

# New attributes of phy-togenic feed additives



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**M**icroencapsulation is a procedure where a liquid, gaseous or solid substance is packed by a tiny millimetric capsule. In other words it is a technology of protecting a certain substance (core) into a sealed (coated, walled or shelled) capsule. The encapsulation process was discovered accidentally in the 1950s when Barret Green of the National Cash Register Company attempted to create a carbonless copy paper that would provide multiple copies.

The pharmaceutical industry later improved the encapsulation methods to safeguard, control the release and target the delivery of medications.

The improvements developed allow choosing the location where the drug needs to be applied and, moreover, release it at gradual or continuous rates in response to a variety of triggers.

Following this tendency, the food industry established constant research to evaluate new materials and methods of encapsulation to avoid degradative reactions followed by loss of feed quality.

Moreover, ongoing research has been reducing the capsule size to 'nano' to improve its efficiency.

Phytogenic compounds such as essential oils are sensitive substances which have a tendency to lose their efficacy and efficiency in reason of quality mitigation due to their susceptibility to high temperatures, dusty inclination and remarkable odour, oxidative and volatile properties.

In the course of ongoing research and innovation, Biomim 'trapped' its phytogenic feed additive into a capsule by a modern matrix-encapsulation process to obtain all the benefits shown by previous encapsulation techniques, also avoiding ingredient losses during feed processing (pelletisation or extrusion) and storage.

This contemporary method of en-

capsulating essential oils ensures uniform capsules with a well defined diameter to enhance the ingredient bioavailability and efficacy. This approach of encapsulating essential oils led to the new generation of phytogenic feed additives, named Biomim® P.E.P. MGE. This new innovation was developed to overcome two major technical issues with powdered essential oil additives: stability and odour.

Furthermore, in contrast to conventional wax or fat coated products, the active ingredients are evenly distributed in a matrix and continuously released in the digestive tract.

The zootechnical performance of 90 piglets was evaluated over 56 days in the experimental facilities of the Biomim Centre of Applied Animal Nutrition. This performance trial compared the addition of matrix-encapsulated essential oils (Biomim® P.E.P. MGE) with conventionally encapsulated essential oils and a control group (no essential oils). Pigs fed matrix-encapsulated essential oils had enhanced weight gain and improved feed conversion as compared with the other treatments, indicating the advantages of this encapsulation technique.

The strategic and innovative encapsulation process is a new benefit which enables the product to have a longer shelf life by protecting it from environmental impacts, partly masking the strong flavour of essential oils whilst maintaining its key features of enhancement of palatability, stability in ration, improved digestion and, consequently, better performance. ■

References are available from Biomim on request

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# What you cannot see, but animals feel



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**S**ilent killers, invisible thieves, undesirable substance, natural poisons – all these names have been given to a group of fungal metabolites called mycotoxins and their sub-clinical effects on animal production are incalculable.

The high frequency of occurrence along with the concentrations at which they occur suggests that mycotoxins are routinely consumed by animals, causing sub-clinical symptoms that subsequently result in production losses. To the animal producer, these subclinical losses are of greater economic importance than losses from acute effects, but again, even more difficult to diagnose.

While growth retardation and reduced productivity are of economic importance, the intrinsic activity of many mycotoxins on the immune system of the animals is of increased concern. The presence of moderate to low amounts of mycotoxins in daily feed rations increases the susceptibility of animals to viral, bacterial and parasitic diseases (Bondy and Pestka, 2000). This increased susceptibility requires increased therapeutic intervention with antibiotics and anti-parasitic drugs, which in turn elevates the costs for animal health care and the use of anti-infective agents. Stoev et al., (2000) demonstrated that ingestion of ochratoxin A (OTA) contaminated feed increased susceptibility to natural infectious disease in pigs. In this experiment, salmonellosis arose spontaneously in all piglets receiving a diet contaminated with 3ppm OTA and in one third of the animals receiving a diet contaminated with 1ppm of toxin. In contrast, none of the animals fed the control diet were affected. In a further experiment, the authors vaccinated the animals against *S. choleraesuis* haemorrhagic diarrhoea. In this

case the mycotoxin contamination led to spontaneous *Serpulina* hydrodysenteriae and *Campylobacter coli* infection (Stoev et al., 2000).

