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Science & Solution

Biomarkers and mycotoxins

The pros and cons

Phytogenics for growth

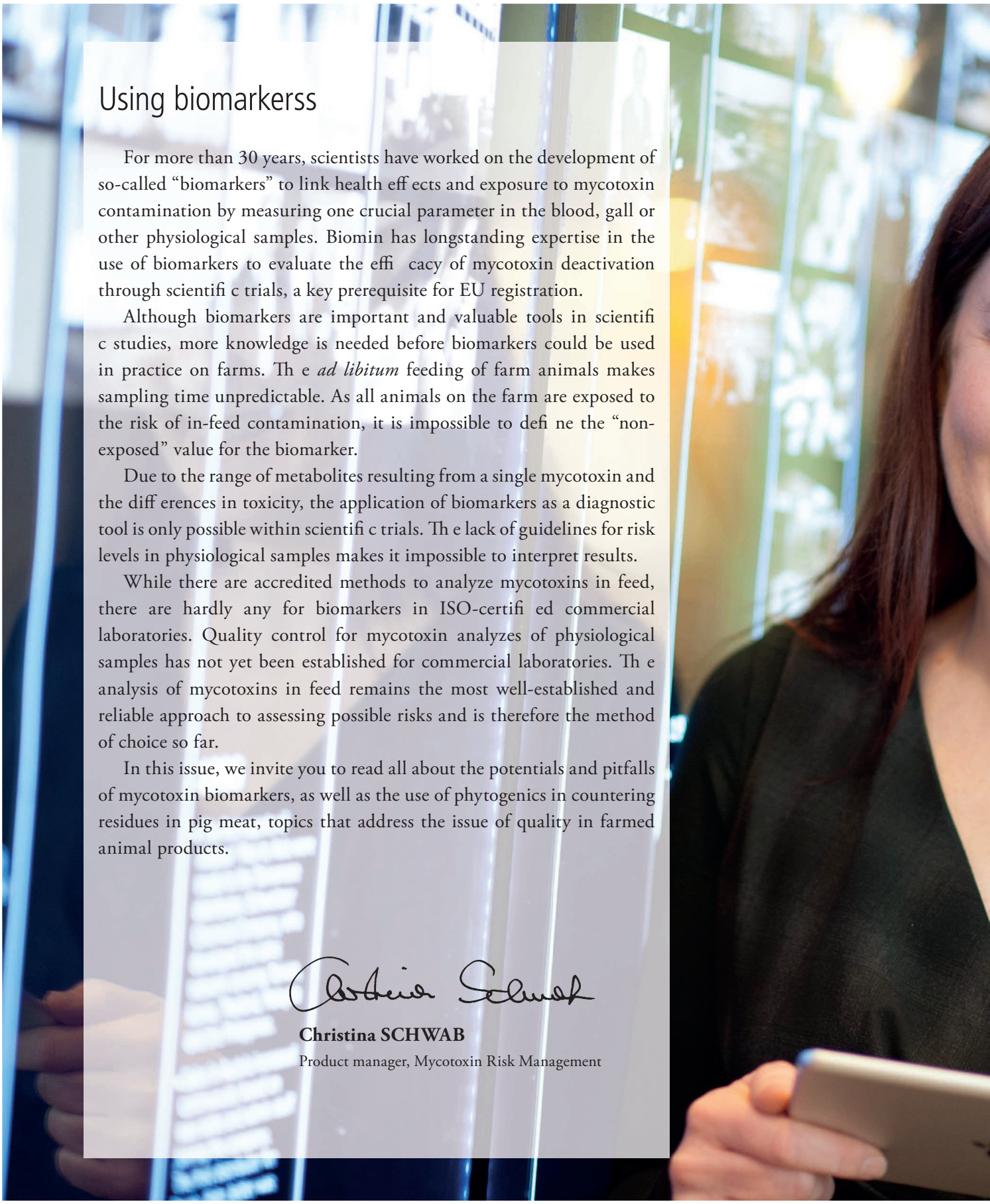
Besides adding fl avor, phytogenics promote pig growth and superior carcass characteristics

WORLD NUTRITION FORUM
2014
SUSTAIN: ABILITY

Swine in focus

Gut health and growth, and market trends in key regions at the World Nutrition Forum

Editorial



Using biomarkerss

For more than 30 years, scientists have worked on the development of so-called “biomarkers” to link health eff ects and exposure to mycotoxin contamination by measuring one crucial parameter in the blood, gall or other physiological samples. Biomin has longstanding expertise in the use of biomarkers to evaluate the effi cacy of mycotoxin deactivation through scientifi c trials, a key prerequisite for EU registration.

