

The Science Behind Phytogenics



Conference Report

Essential oils, secondary plant compounds, phytogenics – all these expressions can be found in the context of animal feed. In the following, Dr. Sabiha Kadari, Regional Technical Director Southeast Asia/Pacific at EW Nutrition, will show the difference between essential oils and phytomolecules and the science behind phytogenics.

Essential oils and phytomolecules- not the same

Let us first show what are essential oils using the example of oregano oil. Essential oils are extracted from plants and unpurified mixes of different phytomolecules. The raw oregano oil extract contains carvacrol, thymol, P-cymene, and several other phytomolecules. The concentration and composition of these phytomolecules can vary significantly, depending on factors such as geographical origin, seasonal variations, plant part, plant growth stage and harvest time, extraction methods, and post-harvest processing. As a result, there can be significant batch-to-batch variations, resulting in differences in animal performance. Furthermore, there is the potential for the presence of undesirable contaminants.

In contrast, **phytomolecules** are the active ingredients in essential oils or other plant materials. They are clearly defined as one active compound (IUPAC name/CAS number) by their unique chemical structures, such as carvacrol. By focusing on specific active compounds, standardized products don't have batch-to-batch variation, enhancing consistent animal performance.

Stringent screening processes

To yield the best phytogetic formulations for animal production, a rigorous screening process is required:

The initial screening process consists of ensuring the bioactives are generally recognized as safe (GRAS) by the US Department of Agriculture and approved by the European Food Safety Authority (EFSA). This step is crucial to ensure that any compounds used in formulations do not pose health risks to animals or humans.

In addition to being selected for their chemical-physical properties, which play a significant role in determining how well the phytogetics will perform in various applications, and a thorough cost-benefit analysis, the phytogetics are mapped for their following biological activities.

Antioxidant

Phytomolecules exert their antioxidant effects through various mechanisms, including scavenging free radicals. The ORAC (Oxygen Radical Absorbance Capacity) test is widely regarded as a gold standard for measuring the antioxidant potential of phytomolecules. It quantitatively assesses the ability of compounds to scavenge free radicals, providing a reliable comparison against a known standard, specifically Trolox, a vitamin E analog. Trolox has well-documented antioxidant properties, making it a reliable benchmark for evaluating the effectiveness of other antioxidants.

Antimicrobial

Incorporating a comprehensive approach to testing the antibacterial properties of phytogetics is essential for developing effective feed additives. The antibacterial properties should not only be tested against harmful enteropathogenic bacteria, such as *Clostridium perfringens*, *E. coli*, and *Salmonella*. It should also be evaluated if beneficial species such as *Lactobacilli*, the proliferation of which is wanted, are preserved.

By evaluating both pathogenic and beneficial bacteria, researchers can ensure that phytogetic formulations support optimal gut health and reduce the reliance on antibiotics.

Anti-inflammatory

Anti-inflammatory properties also help to modulate the gut-associated immune system and mitigate excessive immune response so that animals can allocate more energy towards growth and production. This shift is vital for optimizing feed conversion ratios and overall performance.

Dr. Kadari noted that “EW Nutrition uses nuclear factor kappa beta (NFκB), which regulates the expression of various pro-inflammatory cytokines, and interleukin 6 (pro-inflammatory) and 10 (anti-inflammatory) cytokines as biomarkers, for measuring anti-inflammatory activity. A reduction in NFκB and the ratio of IL-6/ IL-10 indicates a decrease in inflammatory response.”

Anti-conjugation

Conjugation is a common mechanism of horizontal gene transfer that is instrumental in spreading antibiotic resistance between bacteria. “Most resistance genes are found on mobile genetic elements named plasmids and primarily spread by conjugation,” explained Dr. Kadari.

Cell stress of bacteria modulates the conjugation frequency. Among these stressors are antimicrobial phytogetics. The goal is to keep the conjugation frequency below the one that could occur under unchallenged conditions.

Figure 1: High throughput screening allows EW Nutrition researchers to quickly conduct millions of chemical, genetic, or pharmacological tests



Delivery mechanism

Lastly, to optimize the benefit of the selected phytogenics and deliver consistent results, the substances must be protected by, e.g., encapsulation to ensure homogenous distribution in feed and thermostability in pelleted feed. A special delivery system provides for the targeted release of the active ingredients within the organism, specifically ensuring that these compounds are effectively utilized within the body rather than eliminated through the feces. This is crucial for optimizing their benefits in animal production.

Phytomolecules are an essential support in antibiotic reduction

“Phytogenics are increasingly recognized as effective alternatives in antimicrobial reduction programs. The combination of stringent screening processes alongside rigorous *in vitro* and *in vivo* testing is essential for ensuring that phytogenics deliver optimal and consistent performance in animal production,” noted Dr. Kadari.

EW Nutrition’s Swine Academies took place in Ho Chi Minh City and Bangkok in October 2024. Dr. Sabiha Kadari, Regional Technical Director at EW Nutrition SEAP, was one of the highly experienced speakers of EW Nutrition. With expertise in feed cost optimization, feed additive management, audits, and lab support, she provides customized technical solutions and troubleshooting challenges for customers.

Consequences of genetic

improvements and nutrient quality on production performance in swine



Conference Report

Achieving high performance and superior meat quality with preferably low investment – and here, we speak about feed costs, which account for up to 70% of the total costs – is a considerable challenge for pig producers. The following will focus on the effects of genetic enhancements and nutrient quality on overall pig performance.

Effect of body weight and gender on protein deposition

Based on Schothorst Feed Research recommendations for tailoring nutritional strategies to enhance feed efficiency and overall productivity, the following facts must be considered:

- Castrates, boars, and gilts have significantly different nutritional requirements due to variations in growth rates, body composition, and hormonal influences. For instance, testosterone significantly impacts muscle development and protein metabolism, increasing muscle mass in males. In contrast, ovarian hormones may inhibit muscle protein synthesis in females, contributing to differences in overall protein deposition. Boars, therefore, require higher protein levels to support muscle growth. Castrates typically have a higher FCR compared to gilts and

boars due to higher feed intake. Split-sex feeding allows for diet adjustments to optimize growth rates and reduce feed costs per kilogram gained.

