Overcoming Challenges of Xylanase Inhibitors in Animal Feeds

By Dr. Ajay Awati, Global Director Enzymes, EW Nutrition

In recent years, the scientific understanding of xylanase inhibitors (XIs) and their impact on animal nutrition has grown significantly. Xylanase, a crucial enzyme used to enhance nutrient availability in feed, can face challenges from XIs present in cereal grains. This article explores the evolution of plant protection mechanisms, the economic impact of XIs, and the development of a novel xylanase, Axxess XY, resistant to these inhibitors.

Xylanase inhibitors – an evolutionary protection mechanism of plants

Xylanase inhibitors (XI) are a classic example of the evolutionary development of protection mechanisms by cereal plants against pathogens. Microorganisms, such as fungal pathogens, involve the degradation of xylan as one of the mechanisms in pathogenesis (Choquer et al., 2007). There are also other mechanisms by which microorganism-produced xylanases affect plants.

To protect themselves, plants evolved xylanase inhibitors to prevent the activities of xylanases. XIs are plant cell wall proteins broadly distributed in monocots. There are three classes of XIs with different structures and inhibition specificities (Tundo et al., 2022):

1. Triticum aestivum xylanase inhibitors (TAXI)
2. Xylanase inhibitor proteins (XIP), and
3. Thaumatin-like xylanase inhibitors (TLXI).

Xylanase inhibitors have an economic impact

In animal nutrition, xylanases are widely used in diets containing cereal grains and other plant materials to achieve a higher availability of nutrients. The inhibitory activity of XIs prevents this positive effect of the enzymes and, therefore, makes them economically relevant. Studies have reported that higher levels of XIs negatively impact broiler performance. For example, in one of the studies, broilers fed with grains of a cultivar with high inhibitory activity showed a 7% lower weight on day 14 than broilers fed with grains of a cultivar with less inhibitory activity (Madesen et al., 2018). Another study by Ponte et al. (2004) also concluded that durum wheat xylanase inhibitors reduced the activity of exogenous xylanase added to the broiler diets.

Xylanase inhibitors can withstand high temperatures

Even though XIs can impact the performance of exogenous xylanase in different ways, only minor attention was paid to the reduction of xylanase's susceptibility to xylanase inhibitors during the xylanase development in the last decades. Firstly, the issue was ignored mainly through the assumption that XIs are denatured or destroyed during pelleting processes. However, Smeets et al. (2014) showed that XIs could sustain significant temperature challenges. They demonstrated that after exposing wheat to pelleting temperatures of 80°C, 85°C, 92°C, and 95°C, the recovery of inhibitory activity was still 99%, 100%, 75%, and 54%, respectively. Furthermore, other studies also confirmed that conditioning feed at 70-90°C for 30 sec followed by pelleting had little effect on the XI activity in the tested feed, showing that xylanase inhibitors are very likely present in most xylanase-supplemented feeds fed to animals.

Do we only have the problem of xylanase inhibitors in wheat?

No. After first reports of the presence of xylanase inhibitors in wheat by Debyser et al. (1997, 1999), XIs were also found in other cereal grains (corn, rice, and sorghum, etc.), and their involvement in xylanase inhibition and plant defense has been established by several reports (Tundo et al., 2022).

In most of the countries outside Europe, exogenous xylanase is used not only in wheat but also in corn-based diets. Besides broiler feeds, also other animal feeds, such as layer or swine feed being part of more mixed-grain diets, are susceptible to the inhibitory activity of XIs. Nowadays, the situation is getting worse with all the raw material prices increasing and nutritionists tending to use other feed ingredients and locally produced cereals. They need a xylanase which is resistant to xylanase inhibitors.

Xylanases' resistance to XIs is crucial –

Axxess XY shows it

To prevent xylanases from losing their effect due to the presence of xylanase inhibitors, the resistance of new-generation xylanases to these substances is paramount in the development process, including enzyme discovery and engineering.

In the past 25 years, scientists have learned much about XI-encoding genes and discovered how xylanase inhibitors can block microbial xylanases. Additionally, there has been a significant increase in understanding the structural aspects of the interaction between xylanases and XIs, mainly how xylanase inhibitors interact with specific xylanases from fungi or bacteria and those in the GH10 or GH11 family. With such understanding, a new generation xylanase, Axxess XY, was developed. Besides showing the essential characteristics of intrinsic thermostability and versatile activity on both soluble and insoluble arabinoxylan, it is resistant to xylanase inhibitors.

Axxess XY takes xylanase application in animal feeds to the next level.

Axxess XY outperforms other xylanases on the market

Recent scientific developments (Fierens, 2007; Flatman et al., 2002; Debyser, 1999; Tundo et al., 2022; Chmelova, 2019) *and* internal research can be summarized as follows:

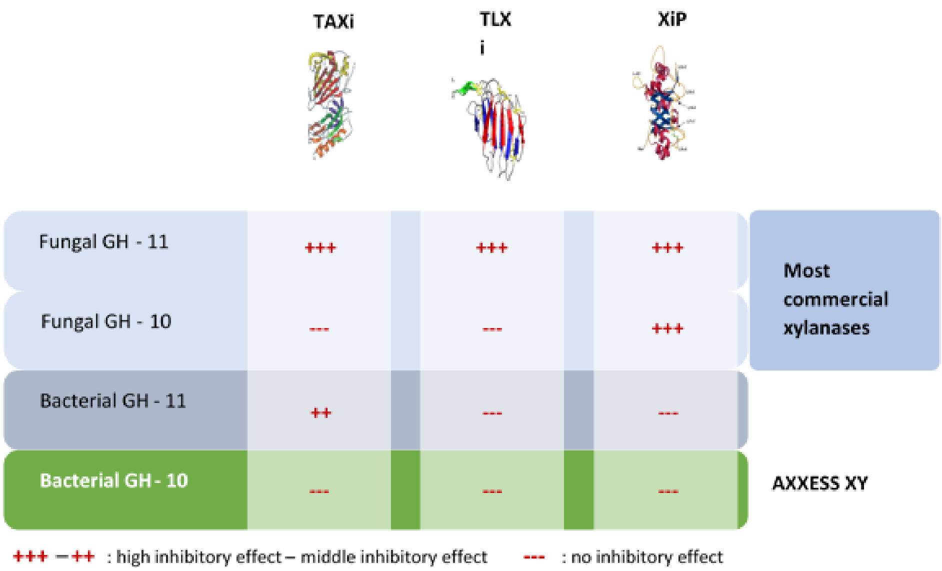

Figure 1: Schematic summary of the susceptibility of different xylanase to xylanase inhibitors from three main groups.

The high resistance to xylanase inhibitors is one of the reasons that a novel xylanase with bacterial origin and from the GH-10 family was chosen to be Axxess XY. EWN innovation, together with research partners, made an interesting benchmark comparison between xylanases that are commercially sold by different global suppliers and Axxess XY. For these trials, all xylanase inhibitors from wheat were extracted. The inhibitors, together with the respective xylanase, were incubated at 40^{0}C (to mimic birds’ body temperature) for 30 mins. Then, the loss of xylanase activity was calculated by analyzing remaining activity after incubation. Results are shown below in Figure 2. There were varying levels of activity loss observed in the different commercially sold xylanases. In some xylanases, the losses were alarmingly high. However, Axxess XY was not inhibited at all.

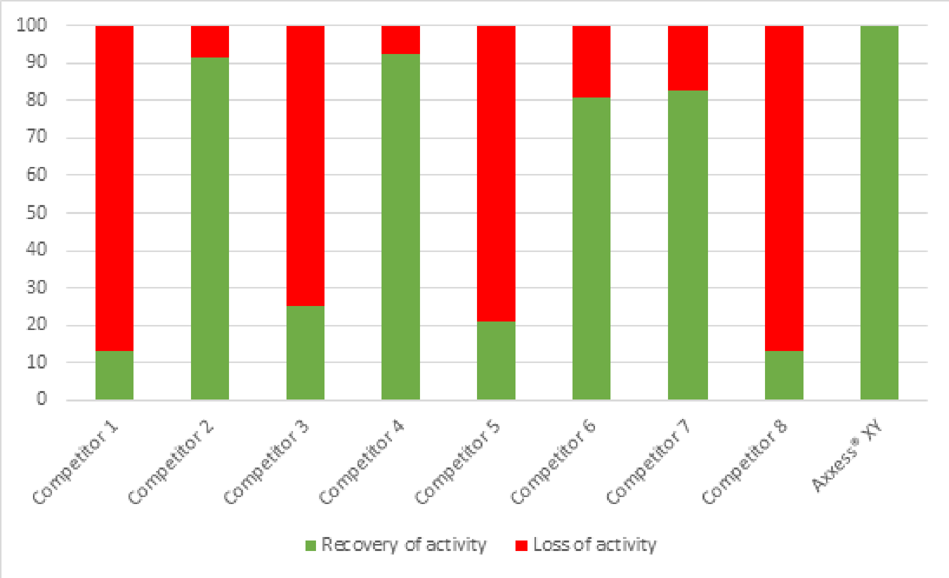

Fig. 2: Extracted total xylanase inhibitors from wheat incubated with the respective xylanase at 40°C for 30 mins. – Loss of activity after incubation with xylanase inhibitors

Conclusion:

Xylanase inhibitors are present in all cereal grains and, unfortunately, heat tolerant (up to 90^{0}C, still 75% of inhibition activity was retained). Regardless of the diets used, there is a possibility that the xylanase used may come across xylanase inhibitors, resulting in a loss of activity. More importantly, this can lead to inconsistent performance.

For effective, consistent, and higher performance of NSP enzyme application, it is a must to use xylanase that is resistant to xylanase inhibitors.

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Organic acids can play a crucial

role in zinc oxide replacement

Dr. Inge Heinzl, Editor EW Nutrition &
Juan Antonio Mesonero Escuredo, GTM Swine/GPM Organic Acids EW Nutrition

The use of high levels of Zinc Oxide (ZnO) in the EU before 2022 was one of the most common methods to prevent postweaning diarrhea (PWD) in pig production. Pharmacologically high levels of ZnO (2000-3000 ppm) increase growth and reduce the incidence of enteric bacterial diseases such as post-weaning diarrhea (PWD)(Carlson et al., 1999; Hill et al., 2000; Hill et al., 2001; Poulsen & Larsen, 1995; De Mille et al., 2019).

However, ZnO showed adverse effects, such as the accumulation of heavy metal in the environment, the risk for antimicrobial resistance (AMR), and problems of mineral toxicity and adverse growth effects when feeding it longer than 28 days (Jensen et al., 2018; Cavaco et al., 2011; Vahjen, 2015; Romeo et al., 2014; Burrough et al., 2019). To replace ZnO in pig production, let us first look at its positive effects to know what we must compensate for.

ZnO has a multifactorial mode of action

ZnO shows several beneficial characteristics that positively influence gut health, the immune system, digestion, and, therefore, also overall health and growth performance.

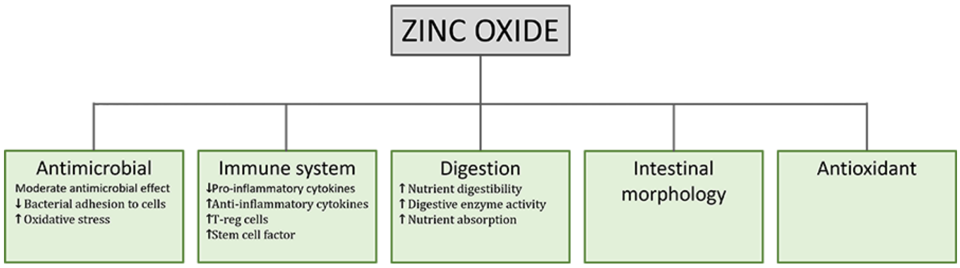


Figure 1. Beneficial effects and ZnO mode of action in postweaning piglets

1. ZnO acts as an antimicrobial

Concerning the antimicrobial effects of ZnO, different possible modes of action are discussed:

- ZnO in high dosages generates reactive oxygen species (ROS) that can damage the bacterial cell walls (Pasquet et al., 2014)
- The death of the bacterial cell due to direct contact of the metallic Zn to the cell (Shearier et al., 2016)
- Intrinsic antimicrobial properties of the ZnO^{2+} ions after dissociation. The uptake of zinc into cells is regulated by homeostasis. A concentration of the ZnO^{2+} ions higher than the optimal level of 10^{-7} to 10^{-5} M (depending on the microbial strain) allows the invasion of Zn^{2+} ions into the cell, and the zinc starts to be cytotoxic (Sugarman, 1983; Borovanský et al., 1989).