Induction of cellular oxidative stress, resulting from an increase in the production of oxygen and hydrogen radicals, and a depletion of cellular defence mechanisms such as glutathione, is common following exposure to many mycotoxins (Surai, 2002). Cellular oxidative stress and enhanced radical production cause lipid peroxidation and cellular necrosis. Many mycotoxins also affect the transport of vitamins and provitamins, which encourages lipid peroxidation, impairs vital cellular function and induces programmed cell death (apoptosis) followed by cell necrosis (Fink Gremmels, 2008).

Incorporation of mycotoxins into membrane structures causes various detrimental changes which are associated with peroxidation of long chain polyunsaturated fatty acids (PUFAs) (lipid peroxidation). Moreover, currently it is not clear if mycotoxins stimulate lipid peroxidation directly by enhancing free radicals production, or if the increased tissue susceptibility to lipid peroxidation is a result of a compromised antioxidant system (Surai, 2006).

It is clear that there are no safe levels of mycotoxins in animal feed. Unfortunately it is not possible to entirely prevent the production of mycotoxins before harvest of agricultural crops, in storage, or during processing operations. The addition of feed additives, based on adsorptive and, more recently, enzymatic modes of action are widely used strategies to reduce mycotoxin-induced performance impairment (Binder, 2007). ■

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# Combining organic acids and essential oils



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Numerous substances deriving from natural sources suitable to replace antibiotic growth promoters (AGP) exist. Amongst them are organic acids and essential oils.

Organic acids work to improve feed and water hygiene, through the reduction of pH and buffer capacity, and control of feed contaminants such as *Salmonella* sp. and *E. coli*. The reduction in pH creates unfavourable conditions for possibly harmful bacteria in feed inhibiting their growth.

This leads to a reduced uptake of pathogenic bacteria by the animal and reduces the competition of undesirable micro-organisms and the host for nutrients.

However, the effectiveness of organic acids in improving feed hygiene highly depends on type of acids, acid concentration and chemical form (salts of acids or free acids) used. Due to reduction of microbial burden in the animal's gastrointestinal (GI) tract, beneficial microflora is maintained, which ensures a healthy gut and leads to improved growth performance. Free organic acids also reduce the buffering capacity in feed. This is an important effect, especially when it comes to the nutrition of young animals and animals fed diets high in protein, minerals and calcium having a high buffering capacity. In turn, lowering buffering capacity has beneficial effects on feed digestion.

On the other hand it has to be mentioned that organic acids are fast absorbed and metabolised in the GI tract. Encapsulation (protection of organic acids with lipid-based matrix) enables active ingredients to be delivered further into the GI tract, but increases production costs of the acidifiers and eliminates the effects of organic acids on feed hygiene resulting in a possibly increased health risk

and a possible reduction in animal performance.

Using essential oils or their constituents in animal feed becomes more and more popular and they are lately often used in combination with other agents, such as organic acids. Organic acids are in particular active in feed, crop and the upper part of the GI tract, whereas essential oils exert their action in the later segments of the GI tract. Therefore, usage of organic acids and essential oils results in a stronger product due to providing beneficial influences throughout the whole GI tract. Besides the anti-bacterial activities essential oils are also recognised as being immuno-modulation agents. Some essential oils are seen to be potent anti-inflammatory compounds. This is beneficial as inflammatory processes are associated with production penalties resulting in reduced growth performance.

However, it was shown that feeding a combination of essential oils and organic acids increased the digestive enzyme activity of the pancreas and intestinal mucosa leading to an increase in growth.

The extent to which organic acids and essential oils individually or in combination affect feed hygiene and animal growth performance depends amongst others on the type of product, as there are many different essential oils and organic acids with different properties commonly used in animal nutrition.

However, the combination of organic acids and essential oils can be considered as a very good solution to improve feed hygiene and enhance performance in pigs and poultry. ■

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# Endotoxins: can we beat these in- sidious enemies?



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**S**eptic shock caused by Gram negative bacteria has been the source of increasing morbidity and mortality among pigs, particularly in the setting of altered immunity.

Endotoxins, lipopolysaccharides (LPS) found in the outer membrane of Gram negative bacteria, have been implicated in the pathogenesis of this syndrome.