Although biomarkers are important and valuable tools in scientifi c studies, more knowledge is needed before biomarkers could be used in practice on farms. Th e *ad libitum* feeding of farm animals makes sampling time unpredictable. As all animals on the farm are exposed to the risk of in-feed contamination, it is impossible to defi ne the “non-exposed” value for the biomarker.

Due to the range of metabolites resulting from a single mycotoxin and the diff erences in toxicity, the application of biomarkers as a diagnostic tool is only possible within scientifi c trials. Th e lack of guidelines for risk levels in physiological samples makes it impossible to interpret results.

While there are accredited methods to analyze mycotoxins in feed, there are hardly any for biomarkers in ISO-certifi ed commercial laboratories. Quality control for mycotoxin analyzes of physiological samples has not yet been established for commercial laboratories. Th e analysis of mycotoxins in feed remains the most well-established and reliable approach to assessing possible risks and is therefore the method of choice so far.

In this issue, we invite you to read all about the potentials and pitfalls of mycotoxin biomarkers, as well as the use of phytogenics in countering residues in pig meat, topics that address the issue of quality in farmed animal products.

Christina SCHWAB
Product manager, Mycotoxin Risk Management

Contents

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Biomarkers for mycotoxins

Potentials and pitfalls

An important part of effective mycotoxin risk management is the regular analysis of feed material for mycotoxins. Contaminated feed ingested by farm animals leads to mycotoxin exposure and further to impaired health effects. For more than 30 years scientists have been working on the development of so called "biomarkers" to link health effects and exposure to contamination by measuring one crucial parameter in blood or other physiological samples. What are the potentials and pitfalls of mycotoxin biomarkers?

Mycotoxins are toxic metabolites produced by filamentous fungi and can be found in almost all types of grains. About 95% of all mycotoxin contamination occur before harvest. Despite the widespread use of preventive measures in good agricultural practice, 81% of more than 4,200 feed samples tested positive for mycotoxins in 2013 (Biomin Mycotoxin Survey, 2013). As the consequences and health effects of mycotoxins differ greatly between single animals, scientists, veterinarians and farmers have been on a persistent search for diagnostically conclusive biomarkers.

What are mycotoxin biomarkers?

Biomarker of exposure

It is important to differentiate between biomarkers of exposure and effect. A good example of a biomarker of exposure is aflatoxin M_1 (AfM_1) in the milk of cows (*see table 1*). Biomarkers of exposure measure the mycotoxin or its metabolites in the blood, milk, urine, feces or other physiological samples. To some extent, the mycotoxins can be detected unchanged in physiological samples while the rest are metabolized.

Table 1. Potential biomarkers of exposure and effect for the main mycotoxins used in scientific studies.

Mycotoxin	Biomarker of exposure	Biomarker of effect
Aflatoxin B_1 (AfB_1)	AfM_1 in milk	• AfB_1-albumin adducts in blood • AfB_1-DNA adducts in urine, tissue
Fumonisin B_1 (FB_1)	FB_1 in blood, urine, feces	Sa/So ratio in blood, tissue
Deoxynivalenol (DON)	DON, deepoxy-DON and other metabolites in urine, tissue, feces	Pro-inflammatory cytokines in blood, tissue
Zearalenone (ZEN)	ZEN, zearalenol, zearalanol and other metabolites in blood, urine, feces	• Glucuronic acid-conjugates in urine, feces • Endocrine disruption in tissue
Ochratoxin A (OTA)	OTA and its metabolites in blood, urine, tissue (kidney)	OTA-DNA adducts in tissue

Source: Biomin, adapted from Baldwin et al., *2011*

Depending on the milk production yield among other factors, it is estimated that 1-6% of ingested AfB_1 can be found in form of AfM_1 (hydroxylated metabolite) in

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The use of biomarkers as a diagnostic tool is ***only possible within*** *and their differences in toxicity. It must also be considered that there*

Photo: Alex Raths, iStockphoto

the milk of cows. Roughly calculated, 0.05 ppb of AfM_1 (EU maximum level for milk) would correlate to a range of AfB_1 contamination from 0.8 to 5 ppb in compound feed (5 ppb is the EU maximum level for compound feed in dairy cattle).