- Different body weight ranges: because puberty is delayed in modern genetics, we can produce heavier pigs without compromising carcass quality. Given that a finisher pig with 80-120 kg bodyweight consumes about half of the total feed of that pig, Dr. Fledderus concluded that extra profit could be realized with an extra feed phase diet for heavy pigs. Implementing multiple finisher diets can help reduce feed costs by allowing for lower nutrient concentrations, such as reducing the net energy and standardized ileal digestible lysine in later phases, without compromising performance.

Decision-making according to feedstuff prices

Least cost formulation is commonly used by nutritionists to formulate feeds for the lowest costs possible while meeting all nutrient requirements and feedstuff restrictions at the actual market prices of feedstuffs. However, diet optimization is more complex. The real question is, "How do you formulate diets for the lowest cost per kilogram of body weight gain?" You must always consider your specific situation, as economic results vary greatly and depend mainly on the prices of pork and feed and pig growth performance (e.g., feed efficiency, slaughter weight, and lean percentage).

How can you optimize your feeding strategy? Reducing net energy (NE) value will result in more fiber entering the diet. This makes sense if fiber by-products are cheaper than cereals. In contrast, an increase in the NE value will increase the inclusion of high-quality proteins and synthetic amino acids. It will use more energy from fat and less from carbohydrates.

The effects of diet composition on meat quality and fat composition also need to be considered.

How can nutrition improve meat quality?

Nutritional strategies not only improve the sensory attributes of pork but also enhance its shelf life, ultimately leading to higher consumer satisfaction and better marketability. Some of the factors Dr Fledderus considered included:

Improving fat quality



The source of dietary fat significantly impacts the quality of pork fat. Saturated fats tend to produce firmer

fat, while unsaturated fats can lead to softer, less stable fat deposits. Diets high in unsaturated fats are more prone to lipid oxidation, negatively affecting shelf life and overall meat quality. The deposition of polyunsaturated fatty acids is only from dietary fat. Saturated fats in pork, partly originates from dietary fat and are also synthesized de novo. So, the amount of polyunsaturated fatty acids in pork depends on the content and composition of dietary fat, which can negatively affect the shelf life and perception of pork meat.

The iodine value (IV) is a measure of the degree of unsaturation in fats. A higher IV indicates a higher proportion of unsaturated fatty acids, leading to softer fat. Pork fat with an IV lower than 70 is considered high quality, as it tends to be firmer and more desirable for processing.

As per the American Oil Chemists Society, IV is calculated as:

$$IV = [C16:1] \times 0.95 + [C18:1] \times 0.86 + [C18:2] \times 1.732 + [C18:3] \times 2.616 + [C20:1] \times 0.785 + [C22:1] \times 0.723$$

(brackets indicate concentration (%) of C16:1 palmitoleic acid, C18:1 oleic acid, C18:2 linoleic acid, C18:3- γ -linoleic acid, C20:1 eicosenoic acid, C22:1 erucic acid per crude fat)

Implications

Dr. Fledderus concluded that the pigs' nutritional requirements are dynamic and influenced by factors such as required meat and fat quality, heat stress, slaughter weight, and genetic developments. Tailoring diets based on gender and body weight is crucial for optimizing protein deposition. Accurate information is essential to formulate diets that achieve optimum economic results, not just the least cost.

Continuous monitoring of feedstuff prices and nutritional content allows for timely adjustments in diet formulations, ensuring that producers capitalize on cost-effective ingredients while maintaining nutritional quality.

EW Nutrition's Swine Academy took place in Ho Chi Minh City and Bangkok in October 2024. Dr. Jan Fledderus, Product Manager and Consultant at the S&C team at Schothorst Feed Research, with a strong focus on continuously improving the price/quality ratio of the diets for a competitive pig sector and one of the founders of the Advanced Feed Package, was a reputable guest speaker in these events.

Recent advances in energy evaluation in pigs



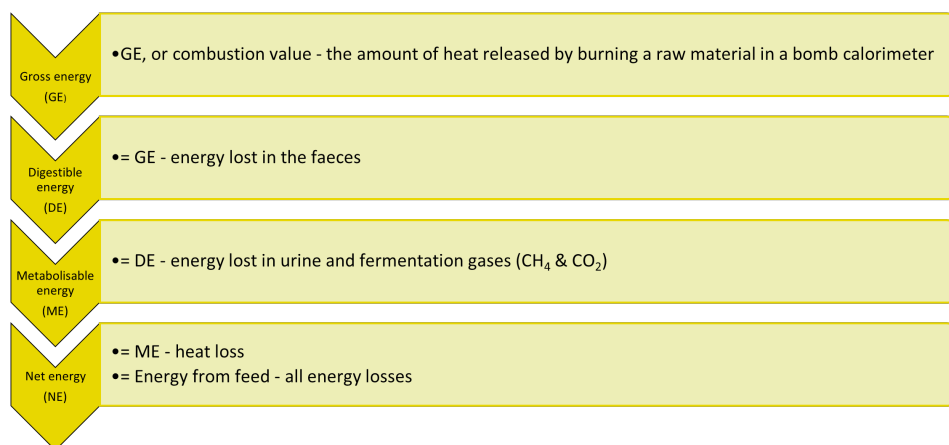
Conference Report

During the recent EW Nutrition Swine Academies in Ho Chi Minh City and Bangkok, Dr. Jan Fledderus, Product Manager and Consultant at Schothorst Feed Research, discussed that much money is involved in a correct energy evaluation system. "Net energy is 70% of feed costs, and feed is about 70% of total costs." Therefore, an accurate energy evaluation system is important as it will give:

- Flexibility to use different raw materials
- Reduction of formulation costs
- Best prediction of pig performance
- Match the available dietary energy requirement of the feed to the pig's requirement

Energy evaluation systems for pigs

The energy value of a raw material or complete feed can be expressed using different energy evaluation systems. Net energy (NE) in pigs refers to the amount of energy available for maintenance and production after accounting for energy losses during digestion, metabolism, and heat production. It is a crucial concept in swine nutrition as it provides a more accurate measure of the energy value of feed ingredients compared to other systems like digestible energy (DE) and metabolizable energy (ME). Diets formulated using NE are lower in crude protein than those using DE or ME because the heat lost during catabolism and excretion of excess nitrogen is considered in the NE system.