Endotoxins are very deceitful substances. On the one hand they stimulate the immune system in a positive way; on the other hand they cause endotoxic shock and death.

Although a lot of research has been done in recent years it has not yet been possible to fully understand the exact transition point of 'good to bad'.

## Associated disease

Of special interest for pig farmers is the involvement of endotoxins in, for example, the Mastitis-Metritis-Agalactia complex of sows or the sudden death syndrome in piglets.

But these are just two examples of endotoxin-associated diseases which can lead to financial losses and increased labour for farmers.

Endotoxins are of bacterial origin. This is of main interest when one imagines that bacteria are part of our lives.

They are everywhere around us in the air, water, food and feed, amongst others, as well as inside the gastrointestinal tract or in other parts of hollow organs.

Classically, an endotoxin is a toxin that is not secreted in soluble form by live bacteria, but instead is a structural component in the bacteria which is released mainly when bacteria are lysed.

As long as there is a balance between growth and death of bacteria every process remains normal

in the organism but what happens when this balance is disturbed?

## Detoxify endotoxins

What happens when more bacteria than those the organism can cope with are produced? In this case bacteria have the chance to liberate their poisonous substances or compete against the body's defences consequently harming it.

As long as the liver (the main detoxification organ) and other organs are 'healthy' the body is able to detoxify endotoxins.

But when the point is reached when the liver cannot cope with all the high endotoxin challenge, many metabolic, immune and endocrine reactions are triggered.

Endotoxins affect complex immune and inflammation cascades in the organism which consequently leads to an overshoot reaction followed by influenza like symptoms and, in worst cases, shock and death.

## Clinical signs

Typical clinical signs in affected pigs are fever, sudden death or nervous symptoms, such as blunting, staggering, ataxia, opisthotonus, subcutaneous oedema particularly in nose, ears, eyelids and larynx (hoarse, squeaky voice).

These out of many more examples were the reason why Biomim started to investigate this topic, allowing a better understanding of the harmful action of endotoxins and the development of a feed additive which helps to keep endotoxins under control. ■

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# Research for economical benefit in animal production



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Acids, phytobiotics and probiotics are traditionally used natural growth promoters (NGP). Those NGPs are thoroughly researched, which makes it difficult to find innovations. With the development of Biotronic Top3, Biomini provides an outstanding and innovative product to the market, as the Biomini Perforizer provides a revolutionary strategy to boost the effects of antimicrobial substances.

Biotronic Top3 contains a blend of organic acids, a phytochemical and the Biomini Perforizer. The benefits of using organic acid blends instead of single organic acids, is well established knowledge. However, if benefits can be seen, and at what extent benefits by using acid blends can be seen, strongly depends on which acids are combined and at which level they are included in the blend.

In house research at Biomini with in vitro studies has proven to have found the optimum blend.

Also the phytochemical used in Biotronic Top3 is an additional powerful feature in combating harmful bacteria due to its special mode of action. Usually phytochemical substances provide positive effects as they are serving as an anti-oxidant.

Cinnamaldehyde, which is a component of Biotronic Top3 plays an important role in the division of pathogenic bacteria, which leads to a reduction in pathogenic bacteria load.

The unique feature in Biotronic Top3 is the Biomini Perforizer. The Biomini Perforizer is a permeabilising substance, which weakens the outer membrane of Gram negative bacteria.

The increased permeability of the outer membrane allows the other active ingredients of the product to easier penetrate into the bacteria cell improving the an-

timicrobial efficiency of the organic acids and the cinnamaldehyde.

The positive effects of the Biomini Perforizer on the weakening of Gram negative bacteria was well investigated by an independent research facility in Scandinavia, which has its expertise in the research on permeabilising substances.

Also in between permeabilising substances differences occur in the strength of weakening the outer membrane of Gram negative bacteria and also the right inclusion level of the permeabilising substance is crucial in order to see the best possible effect on the weakening of the outer membrane.

The fact that in house research at Biomini not only found synergisms by adding the phytochemical to the acid blend but, in particular, by adding the Biomini Perforizer to the acid blend and the phytochemical allows a relatively low concentration of active ingredients.

The positive effects were not only shown on the inhibition of pathogenic bacteria in vitro.

