Organic acids can play a crucial role in zinc oxide replacement

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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.

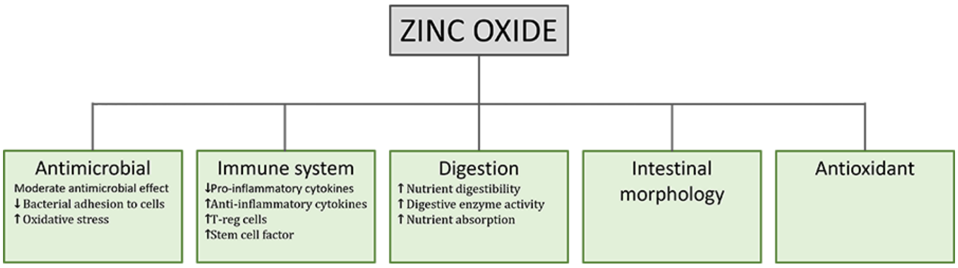

Figure 1. Beneficial effects and ZnO mode of action in postweaning piglets

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

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

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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:

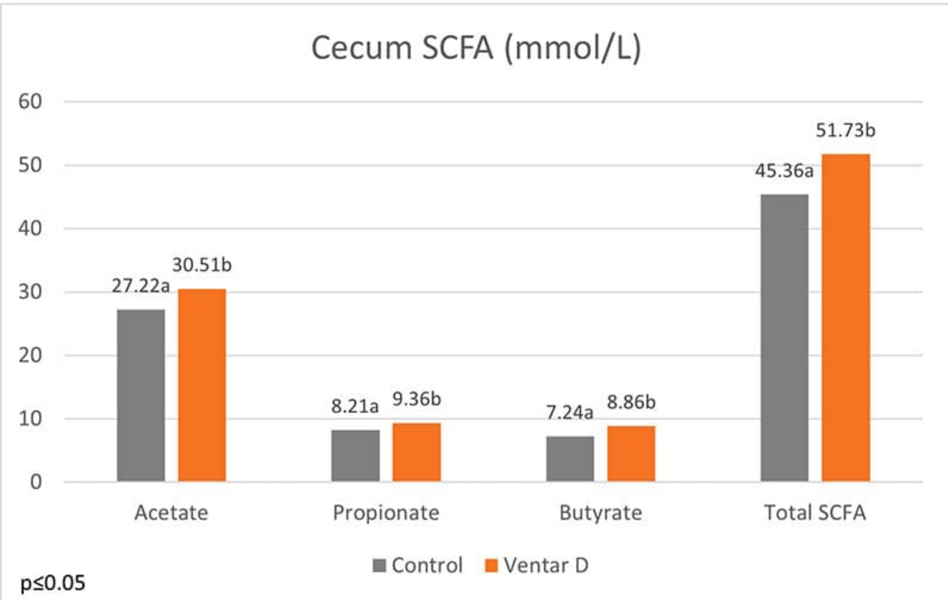


Figure 1: Short-chain fatty acids in the cecum of broilers

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.

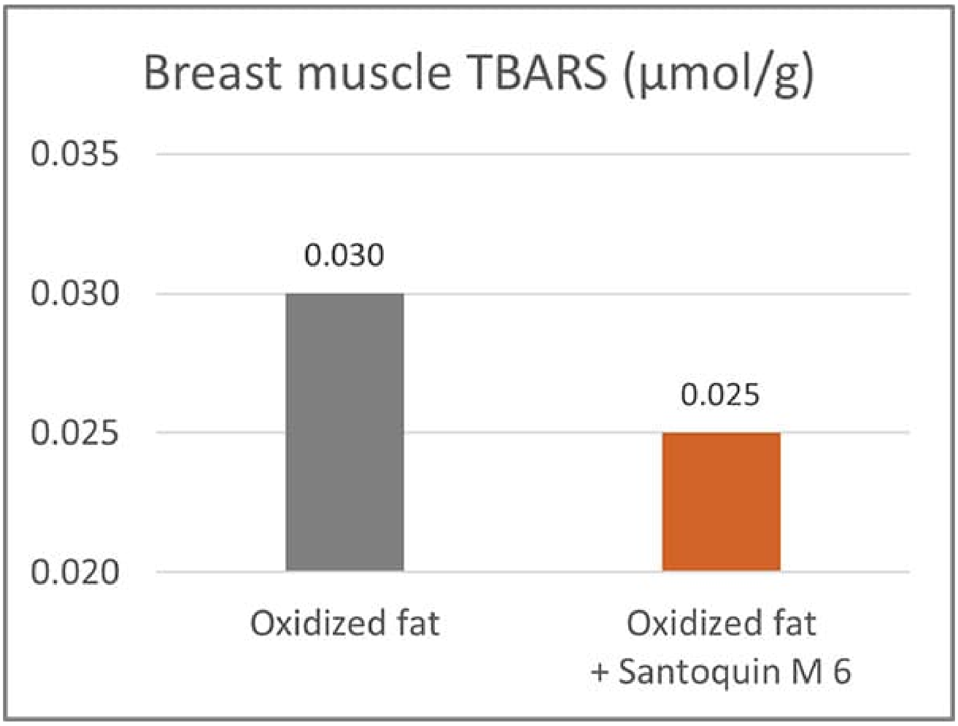


Figure 2: Thiobarbituric acid reactive substances (TBARS) in broiler breast muscles. TBARS are formed as a by-product of lipid peroxidation.

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.

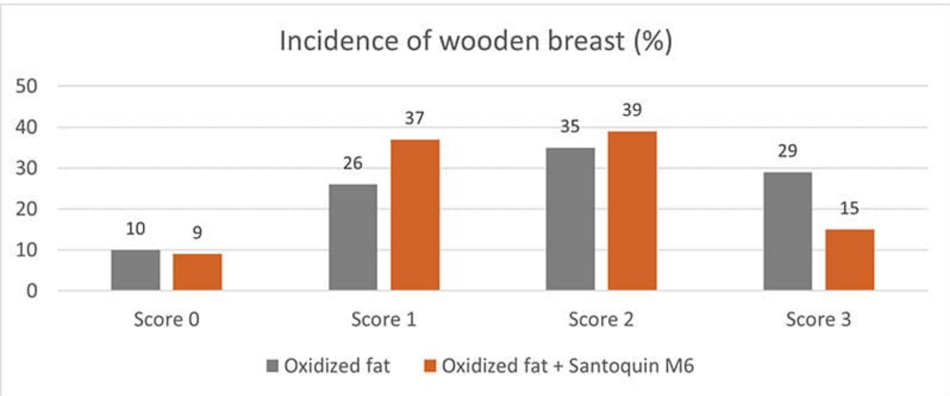


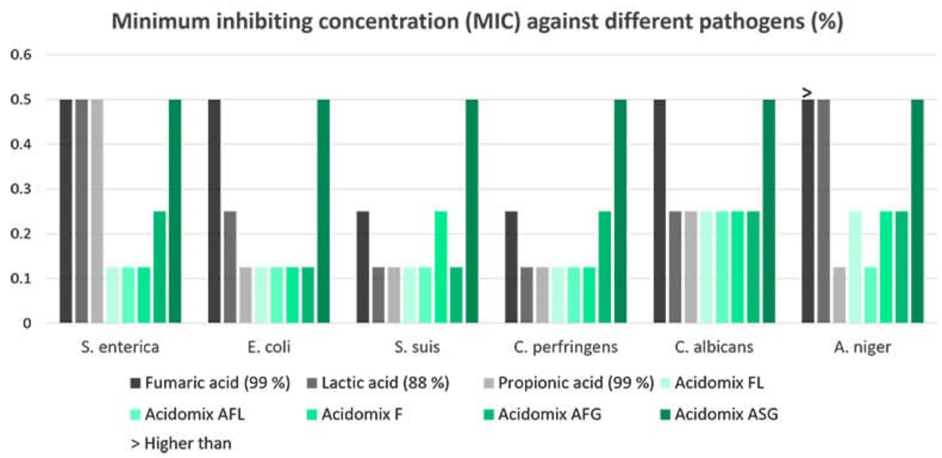
Figure 3: Incidence of wooden breast in broilers

*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.

References

Aaslyng, Margit D., and Lene Meinert. “Meat Flavour in Pork and Beef – from Animal to Meal.” *Meat Science* 132 (2017): 112–17. https://doi.org/10.1016/j.meatsci.2017.04.012.

Alfaig, Ebrahim, Maria Angelovičova, Martin Kral, and Ondrej Bučko. “EFF ECT of Probiotics and Thyme Essential Oil on the Essential Amino Acid Content of the Broiler Chicken Meat.” *Acta Scientiarum Polonorum Technologia Alimentaria* 13, no. 4 (2014): 425–32. https://doi.org/10.17306/j.afs.2014.4.9.

Allen, CD, SM Russell, and DL Fletcher. “The Relationship of Broiler Breast Meat Color and Ph to Shelf-Life and Odor Development.” *Poultry Science* 76, no. 7 (1997): 1042–46. https://doi.org/10.1093/ps/76.7.1042.

