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A magazine of Biomin

Science & Solutions

The winning formula

Mycotoxin deactivation that really works

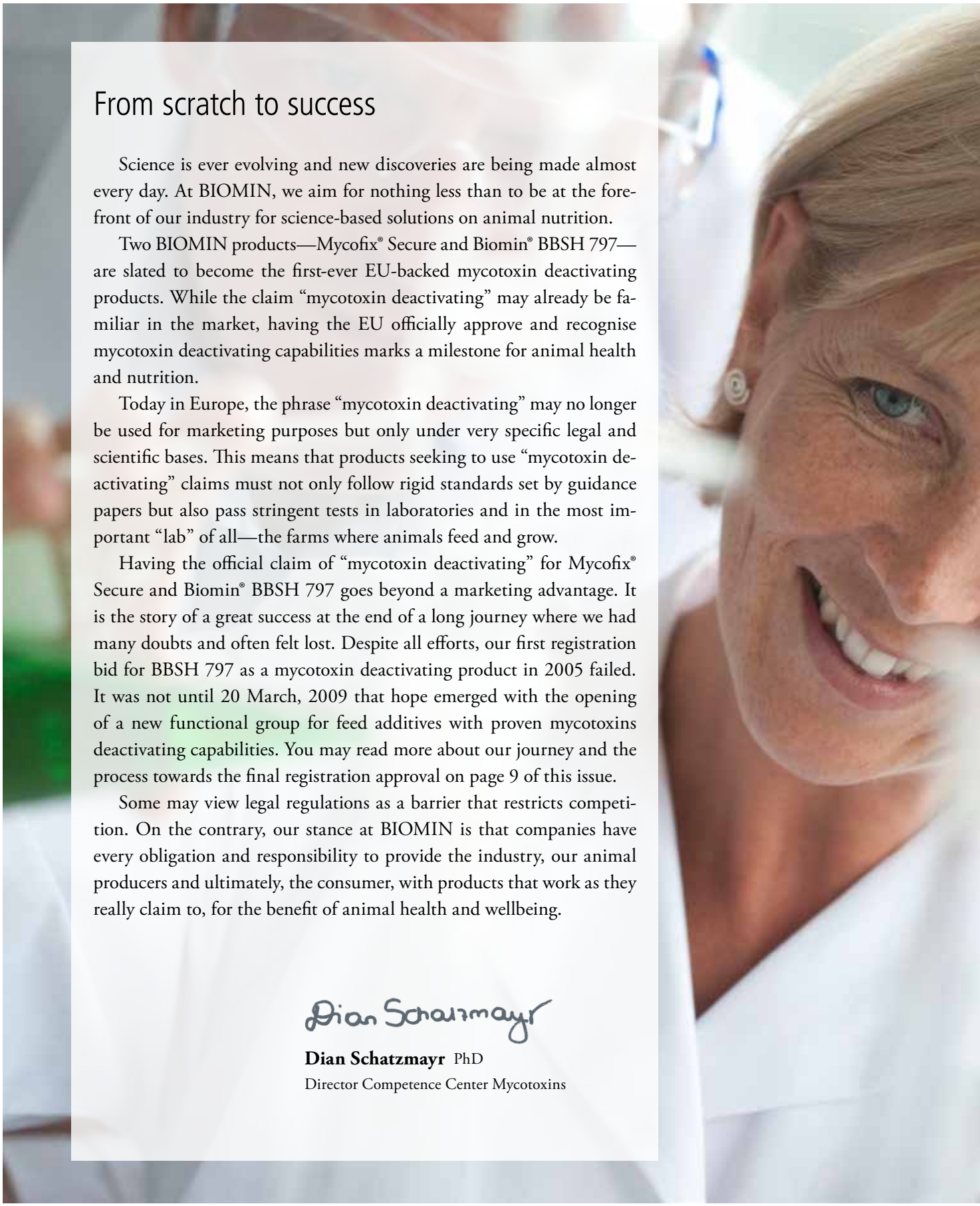
DON in the intestine

How does DON uptake affect chronically exposed pigs?

Weaning right

A successful piggery starts with proper weaning

Editorial



From scratch to success

Science is ever evolving and new discoveries are being made almost every day. At BIOMIN, we aim for nothing less than to be at the forefront of our industry for science-based solutions on animal nutrition.