Effect of energy

Energy is derived from three nutrients: lipids (fats and oils), carbohydrates, and proteins. Using NE values instead of DE or ME values can lead to changes in ingredient ranking when formulating diets. For example:

- Ingredients high in fat or starch may be undervalued in DE systems but receive appropriate recognition in NE evaluations.
- Conversely, protein-rich or fibrous ingredients may be favored in DE systems.

Table 1: Energy values (kcal/kg) of nutrients

Nutrient	Energy	Starch	Protein	Fat
Gross energy	GE	4,486 (100)	5,489 (122)	9,283 (207)
Digestible energy	DE	4,176 (100)	4,916 (118)	8,424 (202)
Metabolizable energy	ME	4,176 (100)	4,295 (103)	8,424 (202)
Net energy	NE	3,436 (100)	2,434 (71)	7,517 (219)
Heat production (kcal/kg)		740	1,861	907
Heat production (% of NE)		22%	76%	12%

Calculation of net energy

Net energy (kcal/kg dry matter) is calculated as:

$$\begin{aligned}
 &= 2,577 \times \text{digestible crude protein} \\
 &+ 8,615 \times \text{digestible crude fat} \\
 &+ 3,269 \times \text{ileal digestible starch} \\
 &+ 2,959 \times \text{ileal digestible sugars} \\
 &+ 2,291 \times \text{fermentable carbohydrates}
 \end{aligned}$$

Factors affecting nutrient digestibility

This raises the obvious question, 'What is the nutrient digestibility of your raw materials?' Dr. Fledderus considered several factors that affect nutrient digestibility and, therefore, NE values, including

- **Age:** as pigs grow, their digestive systems mature, leading to improved nutrient digestibility. Younger pigs typically have lower digestibility rates due to an underdeveloped gastrointestinal tract. Older pigs typically exhibit higher digestibility, especially for fibrous diets, as their digestive systems become more efficient at breaking down complex nutrients.
- **Physiological stage:** the digestibility of diets can vary between pregnant and lactating sows. Digestibility is generally higher for gestating sows; lactating sows may have slightly lower digestibility due to higher feed intake. Also, lactating sows do not consume enough feed to meet their energy needs, leading to body tissue mobilization and weight loss.
- **Feed intake and number of meals per day:** Increased feed intake and more frequent meals can enhance nutrient digestibility. Regular feeding helps maintain gut motility and reduces the risk of digestive disturbances. Studies indicate that pigs fed multiple smaller meals exhibit better nutrient absorption than those fed larger meals less frequently.

- **Use of antibiotics and feed additives:** including exogenous enzymes and other additives can improve nutrient breakdown and overall digestibility of complex feed components, further influencing ingredient rankings within different energy evaluation systems. Antibiotics can lead to dysbiosis, negatively impacting overall gut health and digestion.
- **Feed processing:** gelatinized starch is more easily broken down by digestive enzymes, resulting in higher and faster digestibility compared to raw or unprocessed starch. This increased digestibility leads to a greater proportion of energy being absorbed in the small intestine, contributing positively to the NE value of the feed. As the particle size of feed ingredients decreases, the NE increases. While smaller particles generally improve digestibility, excessively fine grinding can lead to adverse effects such as increased risk of gastric ulcers in pigs.
- **Intestinal health:** a healthy gut is crucial for optimal nutrient absorption. Factors such as the presence of beneficial microbiota and the integrity of the intestinal barrier play significant roles in nutrient digestibility. Conditions like inflammation or dysbiosis can impair nutrient absorption and decrease overall performance.

NE system shows better the “true” energy of the diet

Dr. Fledderus concluded that the NE system offers a closer estimate of pigs’ “true” energy available for maintenance and production (growth, lactation, etc.). This leads to better ingredient rankings, reduced crude protein levels, which decreases nitrogen excretion, and enhanced nutrient utilization, contributing to more sustainable pig production practices. This aligns with increasing demands for environmentally responsible farming methods.

EW Nutrition’s Swine Academy took place in Ho Chi Minh City and Bangkok in October 2024. Dr. Jan Fledderus, Product Manager and Consultant at the S&C team at Schothorst Feed Research, one of the founders of the Advanced Feed Package and with a strong focus on continuously improving the price/quality ratio of the diets for a competitive pig sector, was a reputable guest speaker in these events.

Start right with your piglet nutrition



Conference Report

“A good start is half the battle” can be said if we talk about piglet rearing. For this promising start, piglets must eat solid feed as soon as possible to be prepared for weaning. Dr. Jan Fledderus, Product Manager and Consultant at the S&C team at Schothorst Feed Research, shows some nutritional measures that can be taken to keep piglets healthy and facilitate the critical phase of weaning.

Higher number of low-birth-weight pigs in larger litters

Litter size affects piglet quality. Larger litter sizes from hyperprolific sows often result in higher within-litter variation in birth weights. This variability can lead to a higher proportion of low-birth-weight piglets, which are more susceptible to health issues and have lower survival rates. Additionally, low birthweight pigs have an increased risk of mortality, and an improvement in birth weight from 1kg to 1.8 kg can result in 10 kg more body weight at slaughter.