Balasubramanian, Balamuralikrishnan, Sang In Lee, and In-Ho Kim. “Inclusion of Dietary Multi-Species Probiotic on Growth Performance, Nutrient Digestibility, Meat Quality Traits, Faecal Microbiota, and Diarrhoea Score in Growing–Finishing Pigs.” *Italian Journal of Animal Science* 17, no. 1 (2017): 100–106. https://doi.org/10.1080/1828051x.2017.1340097.

Berri, Céile. “Variability of Sensory and Processing Qualities of Poultry Meat.” *World’s Poultry Science Journal* 56, no. 3 (2000): 209–24. https://doi.org/10.1079/wps20000016.

Cabling, M. M., H. S. Kang, B. M. Lopez, M. Jang, H. S. Kim, K. C. Nam, J. G. Choi, and K. S. Seo. “Estimation of Genetic Associations between Production and Meat Quality Traits in Duroc Pigs.” Asian-Australasian Journal of Animal Sciences 28, no. 8 (2015): 1061–65. https://doi.org/10.5713/ajas.14.0783.

Chen, Binlong, Diyan Li, Dong Leng, Hua Kui, Xue Bai, and Tao Wang. “Gut Microbiota and Meat Quality.” *Frontiers in Microbiology* 13 (2022). https://doi.org/10.3389/fmicb.2022.951726.

Chen, J.L., G.P. Zhao, M.Q. Zheng, J. Wen, and N. Yang. “Estimation of Genetic Parameters for Contents of Intramuscular Fat and Inosine-5′-Monophosphate and Carcass Traits in Chinese Beijing-You Chickens.” *Poultry Science* 87, no. 6 (2008): 1098–1104. https://doi.org/10.3382/ps.2007-00504.

Ding, Rongrong, Ming Yang, Jianping Quan, Shaoyun Li, Zhanwei Zhuang, Shenping Zhou, Enqin Zheng, et al. “Single-Locus and Multi-Locus Genome-Wide Association Studies for Intramuscular Fat in Duroc Pigs.” Frontiers in Genetics 10 (2019). https://doi.org/10.3389/fgene.2019.00619.

Dinh, Thu T., K. Virellia To, and M. Wes Schilling. “Fatty Acid Composition of Meat Animals as Flavor Precursors.” *Meat and Muscle Biology* 5, no. 1 (2021). https://doi.org/10.22175/mmb.12251.

Džinić, N., N. Puvača, T. Tasić, P. Ikonić, and Okanović. “How Meat Quality and Sensory Perception Is Influenced by Feeding Poultry Plant Extracts.” *World’s Poultry Science Journal* 71, no. 4 (2015): 673–82. https://doi.org/10.1017/s0043933915002378.

Džinić, N., N. Puvača, T. Tasić, P. Ikonić, and Okanović. “How Meat Quality and Sensory Perception Is Influenced by Feeding Poultry Plant Extracts.” *World’s Poultry Science Journal* 71, no. 4 (2015): 673–82. https://doi.org/10.1017/s0043933915002378.

Fu, Guilin, Yuxuan Zhou, Yupu Song, Chang Liu, Manjie Hu, Qiuyu Xie, Jingbo Wang, et al. “The Effect of Combined Dietary Supplementation of Herbal Additives on Carcass Traits, Meat Quality, Immunity and Cecal Microbiota Composition in Hungarian White Geese (v0.2)”.” *Peer J.; 11:e15316*, May 8, 2023. https://doi.org/10.7287/peerj.15316v0.2/reviews/3.

Fu, Guilin, Yuxuan Zhou, Yupu Song, Chang Liu, Manjie Hu, Qiuyu Xie, Jingbo Wang, et al. “The Effect of Combined Dietary Supplementation of Herbal Additives on Carcass Traits, Meat Quality, Immunity and Cecal Microbiota Composition in Hungarian White Geese.” *PeerJ* 11 (2023). https://doi.org/10.7717/peerj.15316.

Goodrich, Julia K., Jillian L. Waters, Angela C. Poole, Jessica L. Sutter, Omry Koren, Ran Blekhman, Michelle Beaumont, et al. “Human Genetics Shape the Gut Microbiome.” *Cell* 159, no. 4 (2014): 789–99. https://doi.org/10.1016/j.cell.2014.09.053.

Hazrati, S., V. Rezaeipour, and S. Asadzadeh. “Effects of Phytogenic Feed Additives, Probiotic and Mannan-Oligosaccharides on Performance, Blood Metabolites, Meat Quality, Intestinal Morphology, and Microbial Population of Japanese Quail.” *British Poultry Science* 61, no. 2 (2019): 132–39. https://doi.org/10.1080/00071668.2019.1686122.

He, Linjuan, Jianxin Guo, Yubo Wang, Lu Wang, Doudou Xu, Enfa Yan, Xin Zhang, and Jingdong Yin. “Effects of Dietary Yeast β-Glucan Supplementation on Meat Quality, Antioxidant Capacity and Gut Microbiota of Finishing Pigs.” *Antioxidants* 11, no. 7 (2022): 1340. https://doi.org/10.3390/antiox11071340.

Heinzl, Inge. “Efficient Microbiome Modulation with Phytomolecules.” EW Nutrition, July 6, 2022. https://staging-ewnutritioncom.kinsta.cloud/pushing-microbiome-in-right-direction-phytomolecules/.

Huang, Zengwen, Juan Zhang, Yaling Gu, Zhengyun Cai, Dawei Wei, Xiaofang Feng, and Chaoyun Yang. “Analysis of the Molecular Mechanism of Inosine Monophosphate Deposition in Jingyuan Chicken Muscles Using a Proteomic Approach.” *Poultry Science* 101, no. 4 (2022): 101741. https://doi.org/10.1016/j.psj.2022.101741.

Jiao, Anran, Hui Diao, Bing Yu, Jun He, Jie Yu, Ping Zheng, Yuheng Luo, et al. “Infusion of Short Chain Fatty Acids in the Ileum Improves the Carcass Traits, Meat Quality and Lipid Metabolism of Growing Pigs.” *Animal Nutrition* 7, no. 1 (2021): 94–100. https://doi.org/10.1016/j.aninu.2020.05.009.

Kallus, Samuel J., and Lawrence J. Brandt. “The Intestinal Microbiota and Obesity.” *Journal of Clinical Gastroenterology* 46, no. 1 (2012): 16–24. https://doi.org/10.1097/mcg.0b013e31823711fd.

Khan, Muhammad Issa, Cheorun Jo, and Muhammad Rizwan Tariq. “Meat Flavor Precursors and Factors Influencing Flavor Precursors—a Systematic Review.” *Meat Science* 110 (2015): 278–84. https://doi.org/10.1016/j.meatsci.2015.08.002.

Kroemer, Guido, and Laurence Zitvogel. “Inosine: Novel Microbiota-Derived Immunostimulatory Metabolite.” *Cell Research* 30, no. 11 (2020): 942–43. https://doi.org/10.1038/s41422-020-00417-1.

Kuttappan, Vivek A., Megharaja Manangi, Matthew Bekker, Juxing Chen, and Mercedes Vazquez-Anon. “Nutritional Intervention Strategies Using Dietary Antioxidants and Organic Trace Minerals to Reduce the Incidence of Wooden Breast and Other Carcass Quality Defects in Broiler Birds.” Frontiers in Physiology 12 (2021). https://doi.org/10.3389/fphys.2021.663409

Lei, Jiaqi, Yuanyang Dong, Qihang Hou, Yang He, Yujiao Lai, Chaoyong Liao, Yoichiro Kawamura, Junyou Li, and

Bingkun Zhang. “Intestinal Microbiota Regulate Certain Meat Quality Parameters in Chicken.” *Frontiers in Nutrition* 9 (2022). https://doi.org/10.3389/fnut.2022.747705.

Liu, R., M. Zheng, J. Wang, H. Cui, Q. Li, J. Liu, G. Zhao, and J. Wen. “Effects of Genomic Selection for Intramuscular Fat Content in Breast Muscle in Chinese Local Chickens.” *Animal Genetics* 50, no. 1 (2018): 87–91. https://doi.org/10.1111/age.12744.

Lo, L. L., D. G. McLaren, F. K. McKeith, R. L. Fernando, and J. Novakofski. “Genetic Analyses of Growth, Real-Time Ultrasound, Carcass, and Pork Quality Traits in Duroc and Landrace Pigs: II. Heritabilities and Correlations.” Journal of Animal Science 70, no. 8 (1992): 2387–96. https://doi.org/10.2527/1992.7082387x.

Luna, A., M.C. Lábaque, J.A. Zygadlo, and R.H. Marin. “Effects of Thymol and Carvacrol Feed Supplementation on Lipid Oxidation in Broiler Meat.” *Poultry Science* 89, no. 2 (2010): 366–70. https://doi.org/10.3382/ps.2009-00130.