Two BIOMIN products—Mycofix® Secure and Biomin® BBSH 797—are slated to become the first-ever EU-backed mycotoxin deactivating products. While the claim "mycotoxin deactivating" may already be familiar in the market, having the EU officially approve and recognise mycotoxin deactivating capabilities marks a milestone for animal health and nutrition.

Today in Europe, the phrase "mycotoxin deactivating" may no longer be used for marketing purposes but only under very specific legal and scientific bases. This means that products seeking to use "mycotoxin deactivating" claims must not only follow rigid standards set by guidance papers but also pass stringent tests in laboratories and in the most important "lab" of all—the farms where animals feed and grow.

Having the official claim of "mycotoxin deactivating" for Mycofix® Secure and Biomin® BBSH 797 goes beyond a marketing advantage. It is the story of a great success at the end of a long journey where we had many doubts and often felt lost. Despite all efforts, our first registration bid for BBSH 797 as a mycotoxin deactivating product in 2005 failed. It was not until 20 March, 2009 that hope emerged with the opening of a new functional group for feed additives with proven mycotoxins deactivating capabilities. You may read more about our journey and the process towards the final registration approval on page 9 of this issue.

Some may view legal regulations as a barrier that restricts competition. On the contrary, our stance at BIOMIN is that companies have every obligation and responsibility to provide the industry, our animal producers and ultimately, the consumer, with products that work as they really claim to, for the benefit of animal health and wellbeing.

Dian Schatzmayr

Dian Schatzmayr PhD
Director Competence Center Mycotoxins

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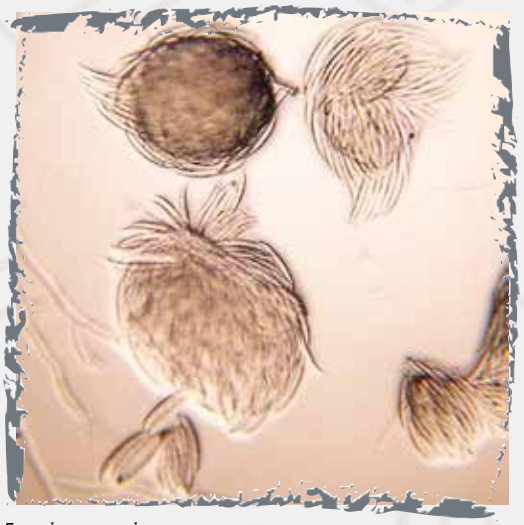

Fusarium graminearum

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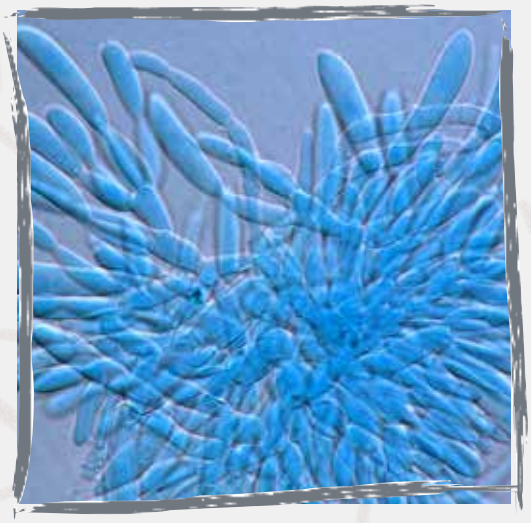
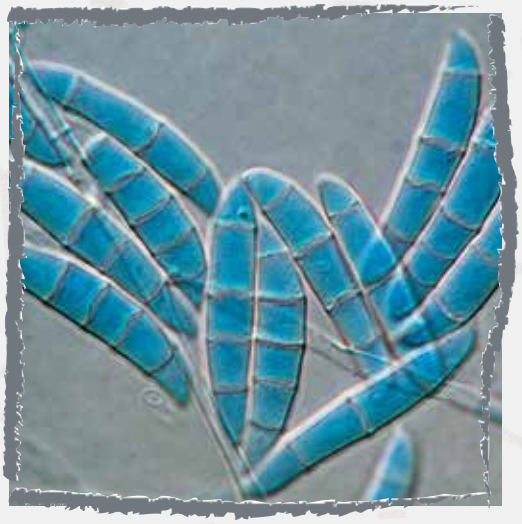