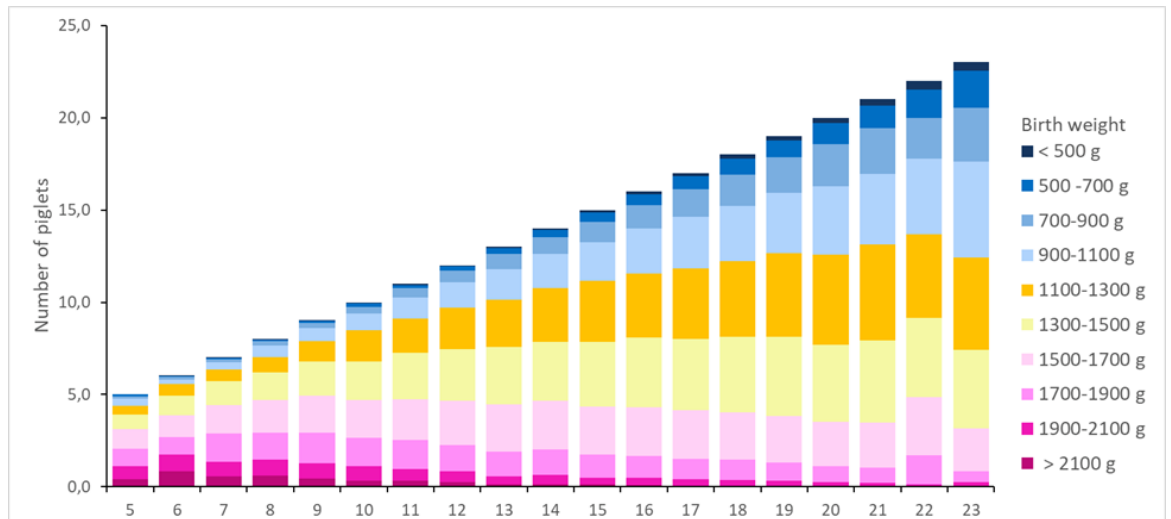


Figure 1: Effect of litter size on birth weight distribution (Schothorst Feed Research Data were collected from 2011 to 2020, based on 114,984 piglets born alive from 7,952 litters).

Implementing management practices for low-birth-weight pigs, such as split suckling, can significantly enhance nutrient intake, support immune function, and ultimately contribute to better survival rates and overall health for these vulnerable piglets.

Weaning age determines intake of creep feed

Pigs that consume creep feed before weaning restart faster to eat, have a higher feed intake, and less diarrhea after weaning. For instance, in a field trial, pigs that consumed feed 10 days before weaning had a 62% incidence of diarrhea, whereas in pigs that consumed feed only 3 days pre-weaning, diarrhea incidence increased to 86%.

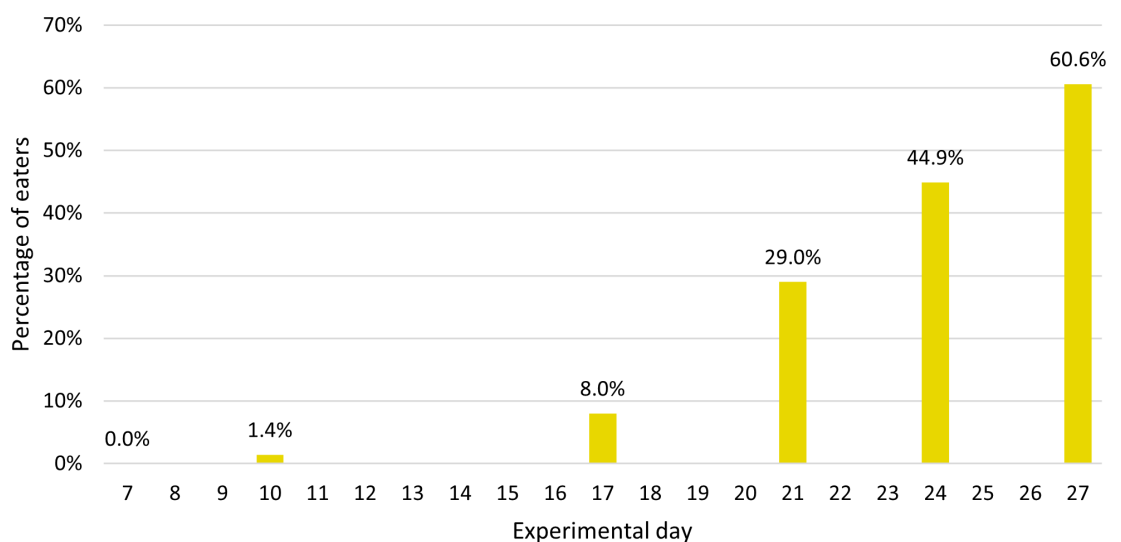


Figure 2: Influence of age on the percentage of pigs consuming creep feed

“As age is the most critical factor for a high percentage of pigs eating before weaning, there is a trend in the EU to increase the weaning age, where some farmers go to 35 days,” remarked Dr. Fledderus.

Furthermore, weaning age is positively correlated with weaning weight. Every day older at weaning improves post-weaning performance and reduces health problems.

Feed management

Creep feed for 7-10 days pre-weaning is essential, not to increase total feed intake, but to train the piglet to eat solid feed to avoid the 'post-weaning dip.' After about 15 days of age, piglets can consume more than is provided by milk alone. Dr. Fledderus strongly recommended creep feeding for at least one week before weaning. "Consuming feed before weaning will result in fewer problems with post-weaning diarrhea," he said.

In addition to creep feeding, a transition diet, from 7 days pre- and 7 days post-weaning, is advised. The composition or form of the transition diet should not be changed.

The key objective of post-weaning diets is to achieve a pH of 2-3.5 in the distal stomach. Pepsin, the primary enzyme responsible for protein digestion, is activated at a pH of around 2.0. Its activity declines significantly at a pH above 3.5, which can lead to poor protein digestion and nutrient absorption.

Fiber as a functional ingredient

Fiber was previously considered a nutritional burden or diluent, but now it is regarded as a functional ingredient. Including dietary fiber, mainly inert fiber such as rice or wheat brans, can increase the retention time of the digesta in the stomach. This extended retention allows for more prolonged contact between digestive enzymes and nutrients, facilitating improved digestion and absorption of proteins and other nutrients. Not only is pH reduced, but because more proteins are hydrolyzed to peptides, there is less undigested protein as a substrate for the growth of pathogenic bacteria and the production of toxic metabolites in the hindgut.

"Size of fiber particles also matters," said Dr. Fledderus. Coarse wheat bran particles (1,088 μm) have been shown to be more effective than finer particles (445 μm) in reducing E. coli levels in the gut. The larger particle size helps prevent E. coli from binding to the intestinal epithelium, allowing these bacteria to be excreted rather than colonizing the gut.

The understanding of dietary fiber's role in pig nutrition has evolved, with recent findings indicating that fiber can actually increase feed intake in piglets, contrary to earlier beliefs that it might decrease intake. High-fiber diets often increase feed intake as pigs compensate for lower energy density. This can help maintain growth rates when formulated correctly.

EW Nutrition's Swine Academy took place in Ho Chi Minh City and Bangkok in October 2024. Dr. Jan Fledderus, Product Manager and Consultant at the S&C team at Schothorst Feed Research, one of the founders of the Advanced Feed Package and with a strong focus on continuously improving the price/quality ratio of the diets for a competitive pig sector, was a reputable guest speaker in these events.