Ma, Cui, Md. Abul Azad, Wu Tang, Qian Zhu, Wei Wang, Qiankun Gao, and Xiangfeng Kong. “Maternal Probiotics Supplementation Improves Immune and Antioxidant Function in Suckling Piglets via Modifying Gut Microbiota.” *Journal of Applied Microbiology* 133, no. 2 (2022): 515–28. https://doi.org/10.1111/jam.15572.

Ma, Jianfeng, Jingyun Chen, Mailin Gan, Lei Chen, Ye Zhao, Yan Zhu, Lili Niu, Shunhua Zhang, Li Zhu, and Linyuan Shen. “Gut Microbiota Composition and Diversity in Different Commercial Swine Breeds in Early and Finishing Growth Stages.” *Animals* 12, no. 13 (2022): 1607. https://doi.org/10.3390/ani12131607.

MacLeod, G. “The Flavour of Beef.” Essay. In *Shahidi, F. (Eds) Flavor of Meat and Meat Products*, 4–37. Boston, MA: Springer, 1994.

Mottram, Donald. “Flavour Formation in Meat and Meat Products: A Review.” *Food Chemistry* 62, no. 4 (1998): 415–24. https://doi.org/10.1016/s0308-8146(98)00076-4.

Okruszek, A. “Fatty Acid Composition of Muscle and Adipose Tissue of Indigenous Polish Geese Breeds.” *Archives Animal Breeding* 55, no. 3 (2012): 294–302. https://doi.org/10.5194/aab-55-294-2012.

Pereira, Ana Lúcia F., and Virgínia Kelly G. Abreu. “Lipid Peroxidation in Meat and Meat Products.” Essay. In *Lipid Peroxidation Research*. London: IntechOpen, 2020.

Puvača, Nikola, Tatjana Peulić, Predrag Ikonić, Sanja Popović, Jasmina Lazarević, Olivera Đuragić, Magdalena Cara, and Nedeljka Nikolova. “Effects of Medicinal Plants in Broiler Chicken Nutrition on Selected Parameters of Meat Quality.” *Macedonian Journal of Animal Science* 9, no. 2 (2019): 45–51. https://doi.org/10.54865/mjas1992045p.

Richards, J. D., J. Gong, and C. F. de Lange. “The Gastrointestinal Microbiota and Its Role in Monogastric Nutrition and Health with an Emphasis on Pigs: Current Understanding, Possible Modulations, and New Technologies for Ecological Studies.” Canadian Journal of Animal Science 85, no. 4 (2005): 421–35. https://doi.org/10.4141/a05-049.

Serra, Valentina, Giancarlo Salvatori, and Grazia Pastorelli. “Dietary Polyphenol Supplementation in Food Producing Animals: Effects on the Quality of Derived Products.” *Animals* 11, no. 2 (2021): 401. https://doi.org/10.3390/ani11020401.

Stewart, S.M., G.E. Gardner, P. McGilchrist, D.W. Pethick, R. Polkinghorne, J.M. Thompson, and G. Tarr. “Prediction of Consumer Palatability in Beef Using Visual Marbling Scores and Chemical Intramuscular Fat Percentage.” *Meat Science* 181 (2021): 108322. https://doi.org/10.1016/j.meatsci.2020.108322.

Sun, Jing, Yan Wang, Nianzhen Li, Hang Zhong, Hengyong Xu, Qing Zhu, and Yiping Liu. “Comparative Analysis of the Gut Microbial Composition and Meat Flavor of Two Chicken Breeds in Different Rearing Patterns.” *BioMed Research International* 2018 (2018): 1–13. https://doi.org/10.1155/2018/4343196.

Thorslund, Cecilie A.H., Peter Sandøe, Margit Dall Aaslyng, and Jesper Lassen. “A Good Taste in the Meat, a Good Taste in the Mouth – Animal Welfare as an Aspect of Pork Quality in Three European Countries.” *Livestock Science* 193 (2016): 58–65. https://doi.org/10.1016/j.livsci.2016.09.007.

Vasquez, Robie, Ju Kyoung Oh, Ji Hoon Song, and Dae-Kyung Kang. “Gut Microbiome-Produced Metabolites in Pigs: A Review on Their Biological Functions and the Influence of Probiotics.” *Journal of Animal Science and Technology* 64, no. 4 (2022): 671–95. https://doi.org/10.5187/jast.2022.e58.

Wang, Cheng, Siyu Wei, Bojing Liu, Fengqin Wang, Zeqing Lu, Mingliang Jin, and Yizhen Wang. “Maternal Consumption of a Fermented Diet Protects Offspring against Intestinal Inflammation by Regulating the Gut Microbiota.” *Gut Microbes* 14, no. 1 (2022). https://doi.org/10.1080/19490976.2022.2057779.

Wen, Chaoliang, Qinli Gou, Shuang Gu, Qiang Huang, Congjiao Sun, Jiangxia Zheng, and Ning Yang. “The Cecal Ecosystem Is a Great Contributor to Intramuscular Fat Deposition in Broilers.” *Poultry Science* 102, no. 4 (2023): 102568. https://doi.org/10.1016/j.psj.2023.102568.

Werkhoff, Peter, Juergen Bruening, Roland Emberger, Matthias Guentert, Manfred Koepsel, Walter Kuhn, and Horst Surburg. “Isolation and Characterization of Volatile Sulfur-Containing Meat Flavor Components in Model Systems.” *Journal of Agricultural and Food Chemistry* 38, no. 3 (1990): 777–91. https://doi.org/10.1021/jf00093a041.

Wu, Choufei, Wentao Lyu, Qihua Hong, Xiaojun Zhang, Hua Yang, and Yingping Xiao. “Gut Microbiota Influence Lipid Metabolism of Skeletal Muscle in Pigs.” *Frontiers in Nutrition* 8 (2021). https://doi.org/10.3389/fnut.2021.675445.

Xiao, Yingping, Kaifeng Li, Yun Xiang, Weidong Zhou, Guohong Gui, and Hua Yang. “The Fecal Microbiota Composition of Boar Duroc, Yorkshire, Landrace and Hampshire Pigs.” *Asian-Australasian Journal of Animal Sciences* 30, no. 10 (2017): 1456–63. https://doi.org/10.5713/ajas.16.0746.

Yu, Qifang, Chengkun Fang, Yujing Ma, Shaoping He, Kolapo Matthew Ajuwon, and Jianhua He. “Dietary Resveratrol Supplement Improves Carcass Traits and Meat Quality of Pekin Ducks.” *Poultry Science* 100, no. 3 (2021): 100802. https://doi.org/10.1016/j.psj.2020.10.056.

Zhao, Guangmin, Yun Xiang, Xiaoli Wang, Bing Dai, Xiaojun Zhang, Lingyan Ma, Hua Yang, and Wentao Lyu. “Exploring the Possible Link between the Gut Microbiome and Fat Deposition in Pigs.” *Oxidative Medicine and Cellular Longevity* 2022 (2022): 1–13. https://doi.org/10.1155/2022/1098892.

Zhou, Xueyan, Xiaosong Jiang, Chaowu Yang, Bingcun Ma, Changwei Lei, Changwen Xu, Anyun Zhang, et al. “Cecal Microbiota of Tibetan Chickens from Five Geographic Regions Were Determined by 16s Rrna Sequencing.” *MicrobiologyOpen* 5, no. 5 (2016): 753–62. https://doi.org/10.1002/mbo3.367.

Zou, Xiaozhuo, Rong Xiao, Huali Li, Ting Liu, Yong Liao, Yuanliang Wang, Shusong Wu, and Zongjun Li. “Effect of a Novel Strain of *Lactobacillus Brevis* M8 and Tea Polyphenol Diets on Performance, Meat Quality and Intestinal Microbiota in Broilers.” *Italian Journal of Animal Science* 17, no. 2 (2017): 396–407. https://doi.org/10.1080/1828051x.2017.1365260.

The Zinc Oxide ban: What led to it, what are the alternatives?

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

In June 2017, the European Commission decided to ban the use of veterinary drugs containing high doses of zinc oxide (3000mg/kg) from 2022. The use of zinc oxide in pig production must then be limited to a maximum level of 150ppm. Companies have been on the lookout for effective alternative strategies to maintain high profitability.

Modern pig production is characterised by its high intensity. In many European countries, piglets are weaned after 3-4 weeks, before their physiological systems are fully developed (e.g. immune and enzyme system). Weaning and thus separation from the mother, as well as a new environment with new germs, means stress for the piglets. Besides, the highly digestible sow's milk, for which the piglets are wholly adapted, is replaced by solid starter feed.

This, associated with the above-mentioned stressors, can result in reduced feed intake during the first week after weaning and therefore in a delayed adaptation of the intestinal flora to the feed. Since the immune system of animals is not yet fully functional, pathogens such as enterotoxic *E. coli* can colonize the intestinal mucosa. This can possibly develop into a dangerous dysbiosis, leading to an increased incidence of diarrhea. Inadequate absorption results in suboptimal growth with worse feed conversion. The consequences are economic losses due to higher treatment costs, lower yields, and animal losses.