Fusarium culmorum



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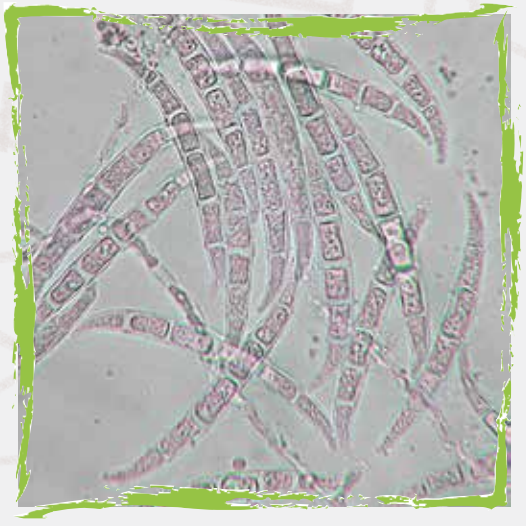


Fusarium graminearum

Christina Schwab PhD
Product Manager
Mycotoxin Risk Management

Deoxynivalenol in the intestine
What actually happens?

Out of 12,947 scientific publications on mycotoxins, more than 3,000 deal with deoxynivalenol. Scientists have only begun to investigate the effects of deoxynivalenol on the intestine of animals in the last decade, and data is still very much limited.

Several *Fusarium* strains are capable of producing deoxynivalenol (DON, vomitoxin) a type-B trichothecene. The common active group of all trichothecenes is the epoxide, which is responsible for the binding of DON to ribosomes inhibiting protein syntheses. The non-toxic de-epoxidated metabolite DOM-1 cannot bind to ribosomes due to the lacking epoxide group.

It is important to know that DON can be further modified into several different metabolites by fungi, plants, animals, and bacteria (see *Table 1*). These DON derivatives are also called masked mycotoxins.

Studies have shown that the amount of DON derivatives, mainly 3/15AcDON and D3G, can account for an additional up to 75% of DON contamination in feed. Newly released wheat cultivars, which are able to more efficiently convert DON to D3G, are more resistant towards the DON producing fungus *Fusarium graminearum*, but can contain up to 10-times more D3G than DON.

What happens to DON and its derivatives when they enter the gastrointestinal tract (GIT) of an animal?

DON after ingestion

The intestinal absorption of DON and its metabolites differ between animals. The localisation of the gut microbiota before the small intestine has a major effect on bioavailability, as DON is mainly absorbed in the small intestine.

In swine, one of the animals most sensitive to DON, the microbial biomass in the stomach—which is located before the small intestine—is only in the range of 10^2-10^3 per mL intestinal fluid (*Figure 1*). About 54–89% of DON can cross the intestinal epithelium and are detected in the blood.

Intestinal bacteria can transform the DON derivatives D3G, 3/15AcDON into DON. The transformation of DON into non-toxic DOM-1 by bacteria such as the active strain in Biomin® BBSH 797 prevents the absorption of DON.

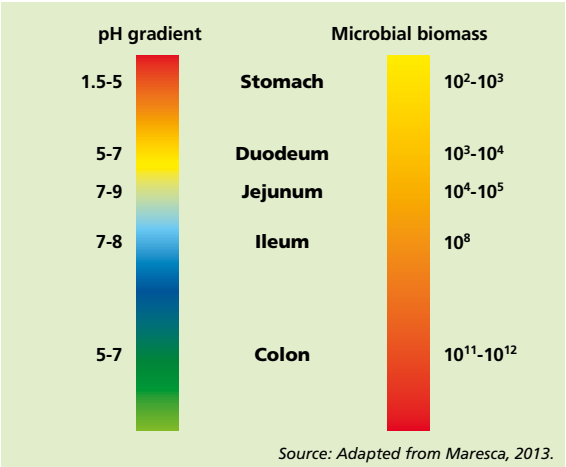
In an experiment with 24 piglets, the concentration of DON in the blood serum was significantly reduced (P<0.05) when Biomin® BBSH 797 was added to DON-contaminated feed (*Figure 2*). DON is then eliminated as glucuronidated DON (D3GA, D15GA) *via* urine.

Table 1. DON can be modified into several different metabolites by fungi, plant, animals and bacteria, influencing its toxic effect.

Metabolised by	DON-metabolites	Abbreviations
Fungi	3-acetyl-DON 15-acetyl-DON	3AcDON 15AcDON
Plants	3-O-glucoside DON	D3G
Animals	DON-3-glucuronide DON-15-glucuronide	D3GA D15GA
Bacteria	De-epoxy-deoxynivalenol	DOM-1

After ingestion of DON-contaminated feed, the intestinal epithelial cells are the first target of DON. Regardless of the amount of DON being absorbed, the intestinal epithelium is exposed to the entire contamination of the feed and therefore non-absorbed toxins can also compromise the entire intestine. Absorbed

Figure 1. Comparison of pH value and microbial density per mL intestinal fluid in swine.