Nutritional strategies to maximize the health and productivity of

SOWS



Conference Report

During lactation, the focus should be on maximizing milk production to promote litter growth while reducing weight loss of the sow, stated Dr. Jan Fledderus during the recent EW Nutrition Swine Academies in Ho Chi Minh City and Bangkok. A high body weight loss during lactation negatively affects the sow's performance in the next cycle and impairs her longevity.

Milk production - 'push' or 'pull'?

"Is a sow's milk production driven by "push" - the sow is primarily responsible for milk production, or "pull" - suckling stimulates the sow to produce milk?" asked Dr. Jan Fledderus at the beginning of his presentation. The answer is that it is primarily a pull mechanism: piglets that suckle effectively and frequently can activate all compartments of the udder, leading to increased milk production. Therefore, the focus should be optimizing piglet suckling behavior (pull) to enhance milk production. This highlights the importance of piglet vitality and access to the udder in maximizing milk yield."

Modern sows are lean

Modern sows have been genetically selected for increased growth rates and leanness, which alters their body composition. This makes traditional body condition scoring (BCS) methods, which rely on subjective visual assessment and palpation of backfat thickness, less effective. This may not accurately represent a sow's true condition, especially in leaner breeds where muscle mass is more prominent than fat. Technology, such as ultrasound measurements of backfat and loin muscle depth, provide more accurate assessments of body condition and can help quantify metabolic reserves more effectively than visual

scoring.

Due to their increased lean body mass, we must consider adjusted requirements for amino acids, energy, digestible phosphorus, and calcium. Their dietary crude protein and amino acid requirements have increased drastically.

Importance of high feed intake for milk production

Sows typically catabolize body fat and protein to meet the demands of milk production. Adequate feed intake helps reduce this catabolism, allowing sows to maintain body condition while supporting their piglets' nutritional needs.

Feeding about 2.5kg on the day of farrowing ensures that sows receive adequate energy to support the farrowing process and subsequent milk production. Sows that are well-fed before farrowing tend to have shorter farrowing durations due to better energy availability during labor.

A short interval between the last feed and the onset of farrowing (3 hours) has been shown to significantly reduce the probability of both assisted farrowing and stillbirths without increasing the risk of constipation. To enhance total feed intake, feeding lactating sows at least three times a day is helpful.

Dr. Fledderus recommended a gradual increase in feed intake during lactation, then from day 12 of lactation to weaning, feeding 1% of sow's bodyweight at farrowing + 0.5 kg/piglet. For example, for a 220kg sow with 12 piglets:

$$(220 \text{ kg} \times 0.01) + (12 \times 0.5 \text{ kg}) = 2.2 + 6 = 8.2 \text{ kg total daily feed intake}$$

Energy source - starch versus fat

The choice between starch and fat as an energy source in sow diets has substantial implications for body composition and milk production.

Starch digestion leads to glucose release, stimulating insulin secretion from the pancreas. Insulin is essential for glucose uptake and utilization by tissues. Enhanced insulin response can help manage body weight loss by promoting nutrient storage and reducing the mobilization of the sow's body reserves.

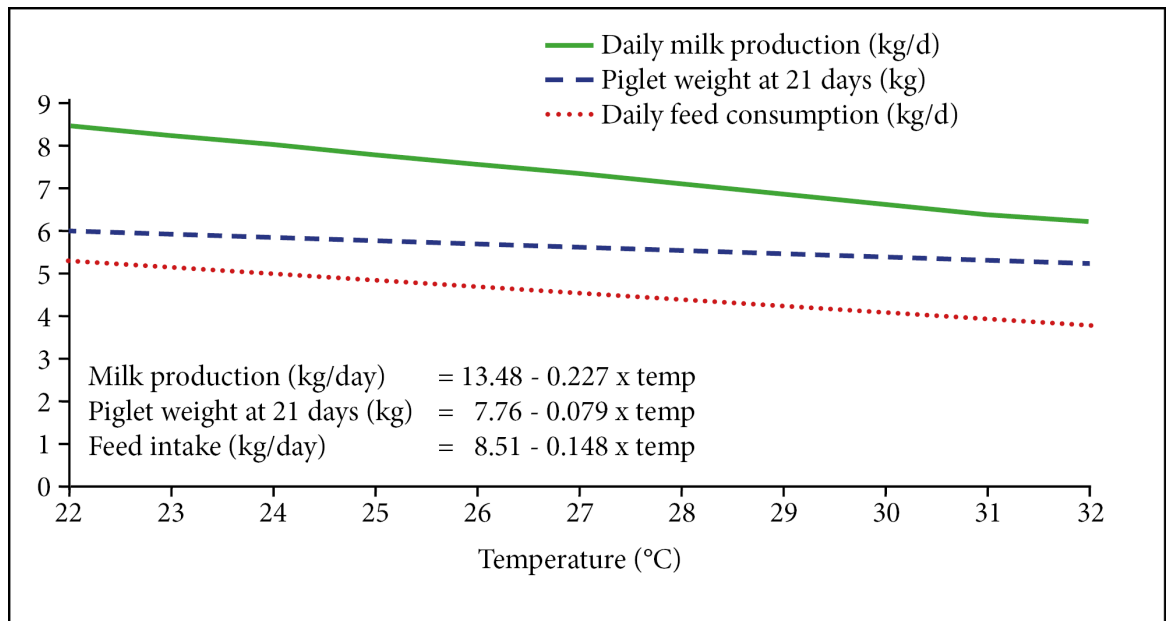
Sows fed diets with a higher fat supplementation had an increased milk fat, which is crucial for the growth and development of piglets.

Table 1: Effect of energy source (starch vs. fat) on sows' body composition and milk yield (Schothorst Feed Research)

	Diet 1	Diet 2	Diet 3
Energy value (kcal/kg)	2,290	2,290	2,290
Starch (g/kg)	300	340	380
Fat (g/kg)	80	68	55
Feed intake (kg/day)	6.7	6.7	6.8
Weight loss (kg)	15	11	10
Weight loss (kg)	3.1	2.7	2.3
Milk fat (%)	7.5	7.2	7.0
Milk fat (%)	260	280	270

Heat stress impacts performance

The optimum temperature for lactating sows is 18°C. A meta-analysis concluded that each 1°C above the thermal comfort range (from 15° to 25°C) leads to a decrease in sows' feed intake and milk production and weaning weight of piglets, as shown below.