Diarrhea is one of the most common causes of economic losses in pig production. In the past, this was the reason antibiotics were prophylactically used as growth promoters. Antibiotics reduce antimicrobial pressure and have an anti-inflammatory effect. In addition to reducing the incidence of disease, they eliminate competitors for nutrients in the gut and thus improve feed conversion.

However, the use of antibiotics as growth promoters has been banned in the EU since 2006 due to increased antimicrobial resistance. As a result, zinc oxide (ZnO) appeared on the scene. A study carried out in Spain in 2012 (Moreno, 2012) showed that 57% of piglets received ZnO before weaning and 73% during the growth phase (27-75 days).

Zinc oxide: the disadvantages outweigh the advantages

What made the use of zinc oxide so attractive? Zinc oxide is inexpensive, available in many EU countries, and as a trace element it can be used in high doses through premixing. In some countries, however, a veterinary prescription is needed; in others, the use is already banned.

Zinc is a trace element involved in cell division and differentiation, and it influences the efficacy of enzymes. Since defence cells also need zinc, a supplementation that covers the demand for zinc strengthens the body’s defences. Through a positive effect on the structure of the gut mucosa membrane, zinc protects the body against the penetration of pathogenic germs.

If ZnO is used in pharmacological doses, it has a bactericidal effect against e.g. staphylococci (Ann et al., 2014) and various types of *E. coli* (Vahjen et al., 2016). Thus, prophylactic use prevents the incidence of diarrhea and the consequent decrease in performance. But the use of zinc oxide also has “side effects”.

Accumulation in the environment

Zinc belongs to the chemical group of heavy metals. For the use as a performance enhancer, it has to be administered in relatively high doses (2000–4000ppm). These high amounts are far above the physiological needs of the animals. With relatively low absorption rates (the bioavailability amounts to approximately 20% (European Commission, 2003)) and subsequent accumulation in manure, zinc can cause substantial contamination of the environment.

Encouraging the development of antibiotic resistance

In addition to the accumulation of zinc in the environment, another aspect also plays an important role: according to Vahjen et al. (2015), a dose of ≥2500mg/kg of food increases the presence of tetracycline and sulfonamide resistance genes in bacteria. In the case of *Staphylococcus aureus*, the development of resistance to zinc is combined with the development of resistance to methicillin (MRSA; Cavaco et al., 2011; Slifierz et al., 2015). A similar effect can be observed in the development of multiresistant *E. coli* (Bednorz et al., 2013; Ciesinski et al., 2018). The reason for this is that the genes that encode antibiotic resistance, i.e. the ones that are “responsible” for the resistance, are found in the same plasmid (a DNA molecule that is small and independent of the bacterial chromosome).

Consequence: no more zinc oxide in the production of piglets from 2022 onwards

The negative effects on the environment and the promotion of antibiotic resistance led to the European Commission’s decision in 2017 to completely ban zinc oxide as a therapeutic agent and as a growth promoter in piglets within five years.

There are effective alternatives to zinc

oxide

By the 2022 deadline, the EU pig industry must find a solution to replace ZnO. It must develop strategies that make future pig production efficient, even without substances such as antibiotics and zinc oxide. To this end, measures should be taken at different levels, such as farm management and biosecurity (e.g. effective hygiene management). The promotion of intestinal health for high animal performance is most important, however.

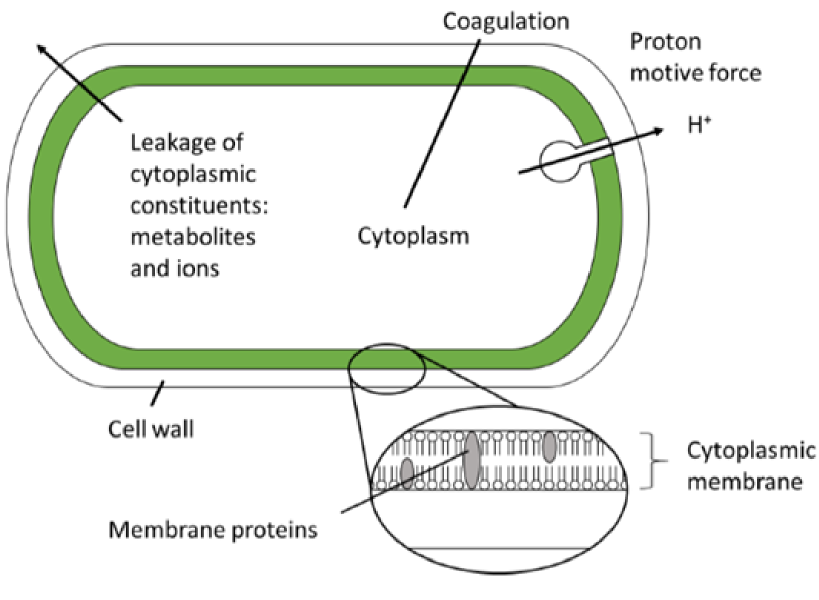
Promotion of gut health through stable gut microbiota

The term eubiosis denotes the balance of microorganisms living in a healthy intestine, which must be maintained to prevent diarrhea and ensure performance. However, weaning, food switching, and other external stressors can endanger this balance. As a result, potentially pathogenic germs can “overgrow” the commensal microbiome and develop dysbiosis. Through the use of functional supplements, intestinal health can be improved.

Phytomolecules – potent compounds created by nature

Phytomolecules, or secondary plant compounds, are substances formed by plants with a wide variety of properties. The best-known groups are probably essential oils, but there are also bitter substances, spicy substances, and other groups.

In animal nutrition, phytomolecules such as carvacrol, cinnamon aldehyde, and capsaicin can help improve intestinal health and digestion. They stabilize the intestinal flora by slowing or stopping the growth of pathogens that can cause disease. How? Phytomolecules, for example, make the cell walls of several bacteria permeable so that cell contents can leak. They also partially interfere with the enzymatic metabolism of the cell or intervene with the transport of ions, reducing the proton motive force. These effects depend on the dose: all these actions can destroy bacteria or at least prevent their proliferation.



Another point of attack for phytomolecules is the communication between microorganisms (quorum sensing). Phytomolecules can prevent microorganisms from releasing substances known as autoinducers, which they need to coordinate joint actions such as the formation of biofilms or the expression of virulence factors.

Medium-chain triglycerides and fatty acids

Medium-chain triglycerides (MCT) and fatty acids (MCFA) are characterised by a length of six to twelve carbon atoms. Thanks to their efficient absorption and metabolism, they can be optimally used as an energy source in piglet feeding. MCTs can be completely absorbed by the epithelial cells of the intestinal mucosa and hydrolysed with microsomal lipases. Hence they serve as an immediately available energy source and can improve the epithelial structure of the intestinal mucosa (Hanczakowska, 2017).

In addition, these supplements have a positive influence on the composition of the intestinal flora. Their ability to penetrate bacteria through semi-permeable membranes and destroy bacterial structures inhibits the development of pathogens such as salmonella and coliforms (Boyen et al., 2008; Hanczakowska, 2017; Zentek et al., 2011). MCFAs and MCTs can also be used very effectively against gram-positive bacteria such as streptococci, staphylococci, and clostridia (Shilling et al., 2013; Zentek et al., 2011).

Prebiotics

Prebiotics are short-chain carbohydrates that are indigestible for the host animal. However, certain beneficial microorganisms such as lactobacilli and bifidobacteria can use these substances as substrates. By selectively stimulating the growth of these bacteria, eubiosis is promoted (Ehrlinger, 2007). In pigs, mannan-oligosaccharides (MOS), fructooligosaccharides (FOS), inulin and lignocellulose are mainly used.

Another element of prebiotics' positive effect on intestinal health is their ability to agglutinate pathogens. Pathogenic bacteria and MOS can bind to each other through lectin. This agglutination prevents pathogenic bacteria from adhering to the wall of the intestinal mucosa and thus from colonizing the intestine (Oyofo et al., 1989).

Probiotics

Probiotics can be used to regenerate an unbalanced gut flora. To do this, useful bacteria such as bifido or lactic acid bacteria are added to the food. They must settle in the gut and compete with the harmful bacteria.

There are also probiotics which target the communication between pathogens. In an experiment, Kim et al. (2017) found that the addition of probiotics that interfere with quorum sensing can significantly improve the microflora in weaned piglets and thus their intestinal health.

Organic acids

Organic acids show strong antibacterial activity in animals. In their undissociated form, the acids can penetrate bacteria. Inside, the acid molecule breaks down into a proton (H^+) and an anion ($HCOO^-$). The proton reduces the pH value in the bacterial cell and the anion interferes with the bacteria's protein metabolism. As a result, bacterial growth and virulence are inhibited.

Conclusion

Today there are several possibilities in piglet nutrition to effectively support the young animals after weaning. The main objective is to maintain a balanced intestinal flora and therefore to sustain intestinal health – its deterioration often leads to diarrhea and hence to reduced returns. Intestinal health is promoted by stimulating beneficial bacteria and by inhibiting pathogenic ones. This can be achieved through feed additives that have an antibacterial effect and/or support the intestinal mucosa, such as phytomolecules, prebiotics, and medium-chain fatty acids. Through a combination of these possibilities, additive effects can be achieved. Piglets receive optimal support and the use of zinc oxide can be reduced.