Source: Adapted from Maresca, 2013.

Christina Schwab PhD
Product Manager
Mycotoxin Risk Management

Regardless of the amount of DON being absorbed, the intestinal epithelium is exposed to the entire contamination of the feed and therefore, non-absorbed toxins can also compromise the entire intestine.

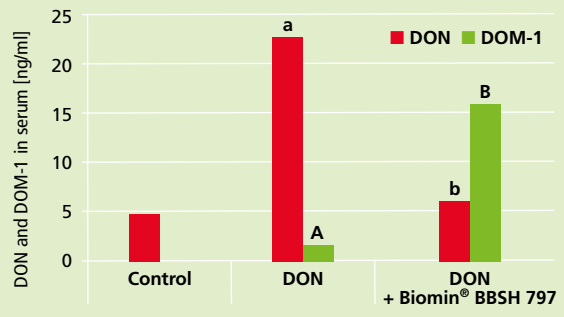


Figure 2. Serum DON and DOM-1 concentrations in piglets fed a diet contaminated with DON (1.8 mg/kg) or the same contaminated diet supplemented with the additive Biomin® BBSH 797 at a dose of 1.7 × 10^8 CFU/kg feed.

Blood was collected before feeding the contaminated diet (control) and 48h after the addition of DON +/- Biomin® BBSH 797.

Different letters (a,b for DON, A,B for DOM-1) above the columns within a given blood collection mean significant differences (P<0.05). *Source: BIOMIN*

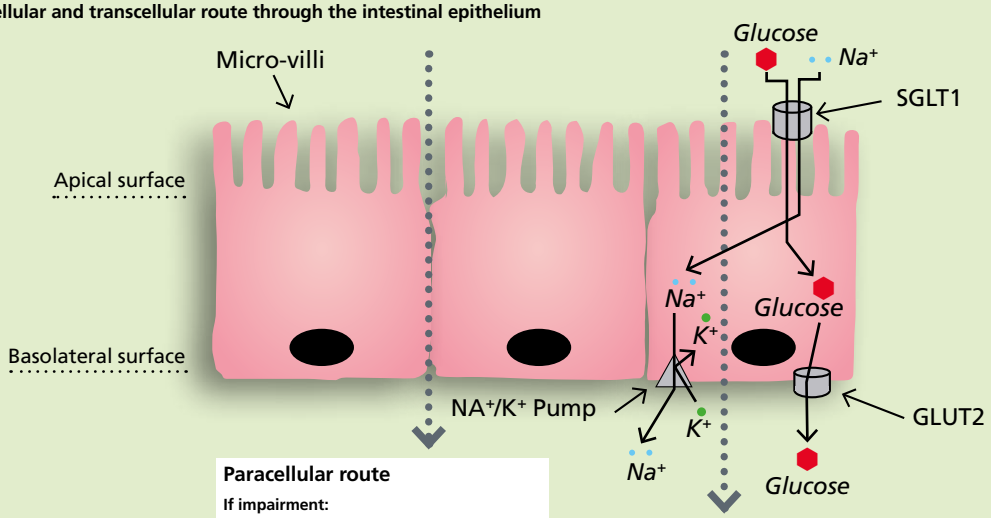
Reduced intestinal barrier function

The intestinal tract represents an important barrier to ingested chemicals, feed contaminants and the first line of defense against intestinal infection. The gut barrier is formed to a large extent by tight junctions that seal the luminal end of the intercellular space.

DON crosses the intestinal mucosa paracellular through the tight junctions. At the same time DON increases the paracellular permeability of the intestine through the opening of the tight junctions. Therefore, chronically exposed animals have a higher DON uptake.

More bacteria can also be translocated across the intestinal epithelium, increasing the risk of intestinal bacterial infections. **Other mycotoxins,** pharmaceuticals, pesticides, allergens, fungi and viruses are also granted an easier passage across the intestinal epithelium.