ZnO shows activity against, e.g., *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Streptococcus pyogenes*, and other enterobacteria (Ann et al., 2014; Vahjen et al., 2016). However, Roselli et al. (2003) did not see a viability-decreasing effect of ZnO on ETEC.

2. ZnO modulates the immune system

Besides fighting pathogenic organisms as described in the previous chapter and supporting the immune system, ZnO is an essential trace element and has a vital role in the immune system. ZnO improves the innate immune response, increasing phagocytosis and oxidative bursts from macrophages and neutrophils. It also ameliorates the adaptative immune response by increasing the number of T lymphocytes (T cells) in general and regulatory T lymphocytes (T-regs) in particular. These cells control the immune response and inflammation (Kloubert et al., 2018). Macrophage capacity for phagocytosis (Ercan and Bor, 1991) and to kill parasites (Wirth et al., 1989), and also the killing activity of natural killer cells depends on Zn (Rolles et al., 2018). By reducing bacterial adhesion and blocking bacterial invasion, ZnO disburdens the immune system (Roselli et al., 2003).

ZnO reduces the expression of several proinflammatory cytokines induced by ETEC (Roselli et al., 2003). Several studies have also shown a modulation effect on intestinal inflammation, decreasing levels of IFN-γ, TNF-α, IL-1ß and IL-6, all pro-inflammatory, in piglets supplemented with ZnO (Zhu et al., 2017; Grilli et al., 2015).

3. ZnO improves digestion and promotes growth

Besides protecting young piglets against diarrhea, the goal is to make them grow optimally. For this target, an efficient digestion and a high absorption of nutrients is essential. Stimulating diverse pancreatic enzymes such as amylase, carboxypeptidase A, trypsin, chymotrypsin, and lipase increases digestibility (Hedemann et al., 2006; Pieper et al., 2015). However, Pieper et al. (2015) also showed that a long-term supply of very high dietary zinc triggers oxidative stress in the pancreas of piglets.

By stimulating the secretion of ghrelin at the stomach level and thereby promoting the release of insulin-like growth factor (IGF-1) and cholecystokinin (CCK), ZnO enhances muscle protein synthesis, cell proliferation, and feed intake (Yin et al., 2009; MacDonald et al., 2000)).

The result of improved digestion is increased body weight and average daily gain, which can be seen, e.g., in a study by Zhu et al. (2017).

4. ZnO protects the intestinal morphology

ZnO prevents the decrease of the trans-endothelial electrical resistance (TEER), usually occurring in the case of inflammation, by downregulating TNF-α and IFN-γ. TNF-α, as well as IFN-γ, increase the permeability of the epithelial tight junctions and, therefore, the intestinal barrier (Al-Sadi et al., 2009).

The enterotrophic and anti-apoptotic effect of ZnO is reflected by a higher number of proliferating and PCNA-positive cells and an increased mucosa surface in the ileum (higher villi, higher villi/crypt ratio)(Grilli et al., 2015). Zhu et al. (2017) also saw an increase in villus height in the duodenum and ileum and a decrease in crypt depth in the duodenum due to the application of 3000 mg of ZnO/kg. Additionally, they could notice a significant ($P<0.05$) upregulation of the mRNA expression of the zonula occludens-1 and occluding in the mucosa of the jejunum of weaned piglets.

In a trial conducted by Roselli et al. (2003), the supplementation of 0.2 mmol/L ZnO prevented the disruption of the membrane integrity when human Caco-2 enterocytes were challenged with ETEC.

5. ZnO acts antioxidant

The antioxidant effect of ZnO was shown in a study conducted by Zhu et al., 2017. They could demonstrate that the concentration of malondialdehyde (MDA), a marker for lipid peroxidation, decreased on day 14 or 28, and the total concentration of superoxide dismutase (SOD), comprising enzymes that transform harmful superoxide anions into hydrogen peroxide, increased on day 14 ($P<0.05$). Additionally, Zn is an essential ion for the catalytic action of these enzymes.

Which positive effects of ZnO can be covered by organic acids (OAs)?

1. OAs act antimicrobial

OAs, on the one hand, lower the pH in the gastrointestinal tract. Some pathogenic bacteria are susceptible to low pH. At a pH<5, the proliferation of, e.g., Salmonella, E. coli, and Clostridium is minimized. The good thing is that some beneficial bacteria, such as lactobacilli or bifidobacteria, survive as they are acid-tolerant. The lactobacilli, on their side, can produce hydrogen peroxide, which inhibits, e.g., Staphylococcus aureus or Pseudomonas spp. (Juven and Pierson, 1996).

Besides this more indirect mode of action, a more direct one is also possible: Owing to their lipophilic character, the undissociated form of OAs can pass the bacterial membrane (Partanen and Mroz, 1999). The lower the external pH, the more undissociated acid is available for invading the microbial cells. Inside the cell, the pH is higher than outside, and the OA dissociates. The release of hydrogen ions leads to a decrease in the internal pH of the cell and to a depressed cell metabolism. To get back to “normal conditions”, the cell expels protons. However, this is an energy-consuming process; longer exposure to OAs leads to cell death. The anion remaining in the cell, when removing the protons, disturbs the cell’s metabolic processes and participates in killing the bacterium.

These theoretical effects could be shown in a practical trial by Ahmed et al. (2014). He fed citric acid (0.5 %) and a blend of acidifiers composed of formic, propionic, lactic, and phosphoric acid + SiO_2 (0.4 %) and saw a reduction in fecal counts of Salmonella and E. coli for both groups.

2. OAs modulate the immune system

The immune system is essential in the pig's life, especially around weaning. Organic acids have been shown to support or stimulate the immune system. Citric acid (0.5%), as well as the blend of acidifiers mentioned before (Ahmed et al., 2014), significantly increased the level of serum IgG. IgG is part of the humoral immune system. They mark foreign substances to be eliminated by other defense systems.

Ren et al. (2019) could demonstrate a decrease in plasma tumor necrosis factor-α that regulates the activity of diverse immune cells. He also found lower interferon-γ and interleukin (Il)-1ß values in the OA group than in the control group after the challenge with ETEC. This trial shows that inflammatory response can be mitigated through the addition of organic acids.

3. OAs improve digestion and promote growth

In piglets, the acidity in the stomach is responsible for the activation and stimulation of certain enzymes. Additionally, it keeps the feed in the stomach for a longer time. Both effects lead to better digestion of the feed.

In the stomach, the conversion of pepsinogen to pepsin, which is responsible for protein digestion, is catalyzed under acid conditions (Sanny et al., 1975)group. Pepsin works optimally at two pH levels: pH 2 and pH 3.5 (Taylor, 1959). With increasing pH, the activity decreases; at pH 6, it stops. Therefore, a high pH can lead to poor digestion and undigested protein arriving in the intestine.

These final products of pepsin protein digestion are needed in the lower parts of the GIT to stimulate the secretion of pancreatic proteolytic enzymes. If they do not arrive, the enzymes are not activated, and the inadequate protein digestion continues. Additionally, gastric acid is the primary stimulant for bicarbonate secretion in the pancreas, neutralizing gastric acid and providing an optimal pH environment for the digestive enzymes working in the duodenum.

As already mentioned, the pH in the stomach influences the transport of digesta. The amount of digesta being transferred from the stomach to the small intestine is related to the acidity of the chyme leaving the stomach and arriving in the small intestine. Emptying of the stomach can only take place when the duodenal chyme can be neutralized by pancreatic or other secretions (Pohl et al., 2008); so, acid-sensitive receptors provide feedback regulation and a higher pH in the stomach leads to a faster transport of the digesta and a worse feed digestion.

4. OAs protect the intestinal morphology

Maintaining an intact gut mucosa with a high surface area is crucial for optimal nutrient absorption. Research suggests organic acids play a significant role in improving mucosal health:

Butyric acid promotes epithelial cell proliferation, as demonstrated in an *in vitro* pig hindgut mucosa study (Sakata et al., 1995). Fumaric acid, serving as an energy source, may locally enhance small intestinal mucosal growth, aiding in post-weaning epithelial cells' recovery and increasing absorptive surface and digestive capacity (Blank et al., 1999). Sodium butyrate supplementation at low doses influences gastric morphology and function, thickening the stomach mucosa and enhancing mucosal maturation and differentiation (Mazzoni et al., 2008).

Studies show that organic acids affect gut morphology, with a mixture of short-chain and mid-chain fatty acids leading to longer villi (Ferrara et al., 2016) and Na-butyrate supplementation increasing crypt depth and villi length in the distal jejunum and ileum (Kotunia et al., 2004). However, the villi length and mucosa thickness in the duodenum were reduced. Dietary sodium butyrate has been linked to increased microvilli length and cecal crypt depth in pigs (Gálfi and Bokori, 1990).

5. OAs show antioxidant activity

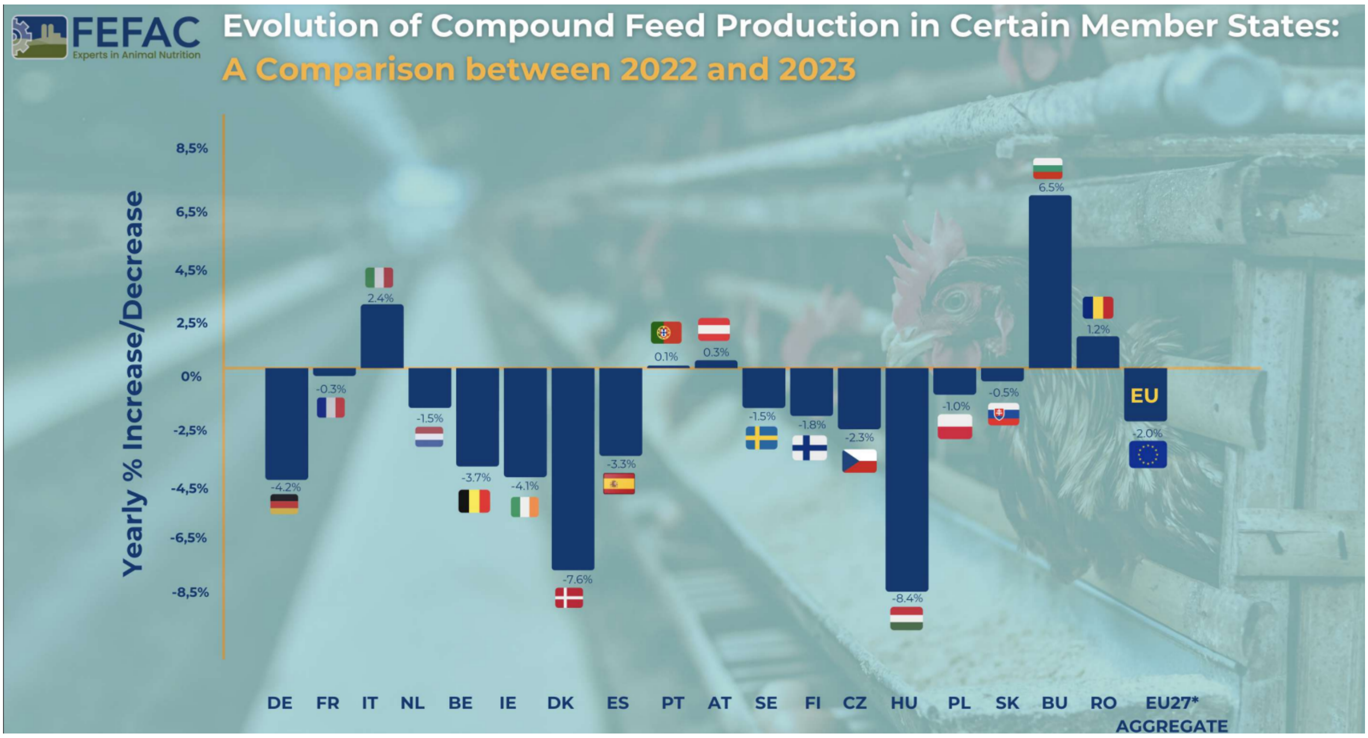
The last characteristic, the antioxidant effect, cannot be provided at the same level as with ZnO; however, Zhang et al. (2019) attest to OAs a certain antioxidant activity. Oxalic, citric, acetic, malic, and succinic acids, which were extracted from Camellia oleifera, also showed good antioxidant activity in a trial conducted by Zhang et al. (2020).