Effect of heat stress on lactating sows (according to Ribeiro et. al., 2018 Based on 2,222 lactating sows, the duration of lactation was corrected to 21 days)

To mitigate the effects of heat stress, which reduces feed intake, it is beneficial to schedule feeding during cooler times of the day. This strategy helps maintain appetite and ensures that sows consume sufficient nutrients for milk production. Continuous access to cool, clean water can also enhance feed consumption.

Pigs produce much heat, which must be “excreted”. Increased respiratory rate (>50 breaths/minute) has been shown to be an efficient parameter for evaluating the intensity of heat stress in lactating sows.

When sows resort to panting as a mechanism to dissipate heat, this rapid breathing increases the loss of carbon dioxide, resulting in respiratory alkalosis. To prevent a rise in blood pH level, HCO_3 is excreted via urine, and positively charged minerals (calcium, phosphorous, magnesium, and potassium) are needed to facilitate this excretion. However, these minerals are crucial for various physiological functions. As their loss can lead to deficiencies that affect overall health and productivity, this mineral loss must be compensated for.

Implications for management

Implementing effective nutritional strategies together with robust management practices is crucial for maximizing the health and productivity of sows. The priority is to stimulate the sow to eat more. This not only enhances milk production and litter growth but also supports the overall well-being of the sow. Regularly assessing sow performance metrics – such as body condition score, feed intake, and litter growth – can help identify areas for improvement in nutritional management.

EW Nutrition's Swine Academy took place in Ho Chi Minh City and Bangkok in October 2024. Dr. Jan Fledderus, Product Manager and Consultant at the S&C team at Schothorst Feed Research, with a strong focus on continuously improving the price/quality ratio of the diets for a competitive pig sector and one of the founders of the Advanced Feed Package, was a reputable guest speaker in these events.

Health management of nursery piglets through nutrition



Conference Report

An optimized gut function is essential for pigs' overall health and performance. When managed correctly, gut health can significantly enhance growth, immunity, and productivity. However, if gut health is compromised, it can lead to lifetime negative impacts on a pig's performance.

Early feed intake enhances GIT development

Dr. Edwards emphasized that good health and performance in the nursery are closely linked to maintaining feed intake, which is essential for developing stomach capacity and function. A larger stomach capacity increases the exposure to digestive enzymes and prolongs stomach dwell time.

Acid output takes time to develop and develops in response to substrate. It directly influences stomach pH and is closely related to pepsin output, which, on its part, influences protein digestibility and the risk of diarrhea.

Protein and immunity

Protein is a double-edged sword, warned Dr. Edwards:

- Excess or undigested protein can create inflammation and oxidative stress in the body. This occurs when the metabolism of surplus protein leads to the production of reactive oxygen species (ROS), which can damage cells and tissues, further exacerbating inflammatory responses. Chronic inflammation may impair immune responses, making pigs more susceptible to infections and diseases.
- On the other hand, a deficiency in amino acids can limit immune response. Amino acids do more than build muscle – they are critical for synthesizing antibodies and other immune-related proteins. Without adequate levels, pigs may struggle to mount effective immune responses, increasing their vulnerability to pathogens.

Table 1: Effects of amino acids on pig gut health and functions (Yang & Liao, 2019)

Amino acid	Functions
Glutamine/glutamate	• Metabolic fuel for rapidly dividing cells, including lymphocytes, enterocytes • maintains or enhances villus height/crypt depth • enhances microbial diversity • is utilized to synthesize GSH and protect against oxidative stress • stimulates both innate and adaptive immunity
Arginine	• promotes intestinal healing and reverses intestinal dysfunction • has anti-inflammatory effects
Cysteine	• is utilized to synthesize GSH (antioxidant) • utilized to synthesize taurine (antioxidant/cell membrane stabilizer) • utilized for mucin synthesis (physical protection)
Threonine	• utilized for mucin synthesis • important component of immunoglobulins • enhances microbial diversity
Glycine	• anti-inflammatory effects • utilized to synthesize GSH (antioxidant)
Methionine	• acts as an antioxidant by protecting other proteins against oxidative damage • important for the proliferation of lymphocytes

Diets should be formulated to all ten essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) while ensuring a ratio of about 50:50 for essential amino acids to non-essential amino acids is optimal for nitrogen retention and utilization in pigs.

During immune challenges, the pig's amino acid requirements, including methionine, cysteine, tryptophan, threonine, and glutamine, increase relative to lysine. Well-known examples are threonine, a key component of mucin (and immunoglobulins), supporting gut health and integrity during stress, and glutamine, a major energy source for rapidly dividing cells in the immune system.

Microbiome evolution and modulation

The microbiota of the pig evolves from birth up to about 20 weeks of age. The alpha diversity (the number of species) and species richness increase with age. The pig microbiome consists of both permanent members that establish stable populations throughout life and transient members that may fluctuate based on dietary changes or environmental factors.

Microbiome modulation through the diet

Diet can influence the rate and maturity of microbiota evolution. For instance, diets rich in fiber and specific carbohydrates can promote the growth of beneficial bacteria such as *Lactobacillus* and

Bifidobacterium. In contrast, diets high in protein can increase potentially harmful bacteria if not appropriately balanced.

Understanding these dynamics is critical for optimizing nutrition strategies that support gut health and overall performance in pigs. Proper management of dietary components can lead to healthier microbiomes, enhancing nutrient absorption and immune responses throughout the pig's life.