References

Ann, Ling Chuo, Shahrom Mahmud, Siti Khadijah Mohd Bakhori, Amna Sirelkhatim, Dasmawati Mohamad, Habsah Hasan, Azman Seeni, and Rosliza Abdul Rahman. “Antibacterial Responses of Zinc Oxide Structures against Staphylococcus Aureus, Pseudomonas Aeruginosa and Streptococcus Pyogenes.” *Ceramics International* 40, no. 2 (March 2014): 2993–3001. https://doi.org/10.1016/j.ceramint.2013.10.008.

Bednorz, Carmen, Kathrin Oelgeschläger, Bianca Kinnemann, Susanne Hartmann, Konrad Neumann, Robert Pieper, Astrid Bethe, et al. “The Broader Context of Antibiotic Resistance: Zinc Feed Supplementation of Piglets Increases the Proportion of Multi-Resistant Escherichia Coli in Vivo.” *International Journal of Medical Microbiology* 303, no. 6-7 (August 2013): 396–403. https://doi.org/10.1016/j.ijmm.2013.06.004.

Boyen, F., F. Haesebrouck, A. Vanparys, J. Volf, M. Mahu, F. Van Immerseel, I. Rychlik, J. Dewulf, R. Ducatelle, and F. Pasmans. “Coated Fatty Acids Alter Virulence Properties of Salmonella Typhimurium and Decrease Intestinal Colonization of Pigs.” *Veterinary Microbiology* 132, no. 3-4 (December 10, 2008): 319–27. https://doi.org/10.1016/j.vetmic.2008.05.008.

Cavaco, Lina M., Henrik Hasman, Frank M. Aarestrup, Members Of Mrsa-Cg: Jaap A. Wagenaar, Haitske Graveland, Kees Veldman, et al. “Zinc Resistance of Staphylococcus Aureus of Animal Origin Is Strongly Associated with Methicillin Resistance.” *Veterinary Microbiology* 150, no. 3-4 (June 2, 2011): 344–48. https://doi.org/10.1016/j.vetmic.2011.02.014.

Ciesinski, Lisa, Sebastian Guenther, Robert Pieper, Martin Kalisch, Carmen Bednorz, and Lothar H. Wieler. “High Dietary Zinc Feeding Promotes Persistence of Multi-Resistant E. Coli in the Swine Gut.” *Plos One* 13, no. 1 (January 26, 2018). https://doi.org/10.1371/journal.pone.0191660.

Crespo-Piazuelo, Daniel, Jordi Estellé, Manuel Revilla, Lourdes Criado-Mesas, Yuliaxis Ramayo-Caldas, Cristina Óvilo, Ana I. Fernández, Maria Ballester, and Josep M. Folch. “Characterization of Bacterial Microbiota Compositions along the Intestinal Tract in Pigs and Their Interactions and Functions.” *Scientific Reports* 8, no. 1 (August 24, 2018). https://doi.org/10.1038/s41598-018-30932-6.

Ehrlinger, Miriam. 2007. “Phytogene Zusatzstoffe in der Tierernährung.“ PhD Diss., LMU München. URN: urn:nbn:de:bvb:19-68242.

European Commission. 2003. “Opinion of the Scientific Committee for Animal Nutrition on the use of zinc in feedingstuffs.” https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed_additives_rules_scan-old_report_out120.pdf

Hanczakowska, Ewa. ”The use of medium chain fatty acids in piglet feeding – a review.” *Annals of Animal Science* 17, no. 4 (October 27, 2017): 967-977. https://doi.org/10.1515/aoas-2016-0099.

Hansche, Bianca Franziska. 2014. „Untersuchung der Effekte von Enterococcus faecium (probiotischer Stamm NCIMB 10415) und Zink auf die angeborene Immunantwort im Schwein. Dr. rer. Nat. Diss., Freie Universität Berlin. https://doi.org/10.17169/refubium-8548

Kim, Jonggun, Jaepil Kim, Younghoon Kim, Sangnam Oh, Minho Song, Jee Hwan Choe, Kwang-Youn Whang, Kwang Hyun Kim, and Sejong Oh. “Influences of Quorum-Quenching Probiotic Bacteria on the Gut Microbial Community and Immune Function in Weaning Pigs.” *Animal Science Journal* 89, no. 2 (November 20, 2017): 412–22. https://doi.org/10.1111/asj.12954.

Oyofo, Buhari A., John R. Deloach, Donald E. Corrier, James O. Norman, Richard L. Ziprin, and Hilton H. Mollenhauer. “Effect of Carbohydrates on Salmonella Typhimurium Colonization in Broiler Chickens.” *Avian Diseases* 33, no. 3 (1989): 531–34. https://doi.org/10.2307/1591117.

Shilling, Michael, Laurie Matt, Evelyn Rubin, Mark Paul Visitacion, Nairmeen A. Haller, Scott F. Grey, and Christopher J. Woolverton. “Antimicrobial Effects of Virgin Coconut Oil and Its Medium-Chain Fatty Acids On Clostridium Difficile.” *Journal of Medicinal Food* 16, no. 12 (December 2013): 1079–85. https://doi.org/10.1089/jmf.2012.0303.

Slifierz, M. J., R. Friendship, and J. S. Weese. “Zinc Oxide Therapy Increases Prevalence and Persistence of Methicillin-Resistant Staphylococcus Aureus in Pigs: A Randomized Controlled Trial.” *Zoonoses and Public*

Health 62, no. 4 (September 11, 2014): 301–8. https://doi.org/10.1111/zph.12150.

Vahjen, Wilfried, Dominika Pietruszyńska, Ingo C. Starke, and Jürgen Zentek. "High dietary zinc supplementation increases the occurrence of tetracycline and sulfonamide resistance genes in the intestine of weaned pigs." *Gut Pathogens* 7, article number 23 (August 26, 2015). https://doi.org/10.1186/s13099-015-0071-3.

Vahjen, Wilfried, Agathe Roméo, and Jürgen Zentek. "Impact of zinc oxide on the immediate postweaning colonization of enterobacteria in pigs." *Journal of Animal Science* 94, supplement 3 (September 1, 2016): 359-363. https://doi.org/10.2527/jas.2015-9795.

Zentek, J., S. Buchheit-Renko, F. Ferrara, W. Vahjen, A.G. Van Kessel, and R. Pieper. "Nutritional and physiological role of medium-chain triglycerides and medium-chain fatty acids in piglets" *Animal Health Research Reviews* 12, no. 1 (June 2011): 83-93. https://doi.org/10.1017/s1466252311000089.

How can you compensate an activated immune system in piglets?

By Technical Team, EW Nutrition

As pig production specialists, we understand that our animals are under constant challenge during their life. Challenges can be severe or moderate, correlated to several factors – such as, for instance, stage of production, environment, and so on – but they will always be present. To be successful, we need to understand how to counter these challenges and support the healthy development of our pigs.

Factors for successful pig production

For years we have been increasing our understanding of how to formulate diets to support a healthy intestine through the optimal use of the supplied nutrients. Functional proteins, immune-related amino acids, and fiber are now applied worldwide for improved pig nutrition.

What lies beyond formulation adjustments?

However, pig producers have also realized that these nutritional strategies alone are not always fully efficient in preventing an “irritation” of the immune system and/or in preventing diseases from happening.

Immune nutrition is gaining a strong foothold in pig production, and the body of research and evidence grows richer every year. At the same time, we see **genetics** continually evolving and bringing production potential to increasingly higher levels. We are also constantly increasing our understanding of the importance of **farm and feed management**, as well as **biosecurity** in this process.

Finally, the importance of a **stable microflora** is now uncontested. Especially around weaning, a stable microflora is necessary to prevent the proliferation of pathogens such as *E.coli* bacteria. Such pathogens can degrade the lysine (the main amino acid for muscle protein production) we have added to our formulations, rendering it useless.

Single molecules (or additives) are able to support the development of gut microflora, boost its integrity, and therefore help the animals use “traditional nutrients” in a more effective way.

The impact of immune system activation on the performance of pigs

Animal performance is influenced by complex processes, from metabolism to farm biosecurity. Environmental conditions, diet formulation and feed management, and health status, among others, directly affect the amount of the genetic potential that animals can effectively express.

Among these so-called *non-genetic* variables, health status is one of the most decisive factors for the optimal performance from a given genotype. Due to the occurrence of (sub-) clinical diseases, the inflammatory process can be triggered and may result in a decrease in weight gain and feed efficiency.

Not so long ago, pig producers believed that a maximized immune response would always be ideal for achieving the best production levels. However, after decades spent researching what this "maximized immune response" could mean to our pigs, studies from different parts of the globe proved that an activated immune system could negatively affect animal performance. The perception is nowadays common sense within the global pig production industry.