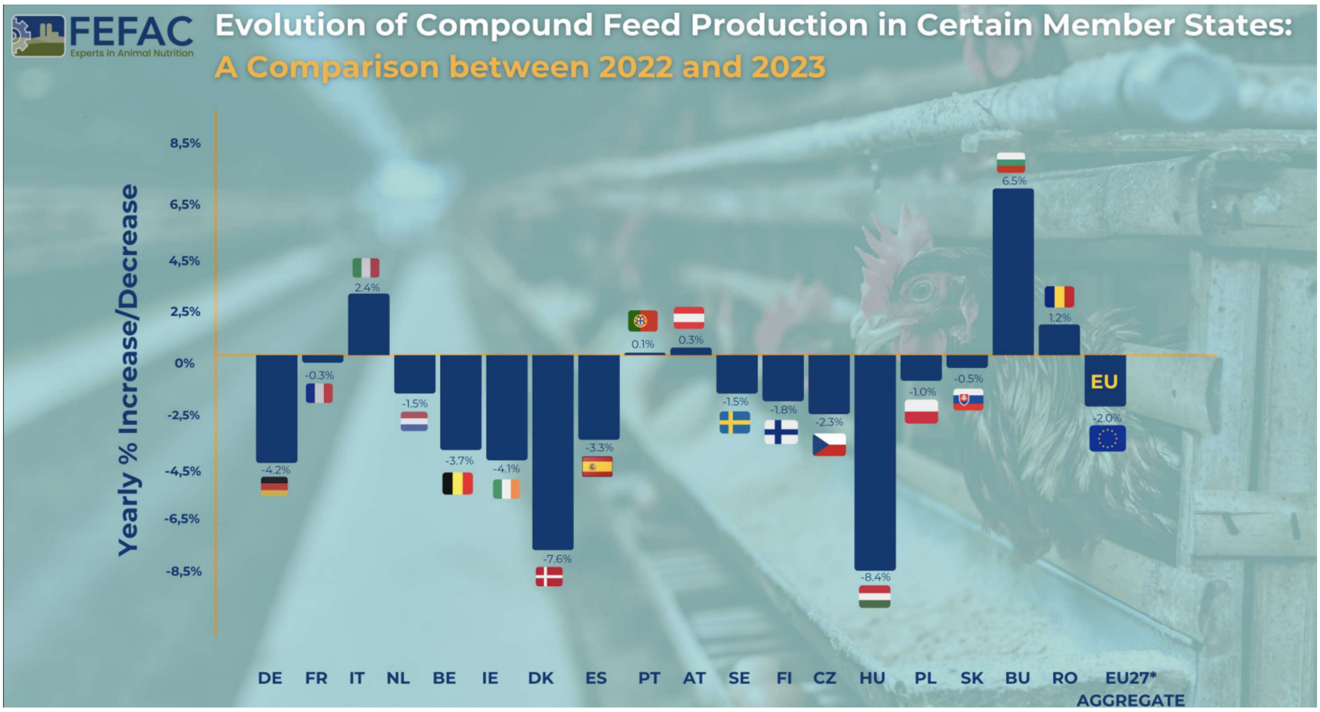
Organic acids are an excellent tool to compensate for the ban on ZnO

The article shows that organic acids have similar positive effects as zinc oxide. They act antimicrobial, modulate the immune system, maintain the gut morphology, fight pathogenic microbes, and also act – slightly – antioxidant. Additionally, they have a significant advantage: they are not harmful to the environment. Organic acids used in the proper pH range and combination are good tools for replacing zinc oxide.

References on request

FEFAC: Quick Overview of 2023 EU Compound Feed Production





Total Production 2023: 144.3 million metric tons for farmed animals

Change from 2022: 2% decrease

Factors Influencing Decrease

Political and Market Pressures: Addressing crises and the shift towards sustainable feed.

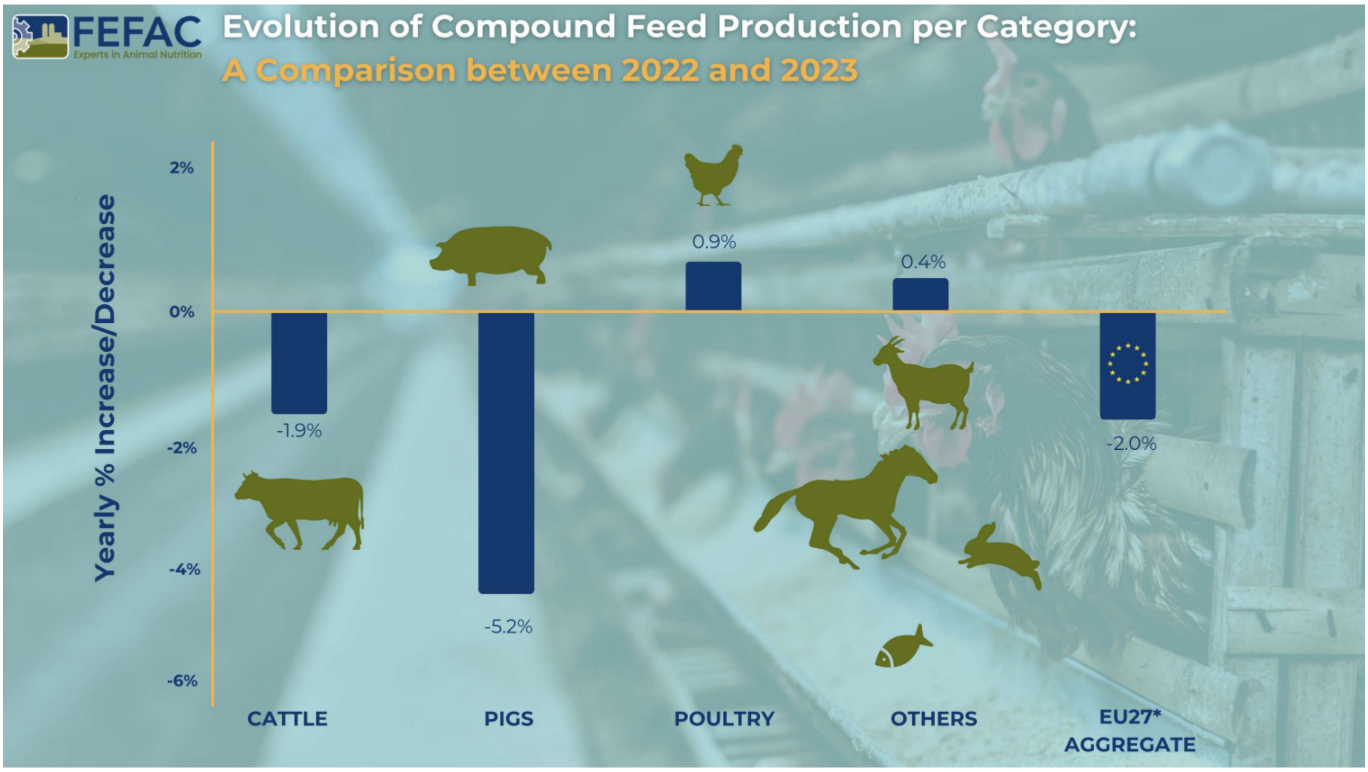
Climate and Diseases: Effects of droughts, floods, Avian Influenza (AI), and African Swine Fever (ASF) on raw material supply and animal production.

National Policies: Initiatives for greenhouse gas and nitrate emission reduction.

Consumer Trends: Food price inflation impacting demand.

Production Variability: Different trends across EU Member States, with notable decreases in countries like Germany, Ireland, Denmark, and Hungary, and slight increases in Austria, Bulgaria, Italy, and Romania.

Sector-Specific Trends



By Species

Pig Feed: Major decline of nearly 2.5 million tons. Key challenges included:

- Loss of export markets, particularly in Asia
- Negative media impact in Germany
- Significant production drop in Denmark (-13.6%) and Spain (loss of 800,000 metric tons)
- Italy’s ongoing struggle with ASF

Poultry Feed: Increase by 0.9 million tons, yet still 700,000 metric tons below 2021 levels. Challenges included declines in Hungary and Czechia due to reduced broiler production.

Cattle Feed: Decrease of 0.8 million tons from 2022.

2024 key factors

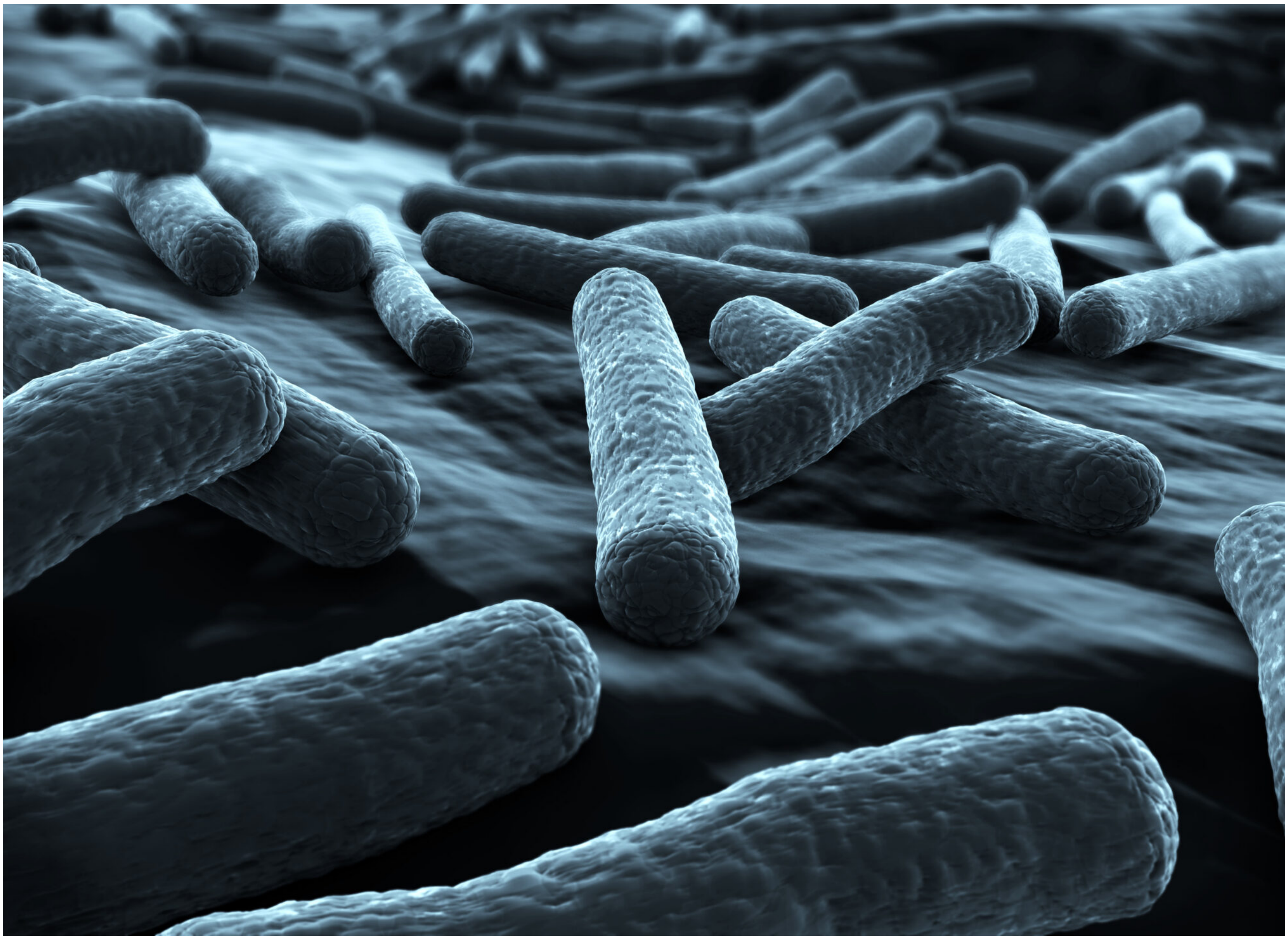
- Animal disease
- Economic instability, persistent food price inflation
- Weather irregularities
- Continued imports of poultry meat from Ukraine
- “Green and animal welfare” policies affecting local production

Summary

The EU’s compound feed production in 2023 faced numerous challenges, leading to an overall decrease. The pig feed sector was most severely hit, while poultry feed showed some recovery. The influence of environmental, economic, and policy factors played a significant role in shaping these trends. Despite the price of feed cereals falling back to the levels seen before Russia’s invasion of Ukraine, these challenges will continue to be felt in 2024.