Paracellular and transcellular route through the intestinal epithelium



Paracellular route

If impairment:

Higher translocation of luminal antigens

- **Commensal flora**
- **Pathogens**
- **Food antigens**
- **Toxins and mycotoxins**

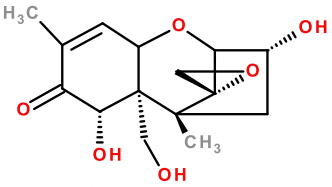
Transcellular route

If impairment:

- **Lower uptake of nutrients such as glucose**
- **Malabsorpbtion of water**

Source: Adapted from Grenier, 2013

Facts about DON



Just small amounts of DON can have a negative effect on intestinal epithelial cells

DON affects the intestinal immune system and harms intestinal villi

DON reduces nutrient uptake and can cause anorexia and feed refusal by directly targeting the brain

mycotoxins can re-enter the intestine through the intestinal epithelium or through enterohepatic circulation (excretion via bile and re-absorption), thereby increasing the exposure time along the GIT.

Poor intestinal function and nutrient uptake

Seventy percent of the immune system is located in the GIT. DON harms the innate immunity by:

- direct activation of signal pathways
- opening of the tight junctions allowing luminal bacterial antigens to trespass
- reduced mucus production

High doses of DON repress the immune response whereas low concentrations promote a rapid mucosal inflammatory response, posing a risk of induced chronic intestinal inflammation, such as inflammatory bowel disease.

DON interferes with the intestinal absorption of nutrients, like glucose and amino acids. The sodium-glucose dependent transporter (SGLT-1) is responsible for glucose uptake. Low concentrations of DON are enough to inhibit SGLT-1 and therefore reduce glucose uptake. SGLT-1 is the most DON-sensitive transporter, followed by GLUT-5, the passive fructose transporter.

SGLT-1 is also responsible for water reabsorption and the blocking of SGLT-1 by DON could be the mechanism behind the oft-occurring DON-induced diarrhoea.

Low doses of DON reduce the height of the intestinal villi, causing villus fusion and atrophy in the duodenum and jejunum of pigs. Villi increase the internal surface area of the intestinal wall and are therefore necessary for effective nutrient absorption.

Feed refusal and anorexia

Two well-known effects of DON are anorexia and feed refusal. The mechanisms behind these effects are complex and scientific evidence shows that within the gut-brain axis, neuroendocrine factors, pro-inflammatory cytokines and bitter taste receptors found throughout the GIT are involved in DON-induced feed refusal. The brain, particularly the hindbrain including the area postrema and hypothalamus, can signal immediate changes in food intake.

As DON is able to cross the blood-brain barrier, about 25–30% of the plasmatic DON can be found in the cerebro-spinal fluid of pigs after 2–60 min. On the other hand DOM-1 cannot cross the blood-brain barrier. A recent study also revealed that DON can alter brain functions and directly target the brain, causing vomiting, anorexia, fever, decreased locomotor activity and social withdrawal.

References are available upon request.

DON is dangerous

Pigs are highly sensitive to DON. About 54–89% of DON can cross the intestinal epithelium in pigs and are detected in the blood.

As intestinal cells are the first cells to be exposed to DON and at much higher concentrations than other tissue, it is of significant interest to investigate how the GIT is being compromised by feed contaminated with DON. A healthy GIT is crucial for an efficient uptake of nutrients, the function of the immune system, and the indigenous microflora.

It should be kept in mind that the effect on the intestine is only one part of the consequences in animals fed DON-contaminated feed and also highlights the importance of implementing a sensitive and effective mycotoxin risk management.

The patented active bacterium in Biomin® BBSH 797 modifies the structure of trichothecenes, a biotransformation process that renders trichothecenes such as DON harmless. This makes Biomin® BBSH 797 a valuable feed additive for pigs, considered the species most susceptible to in-feed DON contamination. Biomin® BBSH 797 is part of the Mycofix® product line.

Diego Padoan DVM
André Van Lankveld Ing. (BSc)
Swine Technical Managers

Winning by weaning right

Successful weaning management requires a holistic approach that considers piglet behaviour, the importance of quality feed nutrition for both post-parturition sows and post-weaning piglets, and the use of feed additives that promote gut health and control pathogenic challenges.

Photo: David Evison/Fotolia

When to wean

A reasonable weaning age runs between day 21 and 28. This would relieve the mother sow from milk production load as well as early weaning stress on the piglet.

Legislation in the EU currently stipulates a mandatory 28 days of lactation. It should also be noted that sow milk production falls dramatically after five weeks.

Farm management, epidemiological evaluations, and the fact that a piglet's immunity is lowest from 14 to 28 days of age are other factors to consider when deciding on the weaning age.