That understanding led us to increasingly search for production systems that will yield the best conditions for the pigs. This means minimum contact with pathogens, reduced stress factors, and therefore a lower need for an activated immune system.

How immune system stimulation works

The immune system has as main objective to identify the presence of antigens – substances that are not known to the body – and protect the body from these "intruders". The main players among these substances are bacteria and viruses. However, some proteins can also trigger an immunological reaction. Specific immune cells are responsible for the transfer of information to the other systems of the body so that it can respond adequately. This response from the immune system includes metabolic changes that can affect the demand for nutrients and, therefore, the animals' growth.

The stimulation of the immune system has three main metabolic consequences:

- behavioral responses
- direct connection with the endocrine system and regulation of the secretions
- release of leukocytes, cytokines, and macrophages

In general, the immune system responds to antigens, releasing cytokines that activate the cellular (phagocytes) and humoral components (antibody), resulting in a decreased feed intake and an increased body temperature/heat production.

When feed formulation is concerned, possibly even more important is to understand that the activation of the immune system leads to a change in the distribution of nutrients. The basal metabolic rate and the use of carbohydrates will have completely different patterns in such an event. For instance, some glucose supplied through the feed follows its course to peripheral tissues; however, part of the glucose is used to support the activated immune system. As a consequence, the energy requirement of the animal increases.

Protein synthesis and amino acid utilization also change during this process. There is a reduction of body protein synthesis and an increased rate of degradation. The nitrogen requirement increases because of the higher synthesis of acute-phase proteins and other immunological cells.

However, increased lysine levels in the diets will not always help the piglets compensate for this shift in the protein metabolism. According to Shurson & Johnston (1998), when the immune system is activated, there is further deamination of amino acids and increased urinary excretion of nitrogen. Therefore we need to understand better which amino acids must be supplied in a challenging situation.

In pigs, the gastrointestinal tract is, to a large extent, responsible for performance. This happens because the gut is the route for absorption of nutrients, but also a reservoir of hundreds of thousands of different microorganisms – including the pathogenic ones.

Understanding Gut Health

Gut health and its meaning have been the topic of several peer-reviewed articles in the last few decades (Adewole et al., 2016, Bischoff, 2011, Celi et al., 2017, Jayaraman and Nyachoti, 2017, Kogut and Arsenault, 2016, Moeser et al., 2017, Pluske, 2013). Despite the valuable body of knowledge accumulated on the topic, a clear and widely-accepted definition is still lacking. Kogut and Arsenault (2016) define it in the title of their paper as “the new paradigm in food animal production”. The authors explain it as the “absence / prevention / avoidance of disease so that the animal is able to perform its physiological functions in order to withstand exogenous and endogenous stressors”.

In a recently published paper, Pluske et al. (2018) add to the above definition that gut health should be considered in a more general context. They describe it “a generalized condition of homeostasis in the GIT, with respect to its overall structure and function”. The authors add to this definition that gut health in pigs can be compromised even when no clinical symptoms of disease can be observed. Every stressful factor can undermine the immune response of pigs and, therefore, the animals’ performance.

All good information on this topic leads us to the conclusion that, without gut balance, livestock cannot perform as expected. Therefore, balance is the objective for which we formulate our pigs’ feed.

Current nutritional strategies for a stable gut microbiota

Feeding: quality of raw materials

The photos included here were taken in the field and show that taking action against this reality is a must for keeping animals healthy.

Much of this action is related to farm management. The most effective way to minimize such situations is to implement a strict control system in the feed production sites, including controlling raw material quality.

Additives can be used to improve the safety of raw materials. As already extensively discussed, everything that goes into the intestine of the animals will affect gut health and performance. Therefore, the potential harmful load of mycotoxins should be taken into account. Besides careful handling at harvest and the proper storage of grains, mycotoxin binders can be applied to further decrease the risk of mycotoxin contamination.

Figure 1. Grain storage in a home pig farm

Figure 2. Feed mixer in a home mixer pig farm

The effect of nutrition on microflora: commercial weaning diet after focusing on gut health

The gut-health-focused formulation of diets must take into account the following essentials:

- decrease of gut pH
- gut wall integrity
- minimization of (pathogenic) microbial growth
- microflora modulation with consequently improved colonization resistance

Gut pH

A lower pH in the stomach slows the passage rate of the feed from the stomach to the small intestine. A longer stay of the feed in the stomach potentially increases the digestion of starch and protein. The secretion of pancreatic juices stimulated by the acidic stomach content will also improve the digestion of feed in the small intestine.

For weaned pigs, it is essential that as little as possible of the substrate will reach the large intestine and be fermented. Pathogens take advantage of undigested feed to proliferate. Lowering these “nutrients” will decrease the risk of bacterial overgrowth.

The same is true where protein sources and their levels are concerned. It is essential to reduce protein content as much as possible and preferably use synthetic (essential) amino acids. The application of such sources of amino acids has been proven long ago, and yet in some cases, it is still not fully utilized. Finally, using highly digestible protein sources should, at this point, be a matter of mere routine.

All these strategies have the same goal: the reduction of undigested substances in the gut. Additionally, the reduction of the protein levels can also decrease the costs of the diets.

Further diet adjustments

Further diet adjustments, such as increasing the sulfur amino acids (SAA) tryptophan and threonine to lysine ratio, must also be considered (Goodband et al., 2014; Sterndale et al., 2017). Although the concept of better balancing tryptophan and threonine are quite clear among nutritionists, SAA are sometimes overestimated. Sulfur amino acids are the major amino acids in proteins related to body maintenance, but not so high in muscle proteins. Therefore, the requirement of SAA must also be approached differently. Unlike lysine, the requirements of SAA tend to be higher in immunologically stimulated animals (Table 1).

Pig weight (kg)	ISA*	SID Lysine (%)	SAA (%)	SAA:Lys
9	High	1,34	0,64	0,48
	Low	1,07	0,59	0,55
14	High	1,22	0,62	0,51
	Low	0,99	0,57	0,58

Table 1. Effect of the immune system activation on the demand for lysine and sulfur amino acids in pigs (Stahly et al., 1998)

*ISA – immune system activation

Vitamins and minerals are classic nutrients to be considered when formulating gut health-related diets. Maybe not so extensive as the amino acids and protein levels, these nutrients have, however, been found to carry benefits in challenging situations. In the past several years, a lot was published on the requirements of pigs facing an activation of the immune system. Stahly et al. (1996) concluded that when the immune system is activated, the phosphorous requirements change.

Parameters	ISA*	
	High	Low
Feed intake (g/d)	674	833
Weight gain (g/d)	426	566
Available P (%)	0,45	0,65

Table 2. Effect of the immune system activation on the performance and phosphorous requirements of pigs (Stahly et al., 1998)

*ISA – immune system activation

Another example is vitamin A. It is involved in the function of macrophages and neutrophils. Vitamin A deficiency decreases the migratory and phagocytic abilities of the immune cells. A lower antibody production is observed in vitamin A deficiency as well. Furthermore, vitamin A is an important factor in mucosal immunity, because this vitamin plays a role in lymphocyte homing in the mucosa (Duriancik et al., 2010).

Phytomolecules: key additives to support gut health

Phytomolecules are currently considered one of the top alternatives to in-feed antibiotics for pigs worldwide. Programs sponsored by the European Union are once more evaluating the effectiveness of these compounds as part of a strategy to produce sustainable pigs with low or no antibiotic use. The EIP-Agri (European Innovation Partnership "Agricultural Productivity and Sustainability") released a document with suggestions to lower the use of antibiotics in feed by acting in three areas:

- improving pig health and welfare
- changing attitudes and human habits
- finding specific alternatives to antibiotics

Under the last topic, the commission recommends plant-based feed additives to be further examined.

Antibiotics have been used for many years for supporting performance in animal production, especially in critical moments. The mode of action consists of the reduction of pathogen proliferation and inflammation processes in the digestive tract. These (soon-to-be-) banned compounds therefore reduce the activation of the immune system, helping keep pigs healthy through a healthy gastrointestinal tract. As potential alternatives to antibiotic usage, phytomolecules should be able to do the same.

The mode of action of phytomolecules

Antimicrobial

Most phytomolecules used nowadays aim to control the number and type of bacteria in the gut of animals. According to Burt (2004), the antimicrobial activity of phytomolecules is not the result of one specific mode of action, but a combination of effects on different targets of the cell. This includes disruption of the membrane by terpenoids and phenolics, metal chelation by phenols and flavonoids, and protective effects against viral infections for certain alkaloids and coumarins (Cowan, 1999).

Digestion support

The antimicrobial efficacy is one of the most important activities of secondary plant compounds, but it also impacts digestion. Windisch et al. (2008) states that growth-promoting agents decrease immune defense stress during critical situations. They increase the intestinal availability of essential nutrients for absorption, thus promoting the growth of the animal.