Source: FEFAC

Endotoxins in 250 words



Dr Inge Heinzl, EW Nutrition

Endotoxins are… toxic, of course. The part "endo" in their name means that they are part of the bacterial cell, or, to specify it, they are part of the outer membrane of Gram-negative bacteria such as *E. coli*, *Salmonella*, *Shigella*, *Klebsiella*, and *Pseudomonas*.

When do they occur?

Always. Endotoxins are released with the lysis of bacteria, e.g., at the end of their life cycle, due to the effective immune defense of the host or treatments. The other possibility is bacterial growth as the membrane gets restructured and the endotoxins (or lipopolysaccharides -LPS-) are liberated.

What is the problem?

The "normal" occurrence (animals and humans always have Gram-negative bacteria in their gut) does not matter, because gut cells do not have receptors to recognize them as a danger in their apical side. However, when the barrier function is compromised, they pass into the bloodstream.

The liver still detoxifies small amounts. The problem comes with higher amounts of endotoxins in the bloodstream. Then, they provoke a strong immune reaction, feed intake drops, and nutritional resources are shifted from growth and production to immunity. These performance drops affect the profitability of the farmer.

What can be done?

Use broad-spectrum toxin binders that

- contain clay minerals showing high affinity and selectivity against endotoxins
- contain yeast cell walls, which, in addition to their binding capacity, support the immune response through macrophage activation and are involved in modulating microflora and bacterial load from the intestine
- provide adequate liver protection.

How to mitigate formulation costs when ingredient prices are high

Conference Report

The price of corn and soybeans dictates the price of all other ingredients, including to some extent amino acids, stated Dr Steve Leeson Professor Emeritus, University of Guelph, Canada at the recent [EW Nutrition] Poultry Academy in Jakarta, Indonesia.

The big question is, when times get tough, can we reduce safety margins and still get good performance?, asked Dr Leeson. “When we formulate diets, we build in some insurance. But so do the breeding companies in their recommendations. For sure, reducing safety margins takes us out of our comfort zones, but we need to be nutritionists, not mathematicians,” he stressed.

Protein and energy are now expensive. As a result of this economic pressure, there is a focus on strategies to reduce feed costs and improving the production efficiency and profitability of poultry enterprises. Feed cost/kg body weight gain is not always at the lowest feed:gain.

To help achieve these targets, Dr Leeson discussed feeding and management strategies that take into account the cost mitigation requirement.

Optimize current digestibility/efficiency

With high feed prices, it is especially important to review the use of feed additives that optimize nutrient release and improve 'digestibility'. The most obvious class of such additives are the various exogenous enzymes that improve the availability of phosphorus, energy, and amino acids. In most instances, these different classes of enzymes are additive in terms of nutrient release, since they have different target substrates or modes of action. All too often, the position is taken that "I take energy uplift from my amylase, so I can't expect energy release from phytase or protease".

The energy release from phytase is invariably net energy related to removal of the phytate molecule, which in effect is an 'antigen' and takes energy to counter its negative effects. The energy release from an amylase, however, is obviously related simply to the improved digestibility of carbohydrate complexes. Similarly, a protease enzyme will always provide energy, since all protein/amino acids are eventually used for energy during protein turnover, hence our use of the often forgotten 'n' in AMEn. We also have the choice of enzyme concentration, especially for phytase, which in the current economic solution is likely to be close to 2 – 2.5 doses, assuming a single dose is around 500-600 FTUs. The economics of super-dosing or mega-dosing is greatly impacted by the cost of the enzyme.

The response of phytase varies with individual amino acids, and with ingredients, with greater responses with ingredients of lower inherent digestibility. Generally, Dr Leeson suggests that a protease will capture 20% of indigestible amino acids. For example:

- 70% digestibility = +6% uplift
- 90% digestibility = +2% uplift

Relax ingredient constraint maximums

Probably the greatest current cost savings can be made from relaxing the maximum levels on ingredients. While corn and soybean meal levels are usually without restriction, we often impose limits on the upper levels of 'alternative' ingredients such as distillers grains, rice by-products and rapeseed/canola meals, etc. When the upper levels are reached in the formula, this suggests cost savings from using higher levels. Current restraints are based on past knowledge of perhaps variable nutrient composition and so the decision to use more of any ingredient must be based on past knowledge of on-going quality control assays. Although we can achieve considerable detail today in such QC assays, monitoring for (consistency of) crude fiber, crude protein, fat, and moisture alone, provide a sound basis for decisions on whether to use more of an individual ingredient.

Source alternate ingredients

Another option is to consider 'new' alternative ingredients. In reality, however, there are no new ingredients as such, since all monogastric nutritionists around the world have only around 19 ingredients available in sufficient quantities to sustain large-scale modern feed mills. There are certainly smaller quantities of specialised local by-products that can be used to advantage, yet these are becoming scarce. Therefore, an ingredient is only novel to you, since inevitably the same ingredient has been used for many years in other regions. As such, there is a wealth of information available on the nutritive value of these 'new' ingredients that can be simply transposed to our formulation matrices.

The bird is very adaptable to new ingredients, in fact it is more responsive to nutrients. Unless there are toxins, antinutritional factors, or other negative factors, it doesn't matter to the bird. Knowing the ingredient composition is the critical feature regarding the success or failure with new ingredients.

Reduce nutrient density

Both layers and meat birds still eat quite precisely to their energy requirements. They are amazingly adaptable to a vast range of nutrient densities, assuming that they can eat enough feed as the lower levels of feed energy are approached. Success in using lower levels of nutrient density is invariably negatively impacted by factors such as high stocking density and a high environmental temperature. Conversely, reducing diet energy usually has the hidden advantage of improved pellet quality.

The key to successful use of lower energy diets lies in prediction of change in feed intake and corresponding adjustment to all other nutrients in the diet.

Flexible cost of Dietary electrolyte balance (DEB)

When first introduced in the 1970s, maintaining DEB around 250MEq was seen to optimize broiler performance, especially leg condition. There is now less emphasis on this, perhaps because of genetic selection for skeletal integrity. DEB, however, may be important during heat stress to stimulate water intake and control manure moisture. Formulating to fixed DEB levels always adds costs. Instead, Dr Leeson suggested to focus on sodium and chloride at a ratio of 1:1.3.

Optimize feed texture (pelleting)

The first consideration is to make a good quality pellet, then worry about pellet size, noted Dr Leeson. He also added he was “a big fan of sunflower meal – it’s great for pellet quality.”

When given a choice in particle sizes, birds invariably show a preference for the largest particles. This situation becomes obvious when ‘fines’ accumulate in the feeder pans over time. As shown below, as pellet size increases, so does the bird’s need to consume fewer pellets. As a result, they need to spend less time at the feeder. Naturally, this idealised pellet size must be balanced against the willingness of mill managers to accommodate the necessary changes in pellet die size. Matching pellet size to bird age becomes critical as stocking density increases.

Impact of pellet size on pellet number consumed by a 30-day-old broiler

Pellet size (diameter)	4 mm length	6 mm length
3 mm	580	390
4 mm	330	220
5 mm	210	140

In the end, cost mitigation should not require complex mathematics. Nutritionists should be able to play with several types of improvements without affecting health and performance.

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EW Nutrition’s Poultry Academy took place in Jakarta and Manila in early September 2023. Dr. Steve Leeson, an expert in Poultry Nutrition & Production with nearly 50 years’ experience in the industry, was the distinguished keynote speaker.

Dr. Leeson had his Ph.D. in Poultry Nutrition in 1974 from the University of Nottingham. Over a span of 38 years, he was a Professor in the Department of Animal &Poultry Science at the University of Guelph, Canada. Since 2014, he has been Professor Emeritus at the same University. As an eminent author, he has more than 400 papers in refereed journals and 6 books on various aspects of Poultry Nutrition & Management. He also won the American Feed Manufacturer’s Association Nutrition Research Award

(1981), the Canadian Society of Animal Science Fellowship Award (2001), and Novus Lifetime Achievement Award in Poultry Nutrition (2011).

Meat quality is a result of genetics, feeding, the microbiome, and the handling of animals and meat

*by **Dr. Inge Heinzl**, Editor EW Nutrition*

Nowadays, nutrition is no longer about pure nutrient intake; enjoyment is also a priority. Consumers attach great importance to the high quality of food and, therefore, also of meat. The genetic selection for faster growth and feeding high-energy diets made meat production more efficient and shortened the raising period. However, this selection may sometimes also result in challenges to meat quality, such as worse water holding capacity, less marbling, less flavor, and reduced storage & processing properties.

The following article will provide detailed information about what meat quality is, how the gut microbiota influences it, and how we can increase meat quality by feeding and modulating the intestinal microflora.

Which factors can contribute to meat quality?

Meat quality is a complex term. On the one hand, meat quality covers measurable parameters such as the content of nutrients, moisture, microbial contamination, etc. On the other hand, and to no small extent, the consumers' preferences are significant. Since meat today is often sold as cuts or in parts (e.g., broiler drumsticks, breast), processing also affects the quality of meat and meat products.

Physical characteristics are objective determinants of meat quality

Physical characteristics are parameters that can be measured. For meat, the following measurable parameters determine meat quality:

1. Fat content and fatty acid composition influence tenderness and taste

Some years ago, the majority of consumers asked for completely lean meat, which, fortunately, has now changed. Fat is a flavor carrier. Especially intramuscular fat (marbling) melts during the preparation, making the meat tender, juicy, and taste good. Fat also transports fat-soluble vitamins.

A further criterion is the composition of the fat, the fatty acids. Geese fat, e.g., is known for its high content of oleic, linoleic, linolenic, and arachidonic acid, all of them derivates of the enzymatic denaturation of stearic acid (Okruszek, 2012).

One exception is cholesterol. Although belonging to the lipids and improving the sensory quality of meat, consumers prefer meat with low cholesterol content.

2. Protein and amino acid content influence the meat value

The content and the composition of protein are important factors in meat quality. Protein is essential for constructing and maintaining organs and muscles and for the functionality of enzymes. The human body needs 20 different amino acids for these tasks, eleven of which it can manufacture by itself. Nine amino acids, however, must be provided by food and are called essential amino acids. Meat is a highly valuable protein source, rich in protein and essential amino acids. The protein quality, therefore, includes the chemical and amino acid score, the index for essential amino acids, and the biological value.

In addition to the pure nutritional value, amino acids contribute to flavor and taste. These flavor amino acids directly influence meat's freshness and flavor and include threonine, alanine, serine, lysine, proline, hydroxyproline, glutamic acid (glutamate is important for the umami taste), aspartic acid, and arginine.

3. Vitamins and trace elements are essential nutrients

Meat is a primary source of B vitamins (B1-B9) and, together with other animal products such as eggs and milk, the only provider of Vitamin B12. Vitamin A is available in the innards, vitamin D in the liver and fat fish, and vitamin K in the flesh.

The most important mineral compounds in meat are zinc, selenium, and iron. Humans can utilize the iron from animal sources particularly well.

4. pH and speed of pH decline decide if the meat is suited for cooking

Since broiler chicken meat nowadays is usually consumed as cut-up pieces or processed products, the appearance at the meat counter or in the plastic box is essential for being sold. The color, seen as an apparent measurement of the freshness and quality of the meat, is influenced by the pH. The muscle pH post-mortem plays an essential role in meat quality. Due to the glycolytic process, the pH post-mortem is a good indication for evaluating physiological meat quality. A rapid pH decline post-mortem to 5.8-6.0 in most cases leads to pale, soft, and exudative (PSE) meat with reduced water retention (Džinić et al., 2015), whereas a high ultimate pH results in dark, firm, and dry (DFD) meat with poor storage quality (Allen et al., 1997)

5. Nobody wants meat like leather

The shear force is a measure of the tenderness of the meat. To determine the shear force, the meat undergoes the process of cooking and chilling. Afterward, standardized meat blocks, with fibers running along the length of the sample, are put into the Warner-Bratzler system. The blade used simulates teeth, and the system measures the force necessary to tear the piece of meat.

6. Microbial contamination is a no-go

The microbial contamination of the meat often occurs during the slaughter process. Let's take a look at salmonella or campylobacter in poultry. The chickens take up salmonella with contaminated feed or water. Campylobacter is transmitted by infected wild birds, inadequately cleaned and disinfected cages, or contaminated water. The bacteria proliferate in the intestine. At slaughter, the intestine's microorganisms can spread onto the meat intended for human consumption.

7. High water holding capacity is necessary to have tender meat

The moisture content contributes to the meat's juiciness and tenderness and improves its quality. If the meat loses its moisture, it gets tough, and quality decreases. Additionally, drip loss reduces the nutritional value of meat and its flavor.