This example shows that conducting mycotoxin analyzes on feed is recommended in order to prevent the economic risk of aflatoxin contaminated milk close to the EU maximum level.

Biomarker of effect

Biomarkers of effect, also called mechanism-based biomarkers, should be directly linked to a specific step in the disruption of metabolic and cellular processes.

For instance, the first step leading towards porcine pulmonary edema in pigs is the disruption of the sphingolipid metabolism by fumonisin B_1 (FB_1). This compound inhibits the ceramide synthase resulting in an elevated sphinganine-to-sphingosine (Sa/So) ratio. The Sa/So ratio is a scientifically recognized biomarker of effect for fumonisins (FUM) in pigs, but not in humans.

Why not ELISA?

Although quick and inexpensive, ELISA can only be used in validated raw materials and is not a suitable method for analyzing non-validated physiological specimens.

Serum and milk samples were analyzed for DON in two different laboratories. While the first laboratory detected concentrations in the range of 69.5-117.5 µg/L by ELISA, levels were below the detection limit measured by HPLC in the second lab. Evidently the results by ELISA were false positive, as this method is not appropriate for mycotoxin analysis of complex matrices like feed, milk and blood.

Table 2. Comparison of ELISA and HPLC for physiological samples.

	DON By ELISA[1]	DON By HPLC[2]
Lactation feed	<134 µg/kg	77 µg/kg
Sow: milk	75 µg/L	<0.5 µg/L
Sow: blood serum	117.5 µg/L	<2.0 µg/L
Piglet: blood serum	69.5 µg/L	<2.0 µg/L

[1] BioCheck GmbH, Leipzig, Germany
[2] S. Dänicke, Institute of Animal Nutrition, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Braunschweig, Germany

Practical challenges

In the case of FUM, the Sa/So ratio applies to scientific trials but not at the farm level. It is difficult to provide controlled feeding and the lack of non-exposed groups on farms makes it impossible to define the cut-off value.

In addition, for a biomarker to have practical relevance, there must be a linear correlation between the exposure and ingestion of the mycotoxin. In some published scientific trials, a linear relationship could be found for DON and its metabolites measured in the blood or urine of swine; however, there are limitations.

Nonetheless, the deviation of individual amounts of mycotoxins detected in physiological samples does not allow any conclusion to be made on the amount of ingested mycotoxins and their health effects in single animals. These are the reasons for the lack of established guidance levels on critical concentrations of DON or other mycotoxins in the blood or other physiological samples of animals, which renders the interpretation of results impossible.

The situation is further complicated by the need for a precise time when sampling for a representative analysis. This is because of the peak in DON and its metabolites in the blood within two hours after ingestion, followed by a

scientific trials due to the range of resulting metabolites are no guidelines for risk levels in physiological specimens.

rapid depletion afterwards. ZEN takes longer to deplete due to the enterohepatic circulation (absorption in the blood, excretion via bile and reabsorption in the blood). Farm animals are usually fed ad libitum which makes sampling time unpredictable, thereby yielding results that are not representative.

Another important aspect is the fact that DON, like other mycotoxins, is converted into metabolites such as DON-glucuronide, deepoxy-DON and also unknown metabolites. The proportion depends on the species, life cycle, gut microbiota and the health status of the animal.

Furthermore, the toxicity of DON metabolites may differ from the parental compound; for example, deepoxy-DON is non-toxic. ZEN can be found as alpha- and beta-zearalenol, alpha- and beta-zearalanol and their glucuronated forms in physiological specimens. The transformation of ZEN into alpha-zearalenol increases estrogenicity. As a result, analyzing for only one individual mycotoxin is not enough.

Analyzing biomarkers

A trend in recent years has been the development of liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS)-based methods, which are highly selective and sensitive enough to detect mycotoxins at very low concentrations. LC-MS/MS offers the possibility to quantify several metabolites in parallel.