Feeding of sows during lactation is a complex matter. Modern sows give birth to a large number of piglets and therefore, adequate preparation is needed during gestation. Post parturition, sows enter a catabolic state. Quality feed nutrition is important for high milk production.

With the help of the right feed additives, sows can obtain the necessary feed requirements even during summer. In addition to a well-planned feeding schedule, biosecurity in the farrowing room helps minimise the presence of pathogens in the sow gut.

Farrowing

Feeding piglets in farrowing crates from day 5 after birth reduces the energy required from the sow and triggers the excretion of enzymes in the newly born. At 21 days post lactation, a piglet suckles a litre of milk on average, which places a high demand on sow milk production.

It is therefore important to carefully evaluate the weaning age of piglets. Early weaning saves the sow energy but poses a big challenge for the piglet. A weaning weight of 6kg is a tremendous threshold for the piglet and anything below this weight requires a high degree of competence from farm attendants, especially when there are a large number of piglets.

Nursery management

Weaning facilities and methods such as separated early weaning (SEW) or medicated early weaning (MEW) have greatly helped in early weaning and the improvement of the herds' health status. All-in-all-out is recognised to be a milestone concept. Pre-weaning size was seen to have a strong positive correlation in gene expression among all genes known to stimulate the appetite.

Once a date is fixed for the end of lactation, the next step would be to prepare the piglet for this. A feeding schedule

and formula must be planned according to the digestive competence of the piglet, with special attention given to palatability, buffering capacity and protein digestibility.

During the first 24-36 hours post weaning, the piglet begins eating to first nourish its gut mucosa. In the initial switch to a solid diet, the piglet's mind has yet to register the concept of eating to fill its belly. At this time, the piglet misses the warmth and comfort of its mother. Regrouping in a new pen, the piglet also faces unknown pen-mates and fights to create a hierarchy.

Lactation

During lactating days, piglets suckle every 1½ hours. It will take some time before piglets begin to feed twice a day. Therefore, leaving feeders empty even for a short period will lead to a mass of semi-digested feed, partly disrupting the stomach pH barrier and function while favouring the growth of pathogens in the gut.

Weaning litters should not exceed 30 to 33 piglets. In disease challenged farms, siblings should be kept together in the same litter. Sorting and regrouping according to weight and sex is also not recommended in such cases. If the temperature is below ideal, laying the barn with some straw and rubber would provide warmth to the belly. Avoid draughts like the devil. *Figure 1* shows the optimal indoor temperatures that would keep piglets in their comfort zone.

Nursery feeding

Several basic concepts would help piglets cope with the sudden change in diet and subsequent metabolic and endocrine changes. After ensuring that all piglets are eating properly, diet choices should be made first according to feed intake, followed by herd consistency, season, breed, feed availability and costs.

Quality raw materials with high biological value such as plasma, potato protein, soy protein concentrates and good quality fishmeal should be included in diets. The FCR in weaning piglets can be as low as 1.3 (for a piglet weighing 6-12 kg). At this stage of growth, the cost of raw materials should be secondary to quality.

Some farmers, to enhance feed intake, adopt long photo periods in the initial days post-weaning, such as leaving the light on for the first 48 hours. A bright barn promotes better toilet behaviour as piglets tend to sleep huddled. If the barn is dark, piglets will use the nearest corners as toilets, which might encourage poor toilet habits.

Nursery treatments

A well-scheduled vaccination program is a great help especially against specific diseases which cannot be easily eradicated from one farm or area. Farmers are used to the immediate effectiveness of antibiotics whereas prevention measures are much more complex. It is thus easy to fall

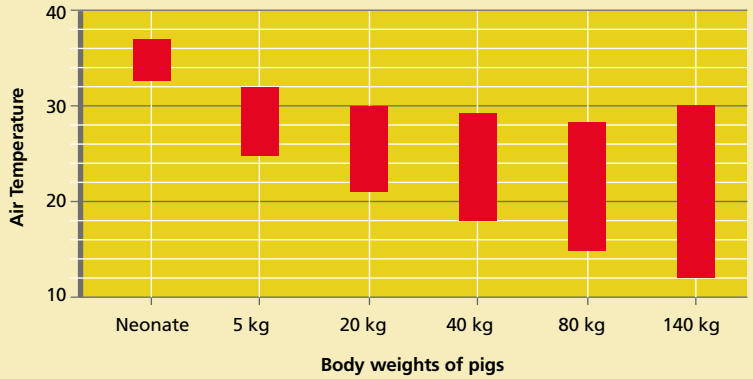
The time of day matters

Weaning in the afternoon has been shown to reduce dominant hierarchical behaviour. The establishment of the hierarchy occurs within 24 hours of mixing but the level of aggression drops dramatically after about one hour.