8. Fat oxidation makes meat rancid, and oxidative stress can cause myopathies in broiler breasts

Rancidity of meat occurs when the fat in the flesh gets oxidized. There are different signs of meat rancidity: bad odor, changed color, and a sticky, slimy texture. Poultry meat is considered more susceptible to the development of oxidative rancidity than red meat. This can be explained by its higher content of phospholipids, PUFAs, especially in the thighs. The breast meat, however, has a relatively low level of intramuscular fat (up to 2 %) and, additionally, myoglobin is a natural antioxidant.

But oxidative stress in broiler breasts – and this more and more happens due to a selection of always bigger breasts – can lead to muscle myopathies such as white stripes or wooden breasts, making the meat only usable for processed products.

Sensory meat quality addresses the human senses

Besides physical quality, the sensory and chemical characteristics are essential to meat's economic importance. All attributes of meat that stimulate the human senses (vision, smell, taste, and touch) belong to the sensory quality. It, therefore, is more subjective and hard to determine. The most important features for the consumer include color (attractive or unattractive), texture (tenderness, juiciness, marbling, drip loss), and taste/ flavor (Thorslund et al., 2016).

The appearance is the first impression

Nowadays, meat is often sold as cuts lying in polystyrene or clear plastic trays, over-wrapped with transparent plastic films, so the appearance is paramount. The meat must show an attractive color. Muscle myopathies, such as the ones occurring in chickens, would not meet consumers' needs.

How does the flavor of meat develop?

There is a reaction between reducing sugars and amino acids when meat is cooked (Mottram, 1998). This Maillard reaction, along with the degradation of vitamins, lipid oxidation, and their interaction, is responsible for the production of the volatile flavor components forming the characteristic aroma and flavor of cooked meat (MacLeod, 1994). Werkhoff et al. (1990) consider cysteine and methionine the most significant contributors to meat flavor development. One factor deteriorating this quality characteristic is lipid peroxidation, which turns the taste to rancid.

Some sensory characteristics are related to physical ones

The parameters of sensory meat quality can be partly explained by measurable parameters. Water retention, e.g., influences the juiciness of the meat. The palatability increases with higher intramuscular fat or marbling (Stewart et al., 2021), the initial pH and the speed of decline decide if the flesh will be pale, soft, and exudative or normal, and lipid peroxidation is the leading cause of a decrease in meat quality (Pereira & Abreu, 2018).

Processing quality

For the processing quality, muscle structure, chemical ingredient interactions, and muscle post-mortem changes are decisive (Berri, 2000).

Does the microbiome influence the meat quality?

The gastrointestinal tract of monogastric animals disposes of a microbiome of primarily bacteria, mainly anaerobic Gram-positive ones (Richards et al., 2005). With its complex microbial community, the digestive tract is responsible for digesting feed and absorbing nutrients, but also for eliminating pathogens and developing immunity. Gut microbiotas play an essential role in digestion, are decisive concerning the synthesis of fatty acids, proteins, and vitamins, and, therefore, influence meat quality (Chen, 2022).

Intestinal microbiotas vary by species/breeds and age (Ma et al., 2022; Sun et al., 2018), and so does meat quality. For example, Duroc pigs with meat of high tenderness, good flavor, and excellent tastiness show different microbiota than other breeds (Xiao, 2017). Zhao et al.(2022) examined high- and low-fat Jinhua pigs, with the high-fat pigs showing more increased backfat thickness but also a higher fat content in the longissimus dorsi. They found low-fat pigs showed a higher abundance of Prevotella and Bacteroides, Ruminococcus sp. AF12-5, Faecalibacterium sp.OFO4-11AC und Oscillibacter sp. CAG:155, which are all involved in fiber fermentation and butyrate production. The high-fat animals showed a higher abundance of Firmicutes and Tenericutes, indicating that they are responsible for higher fat production of the organism in general but also a better fat disposition in the flesh. Lei et al. (2022) showed that abdominal fat was positively correlated with the occurrence of Lachnochlostridium and Christensenelleceae.

The intestinal microbiota-muscle axis enables us to improve meat quality by controlling intestinal microbiota (Lei, 2022). However, to develop strategies to enhance the quality of meat, understanding the composition of the microbiota, the functions of the key bacteria, and the interaction between the host and microbiota is of utmost importance (Chen et al., 2022).

Different factors influence the microbiome

Apart from that microbiotas are different in different breeds, they are additionally influenced by diseases, feeding (diets, medical treatments with, e.g., antibiotics), and the environment (climate, geographical position). This could be shown by different trials. The genetic influence on microbiota was impressively documented by Goodrich et al. (2014), who detected that the microbiomes of monozygotic twins differ less than the ones of dizygotic twins. Lei et al. (2022) compared the microbiota of two broiler breeds (Arbor Acres and Beijing-You, the last one with a higher abdominal fat rate) and found remarkable differences in their microbiota composition. When raising them in the same environment and with the same feed, the microbiotas became similar. Zhou et al. (2016) contrasted the cecal microbiota of five Tibetan chickens from five different geographic regions with Lohmann egg-laying hens and Daheng broiler chickens. Besides seeing a difference between the breeds, slightly distinct microbiota between the regions could also be noticed.

The intestinal microbiome can actively be changed by

- promoting the wanted microbes by feeding the appropriate nutrients (e.g., prebiotics)
- reducing the harmful ones by fighting them, for example, with organic acids or phytomolecules
- directly applying probiotics and adding, therefore, desired microbes to the microbiome.

An increase in the abundance of Lactobacillus and Succiniclasticum could be achieved in pigs by feeding them a fermented diet, and Mitsuokella and Erysipelotrichaceae proliferated by adding a probiotic containing B. subtilis and E. faecalis to the diet (Wang et al., 2022).

How to change the intestinal microbiome to improve meat quality?

Before changing the microbiome, we must know which microbes are “responsible” for which characteristics. However, the microbiotas do not act individually but as consortia. The following table shows a selection of bacteria that, besides supporting the gut and its functions, influence meat quality in some way.

Metabolites	Producing bacteria	Biological functions and effects on pigs
Short-chain fatty acids (acetate, butyrate, and propionate)	Ruminococcaceae Ruminococcus Lachnospiraceae Blautia Roseburia Lactobacillaceae Clostridium Eubacterium Faecalibacterium Bifidobacterium Bacteroides	Regulate lipid metabolism Improve meat quality
Lactate	Lactic acid bacteria Bifidobacterium	Important metabolite for cross-feeding of SCFA-producing microbiota
Bile acids (primary and secondary bile acids)	Clostridium species Eubacterium Parabacteroides Lachnospiraceae	Regulate lipid metabolism
Ammonia	Amino acid fermenting commensals Helicobacter	By-product of amino acid fermentation Inhibits short-chain fatty acid oxidation
B Vitamins and vitamin K	Bacteroides Lactobacillus	Serve as coenzymes in neurological processes (B vitamins) • Essential vitamin for proper blood clotting (vitamin K)

Table 1: Bacteria influencing meat quality (according to Vasquez et al., 2022)

Fat for meat quality is intramuscular fat

If we talk about increasing fat to improve meat quality, we talk about increasing intramuscular fat or marbling, not depot fat. The fat in meat-producing animals is mostly a combination of triglycerides from the diet and fatty acids synthesized. Fat deposition and composition in non-ruminants reflect the fatty acid composition of the diet but are also closely related to the design of the microbiome; short-chain fatty acids in monogastric, e.g., are exclusively produced by the gut microbiome (Dinh et al., 2021; Vasquez et al., 2022). Intramuscular fat is mainly made of triglycerides but also disposes of phospholipids associated with proteins, such as lipoproteins or proteolipids, influencing meat flavor. The fermentation of indigestible polysaccharides or amino acids results in short-chain or branched-chain fatty acids, respectively. Lactate, produced by lactic acid bacteria, is utilized by SCFA-producing microbiota. An imbalance in the microbiome fosters lipid deposition, as shown by Kallus and Brandt (2012), who found a higher proportion of Firmicutes to Bacteroidetes (50% higher) in obese mice than in lean ones. In a trial described by Zhou et al. (2016), tiny Tibetian chickens with a low percentage of abdominal fat were compared to two breeds (Lohmann layers and Daheng broilers) being large and with a high percentage of abdominal fat. The Tibetan chickens showed a two to four-fold higher abundance of Christensenellacea in the cecal microbiome. Christensenellas belong to the bacterial strain of firmicutes. They are linked to slimness in human nutrition, which was already proven by Goodrich et al. (2014) and is the contrary stated by Lei et al. (2022).

Another example was provided by Wen et al. (2023). They compared two broiler enterotypes distinguished by Clostridia vadinB60 and Rikenellaceae_RC9_gut and saw that the type with an abundance of Clostridia_vadinBB60 showed higher intramuscular fat content but also more subcutaneous fat tissue. The scientists also found another bacterium especially responsible for intramuscular fat: A lower plethora of Clostridia vadimBE97 resulted in a higher intramuscular fat content in breast and thigh muscles but not adipose tissues. Similar results were achieved in a trial with pigs and mice: Jinhua pigs showed a significantly higher level of intramuscular fat than Landrace pigs. When transplanting the fecal microbiota of the two breeds in mice, the mice showed similar characteristics in fat metabolism as their donors of feces (Wu et al., 2021).

According to several studies (e.g., Chen et al., 2008; Liu et al., 2019), intramuscular fat in chicken has a low heritability but may be controlled by feeding up to a certain extent. In pigs, Lo et al. (1992) and Ding et al. (2019) found a moderate to low (0.16 – 0.23) heritability for intramuscular fat, but Cabling et al. (2015) calculated a heritability of 0.79 for the marbling score.

At least, especially the composition of fatty acids can easily be changed in monogastric (Aaslyng and Meinert, 2017). Zou et al. (2017) examined the effect of Lactobacillus brevis and tea polyphenol, each alone or combining both. Lactobacillus is probably involved in turning complex carbohydrates into metabolites lactose and ethanol, but also acetic acid and SCFA. SCFAs are mainly produced by Saccharolytic and anaerobic microbiota, aiding in the degradation of carbohydrates the host cannot digest (e.g., cellulose or resistant polysaccharides into monomeric and dimeric sugars and fermenting them subsequently into short-chain fatty acids). Including fibers and various oligosaccharides was shown to increase the gut microbiome’s fermentation capacity for producing short-chain fatty acids.

In a trial conducted by Jiao et al. (2020), they showed that SCFAs applied in the ileum modulate lipid metabolism and lead to higher meat quality in growing pigs. A plant polyphenol was used by Yu et al. (2021). The added resveratrol, a plant polyphenol in grapes and grape products, to the diet of Peking ducks and could significantly increase intramuscular fat.

Oxidation of lipids and proteins must be prevented

The composition of the fatty acids and occurring oxidative stress in adipose and muscle tissue influences or impacts meat quality in farm animals (Chen et al., 2022). During the last few years, the demand for healthier animal products containing higher levels of polyunsaturated fatty acids has increased. Consequently, the risk of lipoperoxidation has risen (Serra et al., 2021). Solutions are needed to counteract this deterioration of meat quality. As can be seen in table 1, ammonia produced by amino acid-fermenting commensals and Helicobacter inhibits the oxidation of SCFAs. Ma et al. (2022) changed the microbiome of sows by feeding a probiotic from mating till day 21 of lactation and achieved a decreased level of MDA, a sign of reduced oxidative stress. Similar results were achieved by He et al. (2022). In their trial, the supplementation of 200 mg yeast ß-glucan/kg of feed significantly decreased the abundance of the phylum WPS-2 as well as markedly increased catalase, superoxide dismutase (both $p<0.05$) and the total antioxidant activity ($p<0.01$) in skeletal muscle. Another approach was done by Wu et al. (2020) in broilers. They applied glucose oxidases (GOD) produced by Aspergillus niger and Penicillium amagasakiense. Both enzymes did not disturb but improved beneficial bacteria and microbiota. The GOD produced by A. niger reduced the content of malondialdehyde in the plasma.

Another alternative is antioxidant extracts from plants (Džinić, 2015). As consumers nowadays bet more on natural products, they would be good candidates. They are considered safe and, therefore, well-accepted by consumers and have beneficial effects on animal health, welfare, and production performance.

Hazrati et al. (2020) showed in a trial that the essential oils of ajwain and dill decreased the concentration of malondialdehyde (MDA) in quails' breast meat and, therefore, lipid peroxidation and reduced cooking loss. The antioxidant effects of thymol and carvacrol were shown by Luna et al. (2010). The group receiving the essential oils showed lower TBARS in the thigh samples than the control group but similar TBARS to the butylated hydroxytoluene-provided group.