Indeed, phytomolecules are a good tool for stabilizing the gut microbiota. But more can be expected when adding this class of additives into your formulation and/or farm operations. Mavromichalis, in his book "Piglet Nutrition Notes – Volume 2", brings attention to the advantages of using phytomolecules such as capsaicin, which is often related to increased feed intake. Recent research has demonstrated that capsaicin increases the secretion of digestive enzymes that may result in enhanced nutrient digestibility. According to Mavromichalis, this can lead to a better feed conversion rate as more nutrients are available to the animal. Indirectly, this also helps control the general bacterial load in the gut.

Antioxidant support

This results from the polyphenols' capacity to act as metal-chelators, free radical scavengers, hydrogen donators, and inhibitors of the enzymatic systems responsible for initiating oxidation reaction. Furthermore, they can act as a substrate for free radicals such as superoxide or hydroxyl, or intervene in propagation reactions.

This variety of benefits explains at least partially the high level of interest in this group of additives for pigs under challenging conditions. For the production of effective blends, it is crucial to understand the different modes of action of the phytomolecules and the probable existing synergies. Furthermore, the production technology must be considered. For instance, microencapsulation techniques that prevent losses during feed processing are an important consideration.

Not to be discarded: Biosecurity

The recent outbreak of African Swine Fever focused our attention on something that is sometimes neglected on the farm: biosecurity rules. According to the report "Good Practices For Biosecurity In The Pig Sector" (2010), the three main elements of biosecurity are:

- segregation
- cleaning
- disinfection

In general terms, the following steps must be adopted with the clear goal of reducing the challenges that the pigs are facing.

- Farms must be located far from other farms (regardless of the species) and ideally must be protected with natural (forest/woods) or physical barriers.
- Only one entrance must be used to go into the farm (for both vehicles and people) and a disinfection procedure must be in place, either by an automatized system or by manual application of disinfectants. Equipment disinfection systems must also be in place.
- Workers and any other person that enters the facility should adhere to strict biosecurity measures 24/7. The farms must have a visitors' book including relevant data on previous visits

to farms (regardless of the species).
- Trucks and visitors should not have been in contact with other pigs recently (at least 48 hours previous to the visit).
- Only farm workers are allowed to go into the barns unless special approval is given (followed by strict biosecurity measurements prior to the visit).
- The use of clothing and footwear that are worn only in the pig unit (and certainly not during visits to other pig farms) is recommended.
- No materials (e.g. tools) can be moved from one barn to another barn. People that enter a barn should change footwear and wash their hands with soap for at least 10 seconds.

These simple actions can make a big difference to the performance of the pigs, and as a consequence to the profitability of a swine farm.

Take-home messages

Different formulations and reassessed nutritional level recommendations have been on the radar for a couple of years. It is high time to consider using efficient additives to support the pigs' gut health. Phytomolecules appear as one of the most prominent tools to reduce pathogenic stress in pig production. Either via feed or water, phytomolecules are proven to reduce bacterial contamination and therefore reduce the need for antibiotic interventions. Furthermore, a more careful look at our daily activities in the farm is crucial. Paying attention to biosecurity and to feed safety should be standard tools to improve performance and the success of pig production operations.

References are available upon request.

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How phytomolecules support antibiotic reduction in pig production

by Merideth Parke, Regional Technical Manager, EW Nutrition

To contain and reverse antimicrobial resistance, consumers and government regulators expect changes in pork production with the clear goal to reduce antibiotic use. For healthy, profitable pig production with simultaneous antibiotic reduction, a holistic strategy is required: refocusing human attitudes and habits, optimal pig health and welfare, and applying potential antibiotic alternatives.

Corn is often contaminated with Aspergillus fungi that can produce poisonous mycotoxins

Pig producers need to manage

pathogenic pressure while reducing antibiotics

Intensive pig production has stress points associated with essential husbandry procedures such as weaning, health interventions, and dietary modifications. Stress is widely accepted to have a negative impact on immune system effectiveness, enhancing opportunities for pathogenic bacteria to invade at a local or systemic level. The gastrointestinal and respiratory systems are highly susceptible to developing disease as a result of these combined factors. Interventions such as antibiotics are commonly implemented to reduce the impact of pathogens and manage pig health. Processes that minimize the number of pathogens in the environment are the foundation for a successful antibiotic reduction plan. The challenge is to smartly combine strategies to keep the gastrointestinal and respiratory tract intact and robust.

Phytomolecules, the specific active defense compounds found in plants, have been identified as capable of enhancing pig health through antimicrobial (Cimanga et al., 2002, Franz et al., 2010), antioxidative (Katalinic et al., 2006, Damjanovic-Vratnica et al., 2007, Lee et al., 2011), digestion-stimulating and immune-supportive functions. As many thousands of phytomolecules exist, laboratory research has focused on identifying those with the capability of microbial management, facilitating the end goal of reducing the reliance on antibiotics for pig health and welfare and the production of safe pork (Zhai et al., 2018).

Which roles can phytomolecules play in reducing antibiotics?

The gastrointestinal tract benefits from applying phytomolecules such as capsaicin, carvacrol, and cinnamaldehyde, as they:

- support a balanced and stable biome,
- prevent dysbiosis, maintain tight junction integrity (Liu et al., 2018),
- increase secretion of digestive enzymes, and
- enhance gut contractility (Zhai et al., 2018).

Pigs most susceptible and in need of phytomolecule gastrointestinal supportive actions are piglets at weaning and pigs of all ages undergoing stress, pathogen challenges, and/or dietary changes.

Porcine respiratory disease is a complex multifactorial disorder. It frequently requires antibiotics to manage infection pressure and clinical disease to maintain pig health, welfare, and production performance. Causal pathogens may be transmitted by direct contact between pigs in saliva (Murase et al., 2018) or bioaerosols (LeBel et al., 2019), via the nasal or oral cavities (inhalation directly into the airways and lungs), or via an unhealthy gut. Phytomolecules such as carvacrol and cinnamaldehyde have antimicrobial properties. Hence, they may help contain respiratory pathogens in their natural habitat (the upper respiratory tract) or during transit through the oronasal cavity and gastrointestinal tract (Swildens et al., 2004, Lee et al., 2001).

In addition to supporting the gastrointestinal and respiratory systems, phytomolecules such as menthol and 1,8-cineole have been shown to enhance the physical and adaptive immune systems in multiple species (Brown et al., 2017, Barbour et al., 2013). When applied via drinking water, adherence to the oronasal mucosa facilitates the inhalation of the active phytomolecule compounds into the respiratory tract. There, they act as mucolytics, muscle relaxants, and enhancers of the mucociliary clearance mechanism (Başer and Buchbauer, 2020). Phytomolecules have also been documented to positively influence the adaptive immune system, promoting both humoral and cell-mediated immune responses (Awaad et al., 2010, Gopi et al., 2014, Serafino et al., 2008).

How phytomolecules feature in the holistic approach to antibiotic reduction

Antibiotic reduction programs positively enact social responsibility by reducing the risk to farmworkers of exposure to antimicrobial-resistant bacteria. They also help maintain or increase efficiency in safe pork production – pork with minimal risk of antibiotic residues.

Implementation of a successful health program with reduced antibiotic use will require:

- application of strict internal and external biosecurity processes;
- evaluation and monitoring of AMR bacteria;
- partnerships with specialist nutritionists to target a lifetime healthy gut biome; and
- phytomolecule-assisted health management (Figure 1).

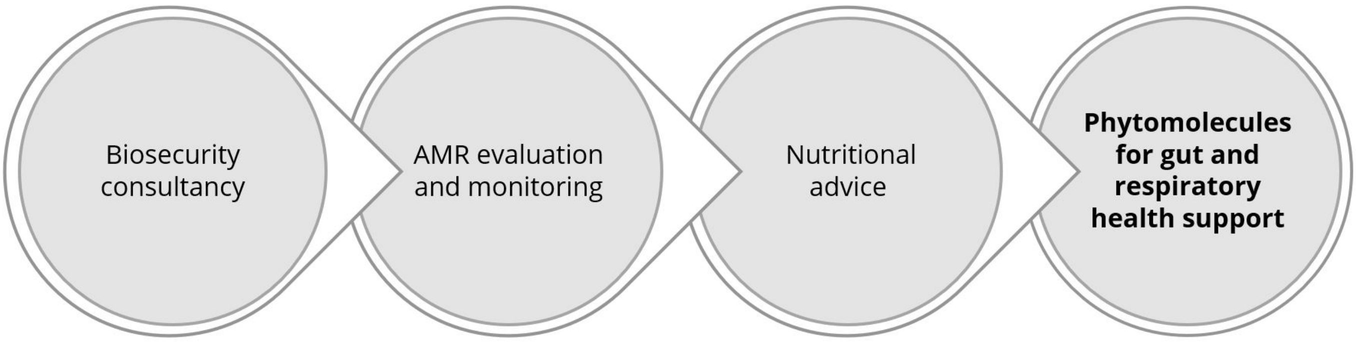

Figure 1: The role of phytomolecules within EW Nutrition's holistic Antibiotic Reduction program

A combination of *in vitro* and *in vivo* studies provides evidence that specific phytomolecules can support both enteric and respiratory systems through biome stabilisation and pathogen management (Bajabai et al., 2020). Antimicrobial activity of thymol, carvacrol, and cinnamaldehyde has been reported against respiratory pathogens including *S. suis, A. pleuropneumoniae*, and *H. parasuis* (LeBel et al., 2019); multi-drug resistant and ESBL bacteria (Bozin et al., 2006); enteric pathogens including *E. coli*, *Salmonella enteritidis*, *Salmonella cholerasuis*, and *Salmonella typhimurium* (Penalver et al., 2005); *Clostridium* spp., *E. coli* spp., *Brachyspira hyodysenteriae* (Vande Maelle et al., 2015); and *Lawsonia intracellularis* (Draskovic et al., 2018). These results have shown phytomolecules to be effective antimicrobial alternatives for incorporation into holistic pig health programs.