In contrast, enzyme-linked immunosorbent assay (ELISA) methods can only serve as a rough screening method as the matrix effects caused by body fluids alter the results. Antibodies used in ELISA tests to quantify mycotoxins have a wide cross-reactivity to related metabolites. For example, most ELISA kits for ZEN also detect alpha-zearalenol but cannot differentiate between the metabolites. The cross-reactivity for the different metabolites is often neither evaluated nor specified precisely in the user manual.

While there exist validated methods to analyze mycotoxins in feed, there are hardly any for biomarkers. In contrast to feed, quality control for mycotoxin analyzes of physiological samples has yet to be established for commercial laboratories.

Although biomarkers are valuable tools in scientific studies, more knowledge is needed on the factors influencing the bioavailability, kinetics and metabolic profile of mycotoxins in animals before biomarkers could be used in practice on farms. There is still a lack of linear correlation for biomarkers. The use of control groups and elaborate sampling is indispensable, which makes the procedure very costly.

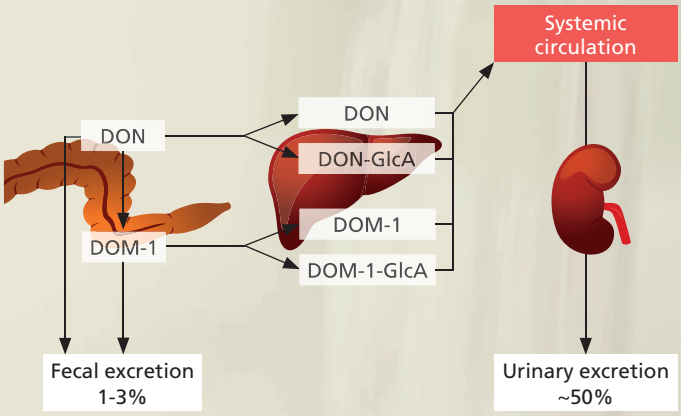
The analysis of mycotoxins in feed is a well-established, reliable approach to assessing possible risks and is therefore the method of choice.

References are available on request.

Metabolic pathway of DON in pigs

Depending on the available gut microbiota, ingested DON is metabolized into non-toxic deepoxy-DON (DOM-1). Further, DON and DOM-1 are partly absorbed into the blood stream and converted in the liver into DON-glucuronide (DON-GlcA) and DOM-1-glucoronide (DOM-1-GlcA).

After systemic circulation, the metabolites are excreted via urine (30-93% of ingested DON). Only small amounts can be found in the feces (1-3%). The missing proportion are unidentified metabolites and further degraded DON. To quantify DON in physiological specimens, all metabolites must be analyzed, and this cannot be achieved under practical conditions.



Phytogenics

Enhancing growth, naturally

Phytogenics are materials of plant origin such as herbs, spices, essential oils or other plant extracts. They have been used for thousands of years for everyday purposes as well as for their specific properties as natural remedies.

Background of beta-adrenergic agonists

Beta-adrenergic agonists (β-agonists) act as repartitioning agents, promoting lean tissue deposition in pigs. In 1999, a specific β-agonist was approved in the US and was subsequently introduced in other countries. Currently, there is only one β-agonist approved by the US Food and Drug Administration for use in swine diets.

Phytogenics express a wide range of biological activities including antimicrobial, anti-inflammatory and antifungal. With the ban on antimicrobial growth promoters (AGPs) in the EU and other countries, the search is on for alternatives. Many studies have been conducted to look into replacements for AGPs.

In the case of phytogenics, the anti-inflammatory and gut microbiota modulating effects in particular were researched and the use of these additives as a replacement for AGPs has increased significantly in recent years.

Benefits of β-agonists in finishing pigs

In finishing-pigs, the dietary use of a certain β-agonist improved growth performance at different feeding durations from six days, whereas carcass composition was

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improved at longer feeding durations. Carcass weight and dressing percentage also increased as a result.

What one has to bear in mind is that in order to have a considerable effect, the nutrient concentrations in the diet must be increased. Further, the protein composition of the diet has to be adjusted. In particular, the first-limiting amino acid, lysine, has to be available to the animal in sufficient amounts for the β-agonist to have an effect on growth performance and leanness. These adjustments ultimately make the feed more expensive.