Protein quality is a question of essential amino acids

Protein with a high content of essential amino acids is one of the most critical components of meat. Alfaig et al. (2014) tested probiotics and thyme essential oil in broilers. They found out that the content of EAAs in breast and thigh muscles numerically increased gradually from the control over the probiotic and a combination of a probiotic up to the thyme essential oil group. A significant ($p<0.05$) increase in all tested amino acids (arginine, cysteine, phenylalanine, histidine, isoleucine, leucine, lysine, methionine, threonine, and valine) could be observed in the samples of the breast and the thigh muscles when comparing the thyme essential oil group with the control. Zou et al. (2017) provided similar results, showing a significant increase in leucine and glutamic acid as well as a numerical increase in lysin, valine, methionine, isoleucine, phenylalanine, threonine, asparagine, alanine, glycin, serin, and proline through the addition of a combination of Lactobacillus brevis and tea polyphenols. They also determined an increase in the beneficial bacteria Lactobacillus and Bacteroides. The experimental results led them to the assumption that both additives may also improve the taste of meat by increasing some of the essential and delicate flavors produced by amino acids.

Tenderness is closely related to drip loss

The already mentioned trial conducted by Lei et al. (2022) with two different broiler breeds (Arbor Acres and Beijing-You) having different microbiota showed a negative correlation between drip loss and the abundance of Lachnochlostridium. They remodeled the Arbor Acres' microbiome by applying a bacterial suspension derived from the Beijing-You breed and decreased drip loss in their meat. He et al. (2022) changed the microbiome by adding yeast ß-glucan to the diet of finisher pigs. They achieved a reduced cooking loss (linear, $p<0.05$) and a lower drip loss ($p<0.05$), together indicating a better water-holding capacity, as well as a decreased lactate content. The addition of a multi-species probiotic to the diet of finishing pigs tended to result in lower cooking and drip loss($p<0.1$) besides modulating the intestinal flora (higher lactobacilli and lower E. coli counts in the feces) (Balasubramanian et al., 2017) and the inclusion of Lactobacillus brevis and tea polyphenol individually or in a synergistic combination improved water holding capacity and decreased drip loss Zou et al. (2017).

Puvača et al. (2019) observed the lowest drip-loss values in breast meat and thigh with drumstick through feeding chickens 0.5 g or 1.0 g of hot red pepper per 100 g of feed, respectively, in the grower and finisher phase. The feeding of resveratrol reduced drip loss of Peking ducks' leg muscles. SCFA infused into the ileum enlarged the longissimus dorsi area and alleviated drip loss (Jiao et al, 2021).

The decrease and increase of the pH after slaughtering determines meat quality

The pH in the muscles of a living animal is about 7.2. With slaughtering and bleeding, the energy supply of the muscles is interrupted. The stored glycogen gets degraded to lactic acid, lowering the pH. Usually, the lowest pH value of 5.4-5.7 in meat is reached after 18 to 24 hours. Afterward, it starts to rise again.

In stressed animals, the stress hormones adrenalin and noradrenalin provoke a rushly occurring and, due to a lack of oxygen, anaerobic metabolism and the quick production of lactic acid. This too rapid decrease in pH leads to the denaturation of proteins in the muscle cells and reduced water-holding capacity. The result is PSE (pale, soft, and exudative) meat.

On the contrary, DFD meat (dark, firm, and dry) occurs if the glycogen reserves, due to challenges, are already used up, and the lactic acid production is insufficient. Especially PSE meat is closely related to breeds – some are more susceptible to stress, others less. However, some trials show that influencing pH in meat is possible to a certain extent.

He et al., 2022 added yeast ß-glucan to the diets of finishing pigs and a higher $pH_{45\ min}$ (linear and quadratic, $p<0.01$) and a higher redness (a*; linear, $p<0.05$) of the meat. Wu et al. (2020) achieved a significantly increased pH_{24h} through the addition of Glucose oxidase produced by Aspergillus niger.

Sensory characteristics are very subjective

In general, the sensory characteristics of meat are seen very individually. Some prefer lean, others fatty meat, some like meat with a characteristic taste, and others with a neutral. However, the typical meat taste of umami is partly determined by the nucleotide inosine monophosphate (IMP), which is regarded as an essential index for evaluating meat flavor and the acceptability of meat products. IMP provides about 40-fold higher umami taste than sodium glutamate (Huang et al. 2022).IMP is the organophosphate of inosin. Inosine, however, according to Kroemer and Zitvogel (2020), is produced by Bifidobacterium pseudolongum, which possibly can be controlled by feeding. Sun et al. (2018) compared Caoke and Partridge Shank chickens and divided them into free-range and cage groups. They found out that, except for acids, the amounts of flavor components were higher in the free-range than in the cage groups. The two housing systems also modified the microbiota, and Sun et al. took it as an indication that meat flavor, as well as the composition and diversity of gut microbiota, are closely associated with the housing systems. Fu et al. (2023) examined the addition of a mixture containing Pulsatilla, Gentian, and Rhizoma coptidis and a mixture with Codonopsis pilosula, Atractylodes, Poria cocos, and Licorice to the feed of Hungarian white geese. They saw that in both groups, the total amino acid levels, especially Glu, Lys, and Asp, increased, with, according to Liu et al. (2018), Glu and Asp directly affecting meat's freshness and flavor. Yu et al. (2021) achieved similar results by adding resveratrol to the diet of Peking ducks. The addition of the herbs additionally led to a higher Firmicutes/Bacteroidetes ratio and an increased level of lactobacilli (Fu et al., 2023).

How can EW Nutrition's feed additives help to improve meat quality?

Meat quality is influenced by the microbiome. So, feed additives that stabilize the microbiome or promote certain beneficial bacterial strains are an opportunity.

Ventar D modulates the microbiome

Ventar D balances the microbiome by promoting beneficial bacteria such as lactobacilli and fighting harmful ones such as Clostridia, E. coli, and Salmonella. (Heinzl, 2022). In another trial with broilers, the addition of Ventar D to all feeds (100 g/t) showed an increase in short-chain fatty acids in the intestine:

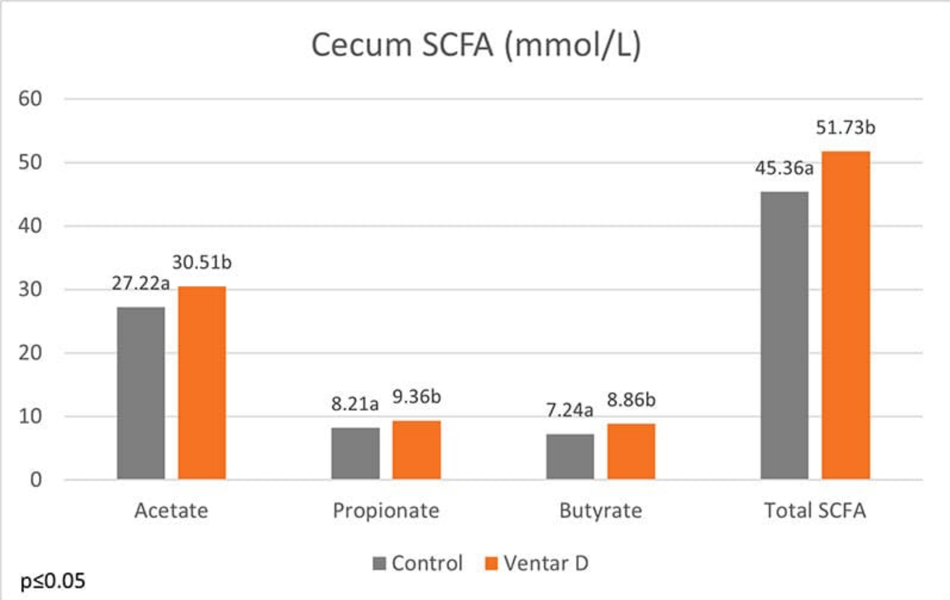

Figure 1: Short-chain fatty acids in the cecum of broilers

Santoquin countersteers oxidation

Another helpful product category is antioxidants. They can prevent the oxidation of lipids and proteins. For this purpose, EW Nutrition offers Santoquin M6*, a product tested by Kuttapan et al. (2021). Santoquin M6 was tested concerning its ability to minimize the oxidative damage caused by feeding oxidized fat. A control group receiving oxidized fat in feed was compared to one receiving oxidized fat plus 188 ppm Santoquin M6 (≙125 ppm ethoxyquin). The main parameters for this study were TBARS in the breast muscle, the incidence of wooden breast, and the live weight on day 48.

Results indicated that the inclusion of Santoquin M6 reduced the production of TBARS in the breast muscles, demonstrating a lower level of oxidative stress in the breast muscles.

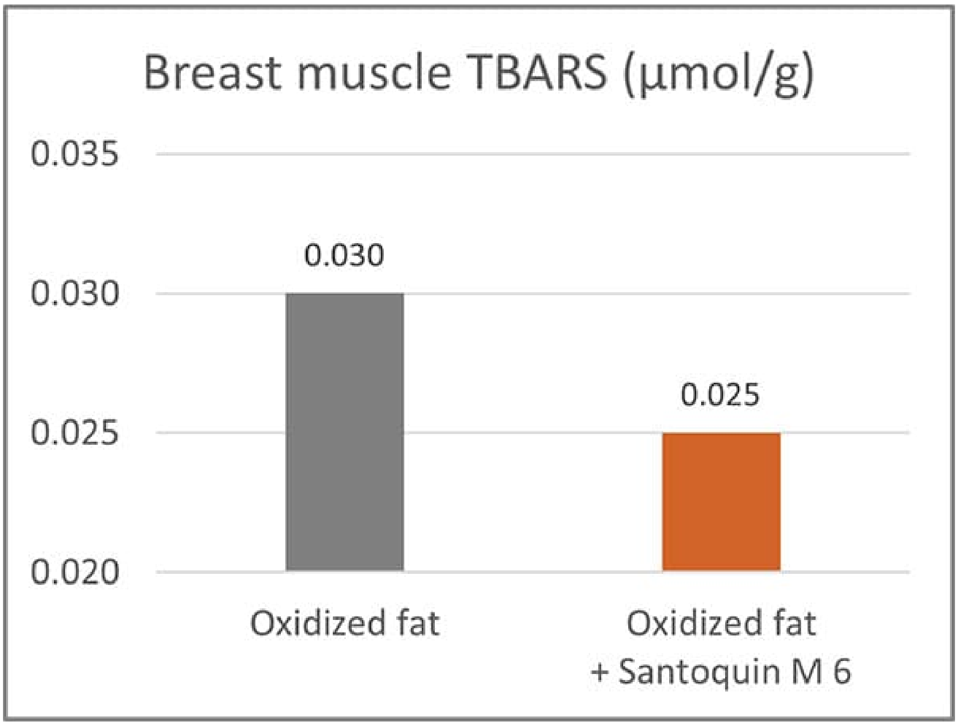

Figure 2: Thiobarbituric acid reactive substances (TBARS) in broiler breast muscles. TBARS are formed as a by-product of lipid peroxidation.

Additionally, it reduced the incidence of severe woody breasts (Score 3) by almost half and helped mitigate the impact of breast muscle degradation due to increased oxidative stress.

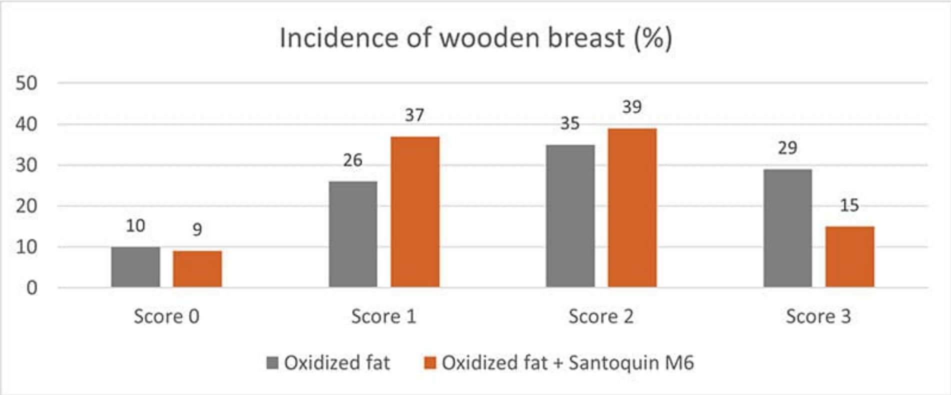

Figure 3: Incidence of wooden breast in broilers

*Usage of ethoxyquin is dependent on country regulations.