In vivo studies also showed positive effects on performance in weaning pigs, as animals compete with the gut bacteria for nutrients and inflammatory responses due to the fight against upcoming diseases caused by pathogenic bacteria consume a lot of energy.

In general up to 6% of the net energy is lost to the gut microflora, which is especially concerning as energy is a limiting factor for the feed intake in pigs and, in turn, influences growth.

With the development of Biotronic Top3, Biomini has added a highly innovative product to its portfolio of natural growth promoters. ■



# Mycotoxin risk management



by Inês Rodrigues, technical manager, Biomini Holding GmbH, Herzogenburg, Austria. ines.rodrigues@biomin.net

In the case of mycotoxicoses, many questions are often posed. Can we rely solely on the symptoms the animals are presenting to diagnose a mycotoxin problem? Usually mycotoxin analyses are a must, but what reasons might there be for the fact that the mycotoxin analysis report shows a low mycotoxin concentration and still, animals show severe mycotoxicoses symptoms?

At the sow barn, females are having reproductive problems and the abortion incidence has increased abruptly. There you go – zearalenone intoxication, without a doubt! However, be careful as things may not be what they look like! Have you ever considered that abortions and infertility alone can be caused by Aujeszky disease, by *Brucella suis* or by perineal contamination?

A correct differential diagnosis allows a practitioner to differentiate mycotoxicoses from poor nutrition, poor management, physical damage to tissues, and infectious diseases. Visual diagnosis is a complex task and often erroneous as same symptoms can be caused by other etiologic agents.

The most precise way to identify a problem involving mycotoxins is by analysing commodities or finished feed for their presence. However, even when this is done and mycotoxin presence is confirmed, results must be cautiously interpreted.

The reason for this relies on the fact that often the sampling process did not allow the withdrawal of what is commonly referred to as a representative sample. Sampling plans must be set up in accordance with the possibilities of the feed mill/farm; however, the essence of a correct procedure relies on three guidelines:

- From a large lot of bulk sample (for example from a truck), as

many increments (points) as possible should be taken at a random fashion.

- Each increment should have approximately 100g. A minimum of 1,000g (1kg) is recommended in case of whole kernel samples. This is called a 'lot sample'.

- From this lot sample (1 to 5kg), the entire collection is ground and homogenised) before weighing out an aliquot for the analytical testing.

As a summary, before blaming mycotoxins for problems in a farm:

- A careful study and diagnosis must be made, taking into attention that there might be different etiological agents for the same symptom.

- Mycotoxin analysis of a representative sample of feed components or finished feed should be performed.

After confirmation of the presence of mycotoxins and if only low levels are found, it is crucial to consider:

- Mycotoxins may interact amongst themselves and their individual effects are increased.

- Several factors may interact and therefore increase the susceptibility of animals to mycotoxins.

- Feed quality has great variations within the year; therefore low contamination levels at one particular period most probably will not reflect the situation throughout the whole year.

All in all, routine analyses of commodities allow people to understand the risk incurred throughout the year, or in other words, which periods of the year are more critical in terms of mycotoxin contamination and therefore require a proactive mycotoxin risk management. ■

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# The consequences of mycotoxins and other interactants



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**W**hat if clinical symptoms are observed in animals even if only a low mycotoxin contamination was analysed in the feed?

In this case the interaction of mycotoxins with other main factors is of importance.

An already well known fact is that the impact of mycotoxins in animals depends on several animal, environmental and toxin related factors.

Young animals are in general more at risk to the effects of mycotoxins.

Animals placed in a hostile setting characterised by, for example, high temperatures, poor ventilation, high humidity, crowding, and viral or bacterial challenges or other stressful conditions are more susceptible to the effects of mycotoxins.

Even small amounts of mycotoxins in the feed can have a detrimental effect on a pig's immune system.

These generally immune suppressive effects of mycotoxins are well studied. Susceptibility to infections and diseases along with reduced efficacy of vaccination programs are practical consequences on pig farms.

The following studies show the susceptibility to different specific diseases in pigs consuming mycotoxins:

- Pigs fed with a diet containing aflatoxin B1 (70-140µg/kg) showed an enhanced susceptibility to infection with *Brachyspira hyodysenteriae* (Joens et al., 1981).