Why β-agonists?

In recent years, China and Russia have banned the import of meat containing residues of certain β-agonists, as it was deemed unfit for human consumption. In 2013, Russia restricted meat imports to those certified free from a particular β-agonist.

Interestingly, China has also banned the use of specific β-agonists, the production of β-agonists and the import of meat containing β-agonists. The reason for the ban in China might be that traditional Chinese dishes commonly use offal in which residues might be higher.

Discussions on the use of β-agonists in pig production and their residues in meat have aroused greater attention due to public concerns as well as reported animal health issues arising from the use of the drug in the feed.

Reason for public concern

The metabolic fate of β-agonists is similar in the target species (pigs and cattle), laboratory animals and humans. Besides the pharmacological effect, β-agonists may cause intoxication. Therefore, any consumption of meat or by-products derived from animals that have consumed β-agonists in feed for growth stimulation may result in clinical effects such as tachycardia, heart rate increase, tremor, headache, muscle spasm and high arterial blood pressure.

The effect of β-agonists on humans is not completely known, but people with cardiovascular diseases are advised not to consume products containing β-agonists.

Beta-agonists used in swine diets metabolize quickly. Although only 84% of the β-agonist is excreted on the first day, no withdrawal period is given. Due to this lack of a withdrawal period, the β-agonist is fed right until slaughter. Hence, residues are still in the body of slaughtered pigs.

Potential side-effects

Independent studies have revealed some negative effects of β-agonists on animals.

After six weeks of feeding a certain β-agonist, pigs spent more time lying and less time walking. Pigs fed β-agonists were more difficult to handle. These differences became apparent very quickly after feeding of the β-agonist had started and continued over an entire four week-period.

The effects of a β-agonist on finishing pigs affected behavior, elevated heart rates and potentially made pigs more susceptible to stress from handling and transport. In a resident-intruder test, a test used to measure aggressiveness, β-agonist-fed gilts performed more attacks in the first 30 seconds. By the end of the test (300 seconds) the dominant control gilts and barrows and dominant but also subordinate β-agonist-fed gilts performed the most attacks ($p<0.05$). This change in behavior may cause major problems under farm conditions because it can lead to associated injuries, social stress and animal losses.

Phytogenics increase digestibility

Several studies have shown that phytogenics have a positive effect on nutrient digestibility, for example on ileal amino-acid digestibility.

Research has shown that phytogenics are able to stimulate digestive secretions, like saliva or bile acids and the activity of digestive enzymes. These effects are proposed to be the core nutritional mode of action of phytogenics. Subsequently, the above-mentioned stimulating effects on secretions and enzyme activity influence the digestibility of nutrients. Accordingly, several studies conducted in different animal species have confirmed higher ileal

Photo: Ugurhan Betin

Besides positive influences on the gastrointestinal tract, selected plant-derived additives are known to improve growth performance and influence carcass composition.

Photo: serezniy_iStockphoto

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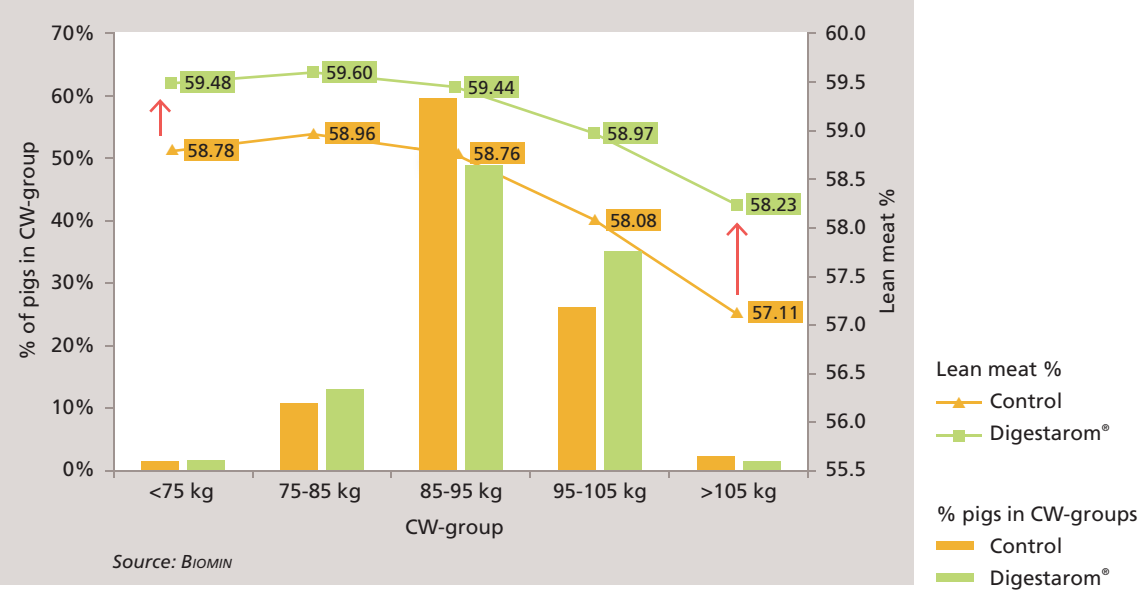
Figure 1. The effect of a PFA on lean meat yield compared to a control period.