The following strategies accelerate the maturation of the microbiome, the gut, and the immune system:

- Promoting and maintaining feed intake: consistent feed intake is crucial for microbial development. Early access to solid feed helps establish a diverse microbiome.
- Raw material continuity: variability in feed composition can disrupt microbial communities, leading to dysbiosis. A step-wise approach to diet changes, with a broad range of ingredients at low inclusion levels, is recommended.
- Regulating digest transit time: the rate at which digesta moves through the gastrointestinal tract affects nutrient absorption and microbial colonization. Strategies to optimize transit time, such as increasing particle size and incorporating insoluble fibers, can enhance nutrient digestibility and promote a healthy microbiome by allowing beneficial microbes to thrive.
- Feeder access: adequate access to feeders encourages regular feeding behavior, supporting consistent nutrient intake and microbial activity. Frequent feeding can help maintain stable gut conditions conducive to microbial growth.
- Inert fiber: helps maintain gut motility and provides substrates for beneficial bacteria, contributing to a balanced microbiome.
- Minimizing stress: stress can negatively impact gut integrity and microbial balance, increasing susceptibility to infections and other health issues.
- Limiting the use of antibiotics helps preserve the natural gut microbiota, which is essential for maintaining health and preventing disease. The use of antibiotics can lead to dysbiosis, making pigs more vulnerable to infections and impairing immune responses.

Limitations in the use of AGPs, Zn, and Cu require rethinking in pig nutrition

Reduced access to in-feed antibiotics and pharmacological levels of zinc and copper have exposed nutritional shortcomings for nursery pigs. Preventive strategies through nutrition, carefully designed diets, and optimal use of eubiotics and functional ingredients are the keys to getting pigs off to a healthy and efficient start.

Nursery nutrition programs should be designed for long-term gut health, efficiency, and functionality. The level of investment will depend on the weaning age/weight, health status, labor quality, etc., noted Dr. Edwards.

EW Nutrition's Swine Academy took place in Ho Chi Minh City and Bangkok in October 2024. Dr. Megan Edwards, an Australian animal nutrition consultant with global research and praxis experience and a keen interest in immuno-nutrition and functional nutrients, was an esteemed guest speaker at this event.

Sustainability will push more by-

products into pig feed - Keep track of mycotoxins!



Mycotoxin Team EW Nutrition

Most grains used in feed are susceptible to [mycotoxin contamination](#), causing severe economic losses all along feed value chains. As skyrocketing raw material prices force producers to include a higher proportion of economical cereal by-products in the feed, the risks of mycotoxin contamination likely increase. This article reviews why mycotoxins cause the damage they do - and how effective toxin-mitigating solutions prevent this damage.

Mycotoxin contamination of cereal by-products requires solutions

Cereal by-products may become more important feed ingredients as grain prices increase. However, from a sustainability point of view and considering population growth, using cereal by-products in animal feed [makes much sense](#). Distiller's dried grains with solubles (DDGS) are a good example of how by-products from food processing industries can become [high-quality animal feed](#).

Share of protein source in EU & UK 2019-2020 (84 mt. of crude protein)

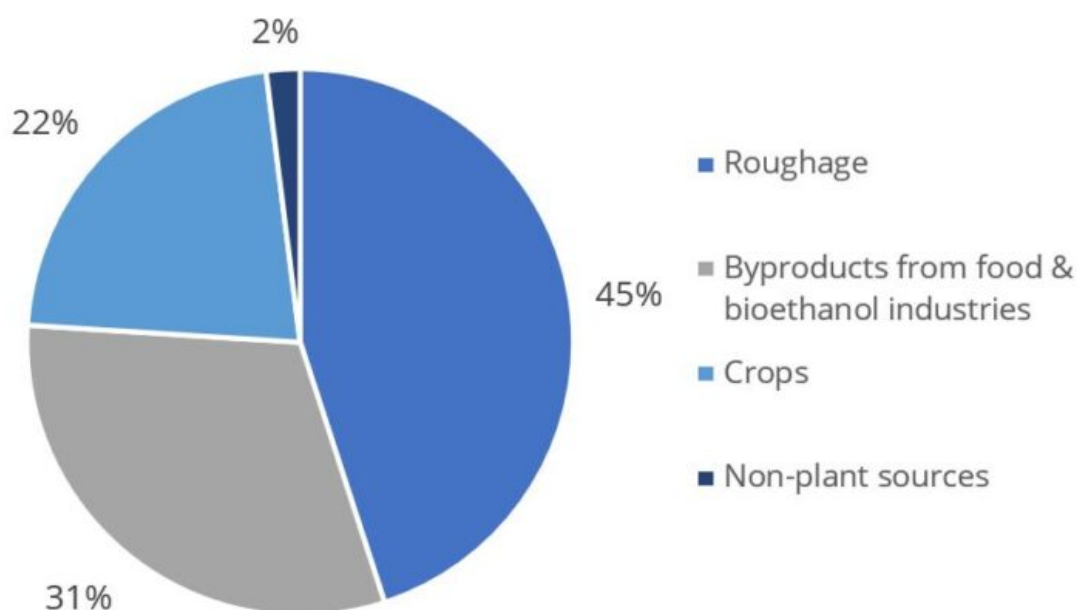


Figure 1: By-products are a crucial protein source (data from FEFAC Feed&Food 2021 report)

Still, research on what happens to mycotoxins during food processing shows that mycotoxins are concentrated into fractions that are commonly used as animal feed (cf. [Pinotti et al., 2016](#); [Caballero and Heinzl, 2022](#)). To safeguard animal health and performance when feeding lower-quality cereals, monitoring mycotoxin risks through regular testing and using toxin-mitigating solutions is essential.

Problematic effects of mycotoxins on the intestinal epithelium

Most mycotoxins are absorbed in the proximal part of the gastrointestinal tract. This absorption can be high, as in the case of aflatoxins (ca. 90%), but also very limited, as in the case of fumonisins (< 1%); moreover, it depends on the species. Notably, a significant portion of unabsorbed toxins remains within the lumen of the gastrointestinal tract.

Importantly, studies based on realistic mycotoxin challenges (e.g., [Burel et al., 2013](#)) show that the mycotoxin levels necessary to trigger damaging processes are lower than the [levels reported as safe](#) by EFSA, the Food Safety Agency of the European Union. The ultimate consequences range from diminished nutrient absorption to inflammatory responses and pathogenic disorders in the animal (Figure 2).