Feed hygiene with Acidomix products minimizes harmful pathogens

The Acidomix product line offers liquid, powdery, and micro-granulated products to be added to feed and water. The organic acids in Acidomix directly act against pathogens in the feed and the water and help keep the intestinal flora in balance.

A trial evaluating the effect of different Acidomix products against diverse pathogens showed lower MICs for most Acidomix products than for single organic acids. The trial was conducted with decreasing concentrations of the Acidomix products (2 – 0.015625 %) and 10^5 CFU of the respective microorganisms (microtiter plates; 50 µl bacterial solution and 50 µl diluted product).

Feeding is the one side, slaughtering the other one

With feeding, the microbiota and some meat characteristics can be changed; however, the last step, handling the animals before and the meat after slaughtering also significantly contributes to a good quality of meat. Stress due to the transport and the slaughterhouse atmosphere, combined with stress-sensible breeds, can lead to PSE meat. Incorrect handling at the slaughterhouse can lead to meat contaminated with pathogens.

Combining feeding measures with professional and calm handling of the animals is the best strategy to achieve high-quality meat.

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DDGS and oxidative damage

Distiller’s dried grains are produced by condensing and drying the stillage left over after starch-fermentation of corn for ethanol production. Solubles left over from the process are usually added before drying, resulting in the DDGS product that has become more and more commonplace in poultry feed formulations.
Historically, this ingredient was used in ruminant diets, as the nutrient content and quality of DDGS is considered to be somewhat variable – not only between, but also within production facilities. However, recent improvements in processing technologies and quality control systems have resulted in more consistent, higher-quality feed products.
Furthermore, newer fractionation processes continue to be implemented by some ethanol plants that are capable of fractioning out protein, fiber and oil portions of the grain, either pre- or post-fermentation, providing a wide variety of co-products which will result from the blending of these fraction streams.

Why is there more danger of oxidative damage today?

A decade ago, typical poultry diets consisted of far fewer feed ingredients than some of today’s rations. Additionally, the use of further-processed by-product meals and fats has also increased due to economic constraints associated with some raw ingredients.
This has created a diet which is highly prone to oxidative degeneration due to the higher rate of thermal, and in some cases, chemical processing of feed ingredients such as DDGS.

Currently, it is not uncommon to see DDGS inclusion rates between 5 and 12% in broiler and turkey diets, depending on bird age and feed prices.
One factor to consider when utilizing DDGS at such levels is the increase in polyunsaturated fatty acid content relative to saturated fatty acids that results from the addition of the 18:2, n-6 -rich corn oil which comprises approximately 10% of most DDGS.
Despite the fact that most modern rations have decreased the total lipid content in recent years, overall increases in the use of vegetable-based by-products invariably change the fatty acid profile of the diet to one which contains higher levels of polyunsaturated fats relative to saturated fats.
Polyunsaturated fatty acids are highly sensitive to oxidation during storage and are likely to turn rancid at high environmental temperatures. The oxidation process involves the generation of fatty acid free radicals, which then react with molecular oxygen to produce peroxide free radicals. This results in a chemical change to the fatty acid which decreases nutrient value and often produces undesirable odor.

…And that’s why risk management of oxidative damage is essential

Oxidative damage in feeds entails economic losses because of the negative impact on feed quality through the transformation of the lipid fraction of feed ingredients, decreased animal growth rate and performance, and decreased meat quality parameters, such as nutritional value and shelf-life. Therefore, lipid stability in feed is important, particularly with regard to the oxidative rancidity that occurs in high-fat ingredients, and prevention and management of oxidative stress is critical.

No revision of the Feed Additives law, says the European Commission

The authorization and marketing of feed additives in the European Union is currently governed by Feed Additives Regulation (EC) No 1831/2003, which came into effect in 2004. In 2021, the European Commission formalized an initiative to revise it, stating as reasons both the focus brought by the Farm to Fork Strategy, as well as inherent complexities in phrasing, process, and more. Representatives of the EC’s responsible unit, DG SANTE Unit G5, have now confirmed to EW Nutrition that, following consultations and analysis, **the revision of the legislation on the authorisation of feed additives will not happen under the current Commission’s mandate**.

The revision was initially deemed necessary on several grounds:

- Not enough focus on sustainable animal farming
- Lack of flexibility in promoting technical and scientific innovation
- A lengthy authorization process
- Unnecessary administrative burden
- Ineffective imports control leading to unfair competition between EU and non-EU operators
- Dependency on imports from third countries for some additives (e.g., vitamins)
- Restrictions on the circulation of feed additives only intended for export
- Insufficient legal clarity and consistency for a few aspects of the Regulation, e.g. use of certain additives in drinking water or labelling provisions for worker safety provisions in various complementary but unclear Regulations
- Extensive, unnecessary labeling regulations that create physical and administrative burdens

Near the end of the two-year assessment process, however, the response of European governmental, supra-national, and non-governmental bodies appears to have been lukewarm. Overall, the conclusion of the EC unit overseeing the process was that "**while a review of the framework would be useful, it does not appear necessary, considering the possibilities already granted by the existing legal framework**." In other words, applicants will have to use the existing mechanisms for applications, with no prospect for change in the near future.

Other strategies and regulations have also fallen through the cracks. For instance, the EU Animal Health Strategy 2007-2013 has not been updated in 10 years and there are no plans to renew the initiative. This is likely because the Green Deal and the flurry of new or upcoming regulations related to it are expected to supplant the framework for protein production in the European Union.

As the mandate of the current EC ends in 2024, there is a slim chance that the feed additive authorization process might be made less cumbersome once a new commission takes over.

From basketball to feed milling: a common tactic for winning in 2023

*By **Ivan Ilic**, Global Manager Technical Product Applications, EW Nutrition*

It has been a rough couple of years for the world. And from climate change to war, all negative impacts have reverberated down to feed millers.

- Climate change affected raw material prices and availability
- COVID-19 impacted shipping costs and manpower
- War impacted energy prices and raw material availability

And that´s without even considering market trends toward sustainability, shifting resources to biofuel, and so on.

With all these challenges going on, working to improve feed mill efficiency has lately kept me extremely busy. I´ve been traveling and talking to customers around the world about SurfAce and how we bring benefits in energy cost savings, process efficiency, moisture optimization, and so on. But when I am at home, I take a walk every evening in the woods near my house. I often use the time to reflect on personal and professional issues.

At some point, I found myself thinking about the European Basketball Championship (in Serbia, basketball is a national sport). Last year, the head coach of the Serbian national team decided not to call one of our best players to the national team. Lots of people criticized this decision, as for the past few years he had been one of the top players in Europe.

So, I started to think about choosing a team over a star. How do you balance your strong points to make sure of a win? (Yes, there is a connection to feed mills. I´m getting there.)

Winning through strategy rather than showmanship

Bozidar Maljkovic is a Serbian legend, who trained several winning teams, among which the European champion team Limoges. This was a French team he picked up mid-season, with moderate resources on the basketball court as well as outside it. The entire 1993 Euro season, Maljkovic chose to play extreme defense and score a very low number of points. In the finals, he played against a big favorite: Benneton Treviso, a wealthier team that, at that time, had a roster of excellent players. He won the game using the same strategy: tight defense, highly tactical game. A championship won not on artistic merit but on strategy.

After that final game, his good friend and well-known coach of Treviso, Petar Skansi, accused Maljkovic that he was destroying the basketball game with that tactic. Maljkovic answered to Skansi in more or less these words: you give me Kukoc (Treviso´s best player) and I´ll win on a different tactic.

When I remembered this episode during my walk, I suddenly saw a pattern in basketball coaching and feedmill management.

Know your objective

As in basketball, in feed milling you must be clear about your target, your main objective. In Maljkovic´s case, the objective was not to make basketball games attractive for the public, just as it was not to his objective to showcase his players. His target was to win the Euro title.

The same goes for the feed mill. Sure, you have several objectives, but there must be a main one. Say your primary objective is to maximize profit. If that is the case, then the next step is to be sure of what the market demands. This way you can avoid spending money for added value on something that the market is unwilling to pay for.

Know your players

Once you know what outcome you can deliver and what the market is prepared to pay for, the next step is analytics.

You must dive deep into your feed mill and get all the data on your “players”: raw materials, technology, people, machines, parameters, logistics etc. You must understand the current status and capabilities of your players, with advantages and limitations. Your job is to use them to the best of their capabilities in order to achieve your objective.

Know the interconnections between players

Just as every player depends on others, also feed mill processes are related and interdependent. If you want to have fine grinding, you will achieve better PDI, but it will cost more energy in milling and the result may not be as good for some categories of animals. Is this efficient and acceptable? It all depends on your main objective.

Balancing between pros and cons and walking that thin line is what efficiency means. With these challenges looming large, finding that balance will be the main task in feed milling.

Be curious

“Be curious” is one of the values of our company, but I would prompt anyone to adopt it. Play with parameters, support operators to do it, and find the point that yields maximum return for your specific objective.

Literature without your own data is fiction. In literature you can find data that says, for instance, that for every 15°C you have 1% more moisture. You can also find literature that says you have 1% more moisture for every 12°C or every 17°C. But what is the ratio in your feedmill? If you do not know, you are still not diving deep enough.

You need to figure out the interconnected factors in your own production. If you calculate by the books and official recommendations, you are adjusting work in some other feed mill, not yours. Yes: guidance is very important to understand relations and to be aware of margins. But inside those margins, you have to find your own numbers.

Find the least opportunity cost

Very often I see goals that are rebels without a cause. Take PDI, for instance. PDI is an important value, no doubt. It has been shown that better PDI correlates with better FCR etc.

However, when you set a target value for PDI you need to be sure that future investment in increasing PDI is relevant to your customers – and that they are willing to pay for that. Even if you are an integrator, first do the math on the benefits and the cost. With rising costs not just for you but also for your end customers, make sure the market can support the premium you are struggling to deliver. If you are sure, then find the most adequate way to win it. You can increase your PDI in lots of different ways, so you will need to calculate the least opportunity cost.

Production is a game of interdependencies. So is any team sport, in fact. When a coach makes a decision to put a star player in the spotlight, there may be a show but not always a win.

In a feed mill, the end game is always played around winning. It is a complex tactic of balancing all players and getting the most in your very specific circumstances. Our job is to identify and maximize these „synergies” in each specific case – and I can confirm that each case is different. In the end, Kukoc may have played the same game in Jugoplastika or Treviso, but no two feed mills are quite the same; even in same feed mill, no two lines will be adjusted the same way.

Masked mycotoxins – particularly dangerous for dairy cows

*By **Si-Trung Tran**, SEAP Regional Technical Manager, EW Nutrition*

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Mycotoxins are secondary metabolites of fungi, commonly found as contaminants in agricultural products. In some cases, these compounds are used in medicine or industry, such as penicillin and patulin. In most cases, however, they are considered xenobiotics that are toxic to animals and humans, causing the disease collectively known as mycotoxicosis. The adverse effects of mycotoxins on human and animal health have been documented in many publications. Aflatoxins (AFs) and deoxynivalenol (DON, vomitoxin) are amongst the most critical mycotoxins affecting milk production and -quality.

Aflatoxins do not only affect cows

Aflatoxins (AFs) are highly oxygenated, heterocyclic difuranocoumarin compounds produced by *Aspergillus flavus* and *Aspergillus parasiticus*. They colonize crops, including many staple foods and feed ingredients. Within a group of over 20 AFs and derivatives, aflatoxin B1 (AFB1), B2, G1, and G2 are the most important naturally occurring compounds.

Among the aflatoxins, AFB1 is the most widespread and most toxic to humans and animals. Concern about mycotoxin contamination in dairy products began in the 1960s with the first reported cases of contamination by aflatoxin M1 (AFM1), a metabolite of AFB1 formed in the liver of animals and excreted in the milk.

There is ample evidence that lactating cows exhibit a significant reduction in feed efficiency and milk yield within a few days of consuming aflatoxin-contaminated feed. At the cellular level, aflatoxins cause degranulation of endoplasmic membranes, loss of ribosomes from the endoplasmic reticulum, loss of nuclear chromatin material, and altered nuclear shapes. The liver, as the organ mainly dealing with the decontamination of the organism, gets damaged, and performance drops. Immune cells are also affected, reducing immune competence and vaccination success (Arnold and Gaskill, 2023).