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digestibility of amino acids and better nutrient utilization as a result.

Enhanced digestibility entails a general improvement in feed conversion ratio (FCR). The higher protein digestion results in better muscle accretion as shown in pigs as well as in broilers. This has to be taken into consideration when discussing the potential of phytogenics as a natural solution to replace β-agonists in livestock.

Wouldn't a natural product be better than β-agonists?

Phytogenics have been shown to improve feed intake, FCR, growth rate and carcass composition. In the following field study, the effect of a phytogenic feed additive (PFA, Digestarom® Finish, Biomin Phytogenics GmbH, Germany) on performance parameters as well as on carcass characteristics of growing-finishing pigs were investigated.

The trial included 5,732 finishing pigs on a total of 10 commercial farms in Austria. The data of lean meat percentage in the different carcass weight (CW) groups is shown in *Figure 1*.

This study compares the effect of a Digestarom® application period to a control period. The average lean meat percentage was higher in all CW-groups of finishing pigs in the Digestarom® group. The heavier the CW, the higher the lean meat percentage through Digestarom® application.

Lean meat yield was 0.68 percentage points higher in the CW-group of 85-95 kg. For pigs over 105 kg, lean meat percentage was 1.12 points higher. This once more indicates better nutrient utilization.

Conclusion

Overall, the side effects of β-agonists on finishing pigs, as well as concerns over residues are important issues that have become a focus of attention. Already banned in many countries, the use of β-agonists in animal production is disputable.

The use of natural substances like PFAs and their beneficial effects on digestibility, especially of feed protein, are promising. The results have shown that PFAs, depending on the balance of natural ingredients in the mixture, can have a positive impact on FCR as well beneficial effects such as higher carcass yield and quality (lean meat percentage) in finishing pig production.

References are available on request.

WORLD NUTRITION FORUM

MUNICH 2014
15-18 October
SUSTAIN: ABILITY

1. Different regions, similar challenges

Swine experts from Europe, America, South Africa and China present the trends in their local swine sectors, highlighting the largely similar issues faced amid unique local production and demand characteristics.

2. Gut for growth

Without a doubt, the best growth promoter is gut health.

This session explores efficient resource management, trends in dietary designs for swine, and anti-inflammatory responses in swine.

Also on the agenda is the use of phytogenics as an alternative to antibiotic growth promoters in swine diets, and their role in in regulating inflammatory processes while boosting the antioxidant activity and immune response of pigs.

A tradition of the **World Nutrition Forum (WNF)** since 2010, the species-specific Breakout sessions address timely topics in swine farming and other animal production sectors.

Each four-hour long Breakout session covers two topics. Sessions for each species are held in parallel on the afternoon of the first day (Thursday, 16 October 2014).

The World Nutrition Forum, sponsored by BIOMIN, is a premier biennial industry event where leading professionals, scientists and decision-makers gather to brain-storm and exchange ideas and strategic prospects on the future of animal nutrition. To be held in Munich, Germany, the WNF 2014 will explore the theme of "sustain:ability".

For up-to-date information, please visit **www.worldnutritionforum.info**.

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