertility, growth, health) and contributes to more economical, environmentally- and animal-friendly production.

Productivity and well-being

Several studies have shown the positive influence of PFAs on sow and litter performance when used in lactation feed. Sows had higher feed intake during lactation, produced more milk, converted the ingested feed more efficiently, minimised body weight loss, and had litters with higher growth rates.

A study was conducted to explore the influence of the continued use of a PFA during lactation and gestation on production parameters. The study was carried out in a large-scale German sow unit with two separate herds, each with an average inventory of more than 4,700 sows of Danish genetics.

Both herds were fed under the same feeding program with the same basal diet. A four-phase feeding concept was employed consisting of lactation-, insemination-, early gestation-, and gestation feeds. Early gestation feed was supplemented by stillage, and gestation feed by spent grains and stillage. Digestarom® Sow, a blend of herbs, spices and essential oils, was applied at a concentration of 150 g/t complete feed (88% DM-base) in all diets of the trial herd.

The study showed that the continuous supplementation of diets with the PFA improved overall sow fertility. Performance parameters such as farrowing rate, piglet index and number of piglets weaned at proper weight were enhanced over all parities.

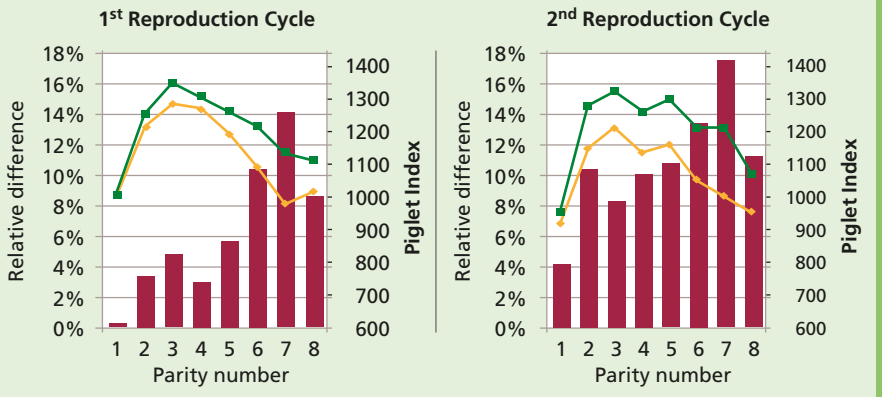
In the first production cycle where the PFA was applied, the trial herd showed higher and more stable piglet indices. These differences were most apparent in the second production cycle when the PFA was applied (*Figure 1*).

High culling rates reduce the average utilisation time of sows, thus affecting lifetime performance, replacement rate and the economics of the herd. However, the culling rate for fertility issues is, among others, an indication of sow stability and health.

More than 30% fewer sows were culled in the trial herd for fertility issues. In particular, sows of early parities were positively influenced by the applied PFA. A reduction in replacement rate of 51.5% in the trial herd was achieved, compared to 54.9% in the control herd.

The results of this study confirmed the positive impact of supplementing diets with the PFA Digestarom® Sow on the zootechnical performance of sows. The phytogenic feed additive also stabilised the health and productivity of higher parity sows, thus contributing to longer productive lifespans, one criterion for animal-friendly production.

Figure 1. Piglet indices of different parity numbers and their relative differences in the first and second reproduction cycle of PFA use (control vs. PFA herd)



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MYCOTOXIN RISK MANAGEMENT

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