Additionally, the inclusion of phytomolecules into pig production systems also enhances production performance by reducing the negative impact of stress on the pig and increasing the positive effects on gut health and nutrient utilization (Franz et al., 2010). Phytomolecules that directly impact digestive actions include capsaicin, which optimizes the production of digestive enzymes and increases serotonin for gut contraction maintenance and improved digesta mixing (Zhai et al., 2018). Cineol's antioxidative activities provide support during times of stress (Cimanga et al., 2002).

Phytomolecules are key to reducing antibiotics in pig production

The pig industry searches for alternatives to therapeutic, prophylactic, and growth-promoting antibiotic applications to keep available antibiotics effective for longer – and to address the social responsibility of mitigating AMR. This search for ways to produce safe pork has made it clear that only a combination of management and antibiotic alternatives can achieve these aligned goals.

Biosecurity, hygiene, stress reduction, and husbandry and nutritional advances form the foundation for the strategic application of specific phytomolecules (Zeng et al. 2016). Supporting pig production and health, this complete holistic solution (EIP-AGRI) moves the pig industry into a future where antibiotic reduction or removal, with equivalent or increased production of safe pork, becomes a reality.

References

Awaard M, Abdel-Alim G, Sayed K, Kawkab, Ahmed1 A, Nada A , Metwalli A, Alkhalaf A. “Immunostimulant effects of essential oils of peppermint and eucalyptus in chickens”. *Pakistan Veterinary Journal* (2010). 2:61-66. http://www.pvj.com.pk/

Bajagai YS, Alsemgeest J, Moore RJ, Van TTH, Stanley D. “Phytogenic products, used as alternatives to antibiotic growth promoters, modify the intestinal microbiota derived from a range of production systems: an in vitro model”. *Applied Microbiology and Biotechnology* (2020). 104:10631-10640. https://doi.org/10.1007/s00253-020-10998-x

Barbour EK, Shaib H, Azhar E, Kumosani T, Iyer A, Harakey S, Damanhouri G, Chaudary A, Bragg RR. “Modulation by essential oil of vaccine response and production improvement in chicken challenged with velogenic Newcastle disease virus”. *Journal of Applied Microbiology* (2013). 115, 1278-1286. https://doi:10.1111/jam.12334

Biljana Damjanovic-Vratnica, Tatjana Dakov, Danijela Sukovic, Jovanka Damjanovic. “Antimicrobial effect of essential oil isolated from Eucalyptus globulus Labill” (2011). *Czech Journal of Food Science* 27(3):277-284. https://www.agriculturejournals.cz/publicFiles/39925.pdf

Bozin B, Mimica-Dukic N, Smin N, Anackov G. “Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils” *Journal of Agriculture and Food Chemicals* (2006). 54:1822-1828 https://pubs.acs.org/doi/10.1021/jf051922u

Brown SK, Garver WS, Orlando RA. “1,8-cineole: An Underappreciated Anti-inflammatory Therpeutic” *Journal of Biomolecular Research &Therapeutics* (2017). 6:1 1-6 https://doi: 10.4172/2167-7956.1000154

Cimanga K., Kambu K., Tona L., Apers S., De Bruyne T., Hermans N., Totte J., Pieters L., Vlietinck A.J. “Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo”. *Journal of Ethnopharmacology* (2002) 79: 213–220. https://doi.org/10.1016/s0378-8741(01)00384-1

Draskovic V, Bosnjak-Neumuller J, Vasiljevic M, Petrujkic B, Aleksic N, Kukolj V, Stanimirovic Z. “Influence of phytogenic feed additive on Lawsonia intracellularis infection in pigs” *Preventative Veterinary Medicine* (2018). 151: 46-51 https://doi.org/10.1016/j.prevetmed.2018.01.002

European Innovation Partnership Agricultural Productivity and Sustainability (EIP-AGRI). https://ec.europa.eu/eip/agriculture/en/european-innovation-partnership-agricultural

Franz C., Baser KHC, Windisch W. “Essential oils and aromatic plants in animal feeding-a European perspective. A review Flavour”. *Flavour and Fragrance Journal* (2010) 25:327-40. https://doi.org/10.1002/ffj.1967

Gopi M, Karthik K, Manjunathachar H, Tamilmahan P, Kesavan M, Dashprakash M, Balaraju B, Purushothaman M. “Essential oils as a feed additive in poultry nutrition”. *Advances in Animal and Veterinary Sciences* (2014) 1:17. https://doi.10.14737/journal.aavs/2014.2.1.1.7

Başer, Kemal Hüsnü Can, and Gerhard Buchbauer. Handbook of Essential Oils Science, Technology, and Applications. Boca Raton: CRC Press, 2020.

Hengziao Zhai, Hong Liu, Shikui Wang, Jinlong Wu, Anna-Maria Kluenter. “Potential of essential oils for poultry and pigs.” *Animal Nutrition* 4 (2018): 179-186. https://doi.org/10.1016/j.aninu.2018.01.005

Katalinic V., Milos M., Kulisic T., Jukic M. “Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols”. *Food Chemistry* (2006) 94(4):550-557. https://doi.org/10.1016/j.foodchem.2004.12.004

LeBel G., Vaillancourt K., Bercier P., Grenier D. “Antibacterial activity against porcine respiratory bacterial pathogens and in vitro biocompatibility of essential oils”. *Archives of Microbiology* (2019) 201:833-840; https://doi.org/10.1007/s00203-019-01655-7

Lee KG, Shibamoto T. “Antioxidant activities of volatile components isolated from Eucalyptus species”. *Journal of the Science of Food and Agriculture* (2001). 81:1573-1597. https://doi.org/10.1002/jsfa.980

Liu SD, Song MH, Yun W, Lee JH, Lee CH, Kwak WG Han NS, Kim HB, Cho JH. “Effects of oral administration of different dosages of carvacrol essential oils on intestinal barrier function in broilers” *Journal of Animal Physiology and Animal Production* (2018) https://doi.org/10.1111/jpn.12944

Murase K, Watanabe T, Arai S, Kim H, Tohya M, Ishida-Kuroki K, Vo T, Nguyen T, Nakagawa I, Osawa R, Nguyen N, Sekizaki T. “Characterization of pig saliva as the major natural habitat of *Streptococcus suis* by analyzing oral, fecal, vaginal, and environmental microbiota”. *PLoS ONE* (2019). 14(4). https://doi.org/10.1371/journal.pone.0215983

Nethmap MARAN report 2018. https://www.wur.nl/upload_mm/7/b/0/5e568649-c674-420e-a2ca-acc8ca56f016_Maran%202018.pdf

Penalver P, Huerta B, Borge C, Astorga R, Romero R, Perea A. “Antimicrobial activity of 5 essential oils against origin strains of the Enterobacteriaceae family”. *Acta Pathologica Microbiologica, et Immunologica Scandinavica* (2005) 113:1-6. AromaticScience, LLC Antimicrobial activity of five essential oils against origin strains of the Enterobacteriaceae family.

Serafino A, Vallebona PS, Adnreola F, Zonfrillo M, Mercuri L, Federici M, Rasi G, Garaci E, Pierimarchi P. “Stimulatory effect of Eucalyptus essential oil on innate cell-mediated immune response” *BioMed Central* (2008). 9:17 https//:doi:10.1186/1471-2172-9-17

Swildens B, Stockhofe-Zurwieden N, van der Meulen J, Wisselink HJ, Nielen M. “Intestinal translocation of Streptococcus suis type 2 EF+ in pigs”. *Veterinary Microbiology* (2004) 103:29-33. https://doi: 10.1016/j.vetmic.2004.06.010

Vande Maele L, Heyndrickx M, Maes D, De Pauw N, Mahu M, Verlinden M, Haesbrouck F, Martel A, Pasmans F, Boyen F. “In vitro susceptibility of *Brachyspira hyodysenteriae* to organic acids and essential oil components”. *Journal of Veterinary Medical Science* (2016). 78(2):325-328. https://doi.org/10/1292/jvms.15-0341

Zeng Z, Zhang S, Wang H, Piao X. “Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review”. *Journal of Animal Science and Biotechnology* (2015) 6:7. https://doi.org?10/1186/s40104-015-004-5