It is really important not to complicate this already nasty situation so some very basic rules are necessary.

These are: ensure the right barn temperature, provide clean water, fresh feed and a feeding space of at least 5 cm per piglet as they like to eat together.

Figure 1. Recommended temperate ranges within pig buildings, according to body weight.



Source: Adapted from Wathes, Jones & Webster, 1983.

Figure 2. Hierarchy model of oxidative stress effects in animals.

At low intensity oxidative stress

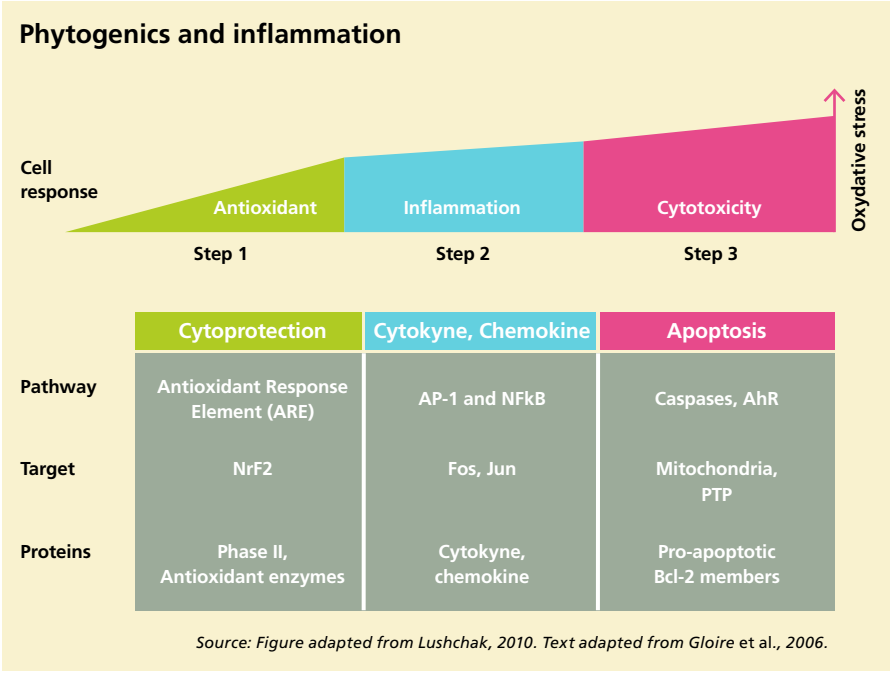
The Keap1/Nrf2 system up-regulates genes encoding antioxidant enzymes to restore redox homeostasis.

At intermediate stress

Antioxidant enzymes are up-regulated inducing inflammation via NF-kB and AP1 pathways.

At higher oxidative stress

Perturbations of mitochondrial pores and destruction of electron transporters occur, culminating in apoptosis and/or necrosis depending on the varying pro/ antioxidant balance in living beings.



Source: Figure adapted from Lushchak, 2010. Text adapted from Gloire et al., *2006.*

back into the simplistic use of antibiotics, which whitewashes what are widely known as PMDSs, or pure management disease syndromes.

Chemotherapy has proven to be limited and many bacterial strains have developed resistances. Without a shrewd and careful use of antibiotics, its effectiveness will be short-lived.

Benefits of acidifiers during weaning

In weaned piglets, citric and/or fumaric acids are recommended for lowering the stomach pH. An even more effective solution is the synergetic association with phytogenics, which permeabilizes the bacterial cell wall to improve acid penetration. Prebiosis and probiosis are relatively new concepts similar to that of vaccination but which are still not very well understood and adopted.

Pre- and probiosis present holistic solutions that focus on accelerating the maturity of the digestive system. Phytogenics can enhance palatability and digestibility challenges faced by food producing animals, as well as promote a positive gut microflora in a way that complements the use of acidifiers. Recent findings on some phytogenics have shown powerful effects that favour cell responses against stress stimuli such as viruses, bacteria and heat stress. Phytogenics also promote an antioxidant reaction via the NrF2 pathway instead of the NFkB inflammation pathway, as shown in *Figure 2*.