- In a study with 20 piglets ( $9.6 \pm 2.1$ kg), it was found that a low oral dose of fumonisin B1 (0.5mg/kg body weight/day, for seven days) containing culture material may predispose piglets to the development of lung pneumo-

nia induced by *Pasteurella multocida* (Halloy et al., 2005).

- Stoev et al. (2000) demonstrated susceptibility to natural infectious disease in 18 young pigs exposed to the immunotoxicity of ochratoxin A (1-3mg/kg feed) as six animals in the 3mg/kg group and two animals in the 1mg/kg group died with clinical and pathological symptoms of salmonellosis and renal ochratoxicosis.

- *Salmonella choleraesuis* was detected in the liver and faeces of animals fed this toxin. In addition, it was discovered that the ingestion of fumonisin B1 alters the cytokine production and decreases the vaccinal antibody response (Taranu et al., 2005).

- Marin et al. (2002) reported that pigs fed low doses of aflatoxins (140 and 280µg/kg) tend to have lower antibody levels of *Mycoplasma agalactiae* than control pigs.

- In another study investigating the effects of deoxynivalenol (1000µg/kg) and zearalenone (250µg/kg), antibody titers of pseudorabies vaccine were impaired in pigs after 6 weeks exposure (Cheng et al., 2006).

Taking all this into account, great awareness must be given to the interaction of mycotoxins with other main factors.

A correct mycotoxin risk management is a key factor for reaching peak performance in animal husbandry. ■

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# Fumonisin: major challenge for porcine immune system



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**F**umonisin are mycotoxins which are produced by several *Fusarium* species, especially *Fusarium verticillioides* and *Fusarium proliferatum*.

Fumonisin have been found worldwide, primarily in maize. More than 10 compounds have been isolated and characterised. Of these, fumonisin B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub> are the major fumonisins produced.

The most prevalent in contaminated maize is fumonisin B<sub>1</sub>, which is believed to be the most toxic. Fumonisin toxicosis in swine has been related to porcine pulmonary oedema (PPE) since 1981, when it was caused by the experimental exposition of pigs to corn which was contaminated with *F. verticillioides*.

This was confirmed by outbreaks of porcine pulmonary oedema in the midwestern and southeastern parts of the United States due to ingestion of corn contaminated with fumonisins.

Nevertheless, before causing such typical clinical symptoms, mycotoxins often act as immunosuppressive agents.

Chronic exposure to fumonisin B<sub>1</sub> can decrease the proliferation of undifferentiated porcine epithelial intestinal cells, altering the integrity of intestinal epithelium and consequently facilitating the entrance of pathogens into the body (Bouhet and Oswald, 2005).

In fact, 1mg of fumonisin B<sub>1</sub>/kg body weight for 10 days in weaned piglets can predispose to a longer shedding of F4+ enterotoxigenic *E. coli* following infection, which deepens intestinal damage (Devriendt et al., 2009).

Step by step, fumonisin B<sub>1</sub> deteriorates the hosts' immune system, affecting recognition and processing of pathogens by the

antigen-presenting cells, avoiding signalling (lower cytokine production) the attraction of effector cells for pathogen elimination, which finally leads to prolonged intestinal infection.

Fumonisin can decrease the clearance of *Pseudomonas aeruginosa* (Haschek et al., 2001).

Also, the phagocytosis of *Salmonella typhimurium* is decreased in alveolar macrophages from pigs fed fumonisin B<sub>1</sub> (Liu et al., 2002).

Consequently, pigs' susceptibility to diseases caused by those pathogens is increased.

Feeding weaning piglets with 8mg fumonisin B<sub>1</sub>/kg during 28 days leads to a significant decrease in antibody titer after vaccination against *Mycoplasma agalactiae* (Taranu et al., 2005).

Feeding pigs fumonisin-contaminated feed could lead to an inappropriate vaccination response, reducing the level of specific antibodies and reducing the period of vaccine protection, or just leaving animals unprotected against this specific disease.

The aforementioned studies describe some of the immunosuppressive effects of fumonisins, and their role as a predisposing factor to disease in pigs.

Still, more information is needed about its mechanisms of action to induce these and other effects.

One thing is certain: fumonisins represent a risk to animal health and performance, and that is why proper mycotoxin risk management is indispensable. ■

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