DON reduces cows’ performance

Another mycotoxin that can also reduce milk quality and affect metabolic parameters, as well as the immune function of dairy cows, is DON. DON is produced by different fungi of the *Fusarium* genus that infect plants. DON synthesis is associated with rainy weather from crop flowering to harvest. Whitlow and co-workers (1994) reported the association between DON and poor performance in dairy herds and showed decreased milk production in dairy cows fed 2.5 mg DON/kg. However, in cows fed 6 to 12 mg DON/kg dry matter for 10 weeks, no DON or its metabolite DOM-1 residues were detected in milk.

Masked mycotoxins hide themselves during analysis

Plants suffering from fungal infestations and thus confronted with mycotoxins convert the harmful forms of mycotoxins into less harmful or harmless ones for themselves by conjugation to sulfates, organic acids, or sugars. Conjugated mycotoxins cannot always be detected by standard analytical methods. However, in animals, these forms can be released and transformed into parent compounds by enzymes and microorganisms in the gastrointestinal tract. Thus, the feed may show a concentration of mycotoxins that is still below the limit value, but in the animal, this concentration is suddenly much higher. In dairy cows, the release of free mycotoxins from conjugates during digestion may play an important role in understanding the silent effects of mycotoxins.

Fusarium toxins, in particular, frequently occur in this “masked form”. They represent a serious health risk for animals and humans.

Aflatoxins first show up in the milk

Masked aflatoxins may also play a role in total aflatoxin contamination of feed materials. Research has harvested little information on masked aflatoxins that may be present in TMR ingredients. So far, metabolites such as Aflatoxin M2 have been identified (Righetti, 2021), which may reappear later in milk as AFM1.

DON-related symptoms without DON?

Sometimes, animals show DON-related symptoms, with low levels detected in the feed or raw materials. Besides sampling errors, this enigma could be due to conjugated or masked DON, which is structurally altered DON bound to various compounds such as glucose, fatty acids, and amino acids. These compounds escape conventional feed analysis techniques because of their modified chemical properties but can be released as their toxic precursors after acid hydrolysis.

Masked DON was first described in 1984 by Young and co-workers, who found that the DON content of yeast-fermented foods was higher than that of the contaminated wheat flour used in their production. The most plausible reason for this apparent increase was that the toxin from the wheat had been converted to a compound other than DON, which could be converted back to DON under certain conditions. Since this report, there has been much interest in conjugated or masked DON.

Silage: masked DON is a challenge for dairy producers

Silage is an essential feed for dairy cows, supporting milk production. Most silage is made from corn and other grains. The whole green plant is used, which can be infected by fungi. Since infection of corn with Fusarium spp. and subsequent DON contamination is usually a major problem in the field worldwide, a relatively high occurrence of this toxin in silage must be expected. The ensiling process may reduce the amount of Fusarium fungi, but the DON formed before ensiling is very stable.

Silage samples show DON levels of concern

It is reasonable to assume that the DON biosynthesized by the fungi was metabolized by the plants to a new compound and thus masked DON. Under ensiling conditions, masked DON can be hydrolyzed, producing free DON again. Therefore, the level of free DON in the silage may not reflect the concentration measured in the plants before ensiling.

A study analyzed 50 silage samples from different farms in Ontario, Canada. Free DON was found in all samples, with levels ranging from 0.38 to 1.72 µg/g silage (unpublished data). Eighty-six percent of the samples contained DON at concentrations higher than 0.5 µg/g. Together with masked DON, it poses a potential threat to dairy cattle.

Specific hydrolysis conditions allow detection

However, in the natural ensiling process, the conditions for hydrolysis of masked DON are not optimal. The conditions that allow improved analysis of masked DON were recently described. This method detected masked DON in 32 of 50 silage samples (64%) along with free DON, increasing DON concentration by 23% in some cases (unpublished data).

Mycotoxins impact humans and animals

Aflatoxins, as well as DON, have adverse effects. In the case of DON, the impact on the animal is significant; in the case of aflatoxin, the possible long-term effects on humans are of higher relevance.

DON has more adverse effects on the animal and its performance

Unlike AFs, DON may be found in milk at low or trace concentrations. It is more associated with negative effects in the animal, altered rumen fermentation, and reduced flow of usable protein into the duodenum. For example, milk fat content was significantly reduced when cows were fed 6 µg DON/kg. However, the presence of DON also indicates that the feed probably contains other mycotoxins, such as zearalenone (ZEA) (estrogenic mycotoxin) and fusaric acid (pharmacologically active compound). All these mycotoxins may interact to cause symptoms that are different or more severe than expected, considering their individual effects. DON and related compounds also have immunosuppressive effects, resulting in increased somatic cell counts in milk. The U.S. FDA has established an action level for DON in wheat and wheat-derived products intended for cows, which is 5µg DON/g feed and the contaminated ingredient must not exceed 40% of the ration.

Aflatoxins decrease milk quality and pose a risk to humans

Aflatoxins are poorly degraded in the rumen, with aflatoxicol being the main metabolite that can be reconverted to AFB1. Most AFs are absorbed and extensively metabolized/hydrolyzed by enzymes found mainly in the liver. This results in the formation of AFM1, a part of which is conjugated to glucuronic acid and subsequently excreted in the bile. The other part enters the systemic circulation. It is either excreted in urine or milk. AFM1 appears within 12-48 hours after ingestion in cow’s milk. The excreted amount of AFM1 in milk from dairy cows usually ranges from 0.17% to 3% of the ingested AFB1. However, this carryover rate may vary from day to day and from one milking to the next in individual animals, as it is influenced by various factors, such as feeding regime, health status, individual biotransformation capacity, and, of course, by actual milk production. Carryover rates of up to 6.2% have been reported in high-yielding dairy cows producing up to 40 liters of milk per day.

In various experiments, AFM1 showed both carcinogenic and immunosuppressive effects. Accordingly, the International Agency for Research on Cancer (IARC) classified AFM1 as being in Group 2B and, thus, possibly carcinogenic in humans. The action level of 0.50 ppb and 0.05 ppb for AFM1 in milk is strictly adhered to by the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), respectively.

Trials show the high adsorption capacity of Solis Max

A trial was conducted at an independent laboratory located in Spain. The evaluation of the performance of Solis Max was executed with the following inclusion levels:

- 0.10% equivalent to 1.0 kg of Solis Max per ton of feed
- 0.20% equivalent to 2.0 kg of Solis Max per ton of feed

A phosphate buffer solution at pH 7 was prepared for the trial to simulate rumen conditions. Each mycotoxin was tested separately, preparing solutions with known contamination (final concentration described in the table below). The contaminated solutions were divided into 3 parts: A positive control, 0.10% Solis Max and 0.20% Solis Max. All samples were incubated at 41°C for 1 hour, centrifuged, and the supernatant was analyzed for the mycotoxin added to determine the binding efficacy. All analyses were carried out by high-performance liquid chromatography (HPLC) with standard detectors.

Mycotoxin	Contamination Level (ppb)
Aflatoxin B1	800

DON	800
Fumonisin B1	2000
ZEA	1200

Results:
The higher concentration of Solis max showed a higher adsorption rate for most mycotoxins. The high dose of Solis Max adsorbed 99% of the AFB1 contamination. In the case of DON, more than 70% was bound. For fumonisin B1 and zearalenone, Solis max showed excellent binding rates of 87.7% and 78.9%, respectively (Figure 1).

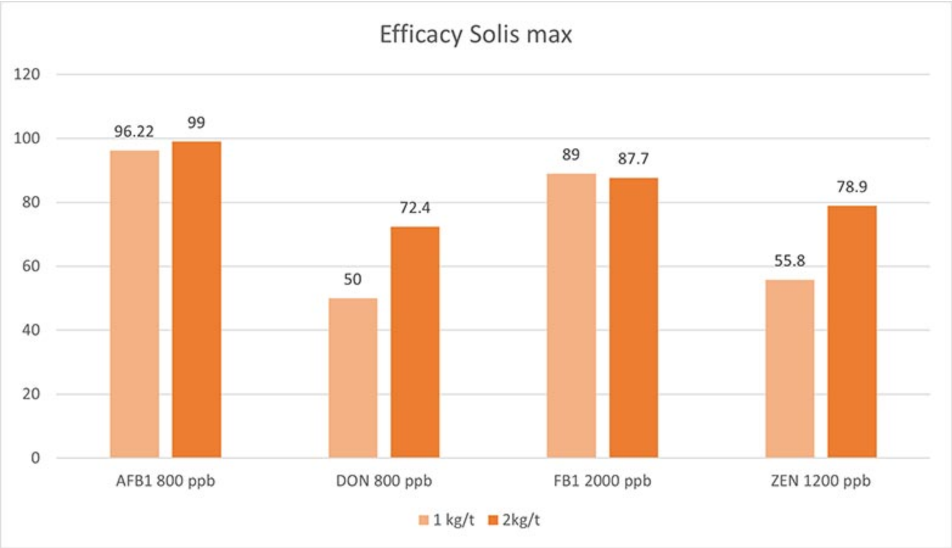

Figure 1: Solis Max showed a high binding capacity for the most relevant mycotoxins

Another trial was conducted at an independent laboratory serving the food and feed industry and located in Valladolid, Spain.

All tests were carried out as duplicates and using a standard liquid chromatography/mass spectrometry (LC/MS/MS) quantification. Interpretation and data analysis were carried out with the corresponding software. The used pH was 3.0, toxin concentrations and anti-mycotoxin agent application rates were set as follows (Table 1):

Mycotoxin	Challenge level	Challenge (ppb)	Solis Plus 2.0 inclusion	Assay time
Aflatoxin	Low	150	0.2%	30 min.
	High	1500	0.2%	30 min.
Fumonisin	Low	500	0.2%	30 min.
	High	5000	0.2%	30 min.
Ochratoxin	Low	150	0.2%	30 min.
	High	1500	0.2%	30 min.

Table 1: Trial set-up testing the binding capacity of Solis Plus 2.0 for several mycotoxins in different contamination levels

Results:

Under acidic conditions (pH3), Solis Plus 2.0 effectively adsorbs the three tested mycotoxins at low and high levels. 100% binding of aflatoxin was achieved at a level of 150ppb and 98% at 1500ppb.In the case of fumonisin, 87% adsorption could be reached at 500ppb and 86 for a challenge with 5000ppb. 43% ochratoxin was adsorbed at the contamination level of 150ppb and 52% at 1500ppb.

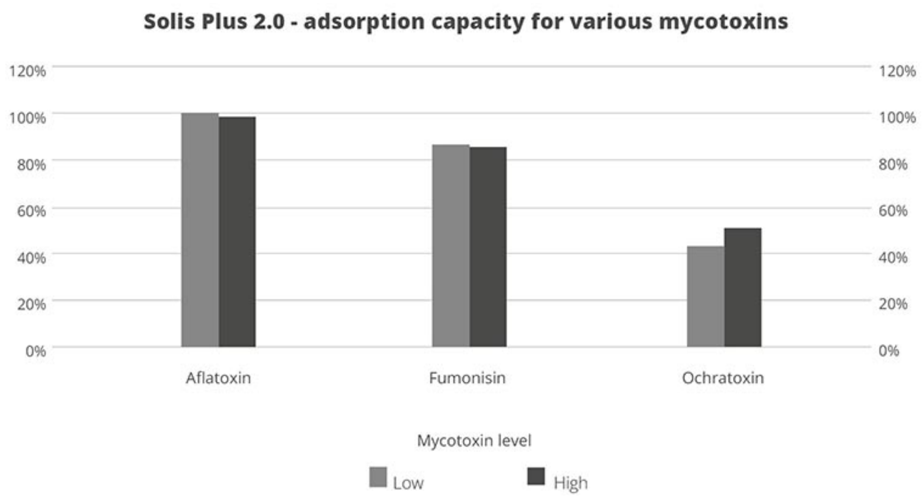

Figure 2: The adsorption capacity of Solis Plus 2.0 for three different mycotoxins at two challenge levels

Mycotoxins – Effective risk management is of paramount importance

Although the rumen microflora may be responsible for conferring some mycotoxin resistance to ruminants compared to monogastric animals, there are still effects of mycotoxins on rumen fermentation and milk quality. In addition, masked mycotoxins in feed present an additional challenge for dairy farms because they are not readily detectable by standard analyses.

Feeding dairy cows with feed contaminated with mycotoxins can lead to a reduction in milk production. Milk quality may also deteriorate due to an adverse change in milk composition and mycotoxin residues, threatening the innocuousness of dairy products. Dairy farmers should therefore have feed tested regularly, consider masked mycotoxins, and take action. EW Nutrition’s MasterRisk tool provides a risk evaluation and corresponding recommendations for the use of products that mitigate the effects of mycotoxin contamination and, in the end, guarantee the safety of all of us.