Zinc oxide is used to prevent scouring but this gives rise to the same problem as antibiotics. Worldwide, legislation and environmental concerns are clamping down on the indiscriminate use of this compound; hence, there is increased attention on the quality of the weaning diet today, with a focus on feed additives to prevent scours.

Weaning it steadily

The weaning phase is a challenging one but correct management sets the stage for producing winning pigs. It is of utmost importance to protect all animals from mycotoxins. Winning the war against mycotoxins requires first a keen focus on proper weaning. One should also bear in mind the most important factor in successful animal production, namely homogeneity in all stages of production from the farrowing crate, through to weaning, fattening and finally, slaughter.

Did you know...

...that poor management can rapidly deteriorate even the best quality silage?

Learn how to assess and maintain silage quality on the field with this BIOMIN video!

Find out more about silage quality evaluation in the next issue of **Science & Solutions**.

Not just a claim . . .

The process

On 20 Sep 2013, Mycofix® Secure and Biomin® BBSH 797 received positive votes in the EU for proven mycotoxin deactivating capabilities. This landmark decision has put the spotlight on a new class of technological feed additives with proven mycotoxin counteracting properties, demonstrating that mycotoxin deactivation is not just about words but actions.
Putting the action back into words, **Science & Solutions** traces the decades-long challenge, from the initial registration right to the eventual vote confirming the official backing of scientific claims.

1999
BIOMIN files the first dossier for Biomin® BBSH 797 as a mycotoxin deactivator with a no-vote result in 2005.
The product group "mycoxotin deactivator" does not exist. FAILED

2005
FEFANA, on the initiative of BIOMIN, establishes the Task Force "Mycotoxins".
The aim of the Task Force is to lobby for the creation of a new functional group that would create a legal basis for the registration of products as "mycotoxin deactivators".

2009
20 Mar: SCFCAH votes for a new functional group "substances for reduction of the contamination of feed by mycotoxins" under Reg (EC) No. 1831/2003.
➜ Companies may now submit registration dossiers on mycotoxin deactivating products.

2010
EFSA publishes the first document stipulating the rules which companies must follow in filing applications for mycotoxin deactivators. An extended guidance is published in 2012.
➜ BIOMIN submits a dossier on Mycofix® Secure for "aflatoxin binding".

2012
➜ BIOMIN submits a dossier on Biomin® BBSH 797 for "trichothecene biodegradation".

2013
➜ BIOMIN submits a dossier on FUMzyme® for "fumonisin biotransformation".
September: SCFCAH passes positive votes on Mycofix® Secure and Biomin® BBSH 797.
October: Final EU authorisation obtained. EU regulation published.

2014
EFSA opinion expected on FUM*zyme*®.

The players

BIOMIN
Instrumental in working with FEFANA on pushing for a new functional group in order to establish a legal basis for the authorisation of mycotoxin deactivating products.
Developed the currently adopted analytical method for characterising the AfB_1-binding capacity of bentonites that has made possible the authorisation of "aflatoxin binding" as an official claim.

EFSA - *European Food Safety Authority*
Assesses all dossiers submitted on behalf of the European Commission.
Draws up rigid parameters for species specificity, safety and efficacy by which all dossiers filed for mycotoxin deactivating products are scrutinised.

FEFANA - *EU Association of Specialty Feed Ingredients and their Mixtures*
Group that lobbies for the interests of specialty ingredients producers in the feed industry in the EU.

SCFCAH - *EU Standing Committee on the Food Chain and Animal Health*
Regulatory committee of the European Commission on matters pertaining to farm-to-fork food safety.

The promise

What does "mycotoxin deactivation" really mean?

Under the EFSA guidance, authorisations for mycotoxin deactivating feed additives must fulfil the following requirements:

Specificity
The target mycotoxin(s) and animal species must be specified. The additive must also not interfere with the analytical determination of mycotoxin levels in feed.

Safety
Additives with binding capabilities should not interact with other feed ingredients.
For additives that modify the chemical structure of mycotoxins, it must be proven that the resulting degraded metabolite is safe for the target animal species and consumers.

Efficacy
Only *in vivo* studies are accepted as the effects of mycotoxins occur only after ingestion. At least three *in vivo* studies in two locations with a minimum trial size are required for adequate evaluation.

Mycofix®
More protective.

MYCOTOXIN RISK MANAGEMENT

Mycotoxins decrease performance and interfere with the health status of your animals.

Mycofix® is **the** solution for mycotoxin risk management.

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Naturally ahead

Biomin®