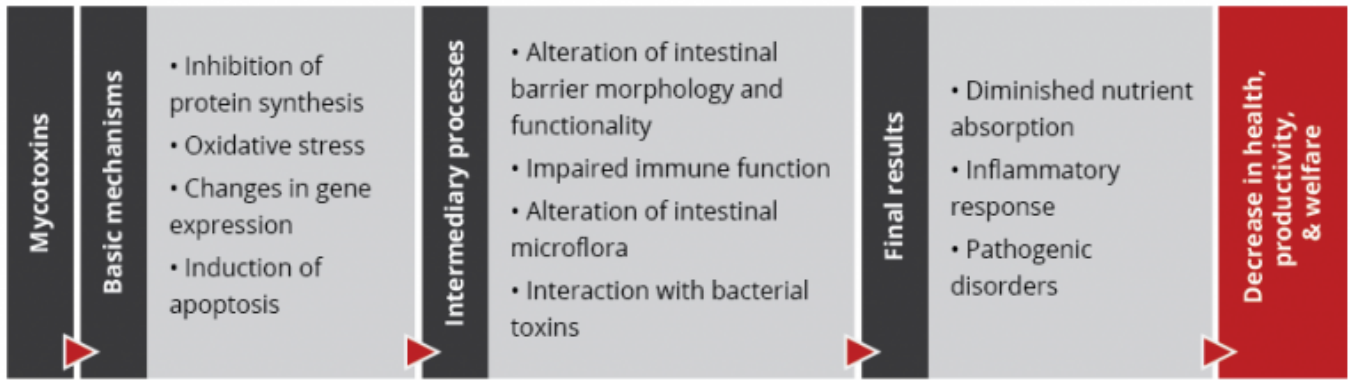


Figure 2: Mycotoxins' impact on the GIT and consequences for monogastric animals

1. Alteration of the intestinal barrier's morphology and functionality

Several studies indicate that mycotoxins such as aflatoxin B1, DON, fumonisin B1, ochratoxin A, and T2, can increase the permeability of the intestinal epithelium of poultry and swine (e.g., [Pinton & Oswald, 2014](#)). This is primarily a consequence of the inhibition of protein synthesis.

As a result, there is an increase in the passage of antigens into the bloodstream (e.g., bacteria, viruses, and toxins). This increases the animal's susceptibility to infectious enteric diseases. Moreover, the damage that mycotoxins cause to the intestinal barrier entails that they are also being absorbed at a higher rate.

2. Impaired immune function in the intestine

The intestine is a very active immune site, where several immuno-regulatory mechanisms simultaneously defend the body from harmful agents. [Immune cells are affected by mycotoxins](#) through the initiation of apoptosis, the inhibition or stimulation of cytokines, and the induction of oxidative stress.

3. Alteration of the intestinal microflora



Recent studies on the effect of various mycotoxins on the intestinal microbiota show that [DON and other trichothecenes favor the colonization of coliform bacteria in pigs](#). DON and ochratoxin A also induce a [greater invasion of *Salmonella*](#) and their translocation to the bloodstream and vital organs in birds and pigs – even at non-cytotoxic concentrations.

It is known that fumonisin B1 may induce changes in the balance of sphingolipids at the cellular level, including for gastrointestinal cells. This facilitates the adhesion of pathogenic bacteria, increases in their populations, and prolongs infections, [as has been shown in the case of *E. coli*](#). The colonization of the intestine of food-producing animals by pathogenic strains of *E. coli* and *Salmonella* also poses a risk to

human health.

4. Interaction with bacterial toxins

When mycotoxins induce changes in the intestinal microbiota, this can increase the endotoxin concentration in the intestinal lumen. [Endotoxins promote the release of several cytokines](#) that induce an enhanced immune response, causing inflammation, thus reducing feed consumption and animal performance, damage to vital organs, sepsis, and death of the animals in some cases.

The synergy between mycotoxins and endotoxins can result in an overstimulation of the immune system. The interaction between endotoxins and estrogenic agents such as zearalenone, for example, generates [chronic inflammation and autoimmune disorders](#) because immune cells have estrogen receptors, which are stimulated by the mycotoxin.

Increased mycotoxin risks through by-products? Invest in mitigation solutions

To prevent the detrimental consequences of mycotoxins on animal health and performance, proactive solutions are needed that support the intestinal epithelium's digestive and immune functionality and help maintain a balanced microbiome in the GIT. This becomes even more important as the current market conditions will likely engender a long-term shift towards including more cereal by-products in animal diets.

Trial data shows that EW Nutrition's toxin-mitigating solution SOLIS MAX 2.0 provides adequate protection against feedborne mycotoxins. The synergistic combination of ingredients in SOLIS MAX 2.0 prevents mycotoxins from damaging the animals' gastrointestinal tract and entering the bloodstream and additionally acts as antioxidant and liver-protecting:

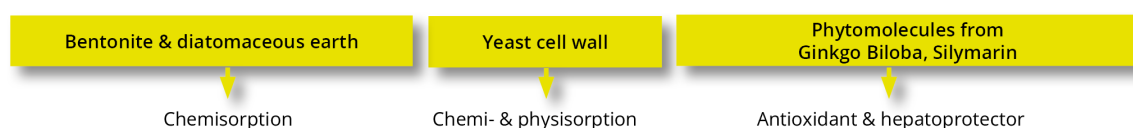


Figure 3: Moa of Solis Max 2.0

In-vitro study shows strong mitigation effects of SOLIS MAX 2.0 against a wide range of mycotoxins

Animal feed is often contaminated with two or more mycotoxins, making it essential for an anti-mycotoxin agent to be effective against a wide range of different mycotoxins. A trial with SOLIS MAX 2.0 was conducted at an independent laboratory in Spain with an inclusion level of the product of 0.10% (equivalent to 1 kg per ton of feed). A phosphate buffer solution at pH 7 was prepared to simulate intestinal conditions in which a portion of the mycotoxins may be released from the binder (desorption). The following mycotoxins were evaluated in the test (see Table 1):

Table 1: Mycotoxin challenges

Mycotoxin	Challenge (ppb)
Aflatoxin B1 (AFB1)	100
Deoxynivalenol (DON)	1,000
Fumonisin B1 (FB1)	2,000
T-2 toxin (T-2)	500
Ochratoxin A (OTA)	500
Zearalenone (ZEA)	1,000

Each mycotoxin was tested separately by adding a challenge to buffer solutions, incubating for one hour at 41°C, to establish the baseline (table). At the same time, a solution with the toxin challenge and Solis Max 2.0 was prepared, incubated, and analyzed for the residual mycotoxin to find the binding efficacy. All analyses were carried out using high-performance liquid chromatography (HPLC) with standard detectors.

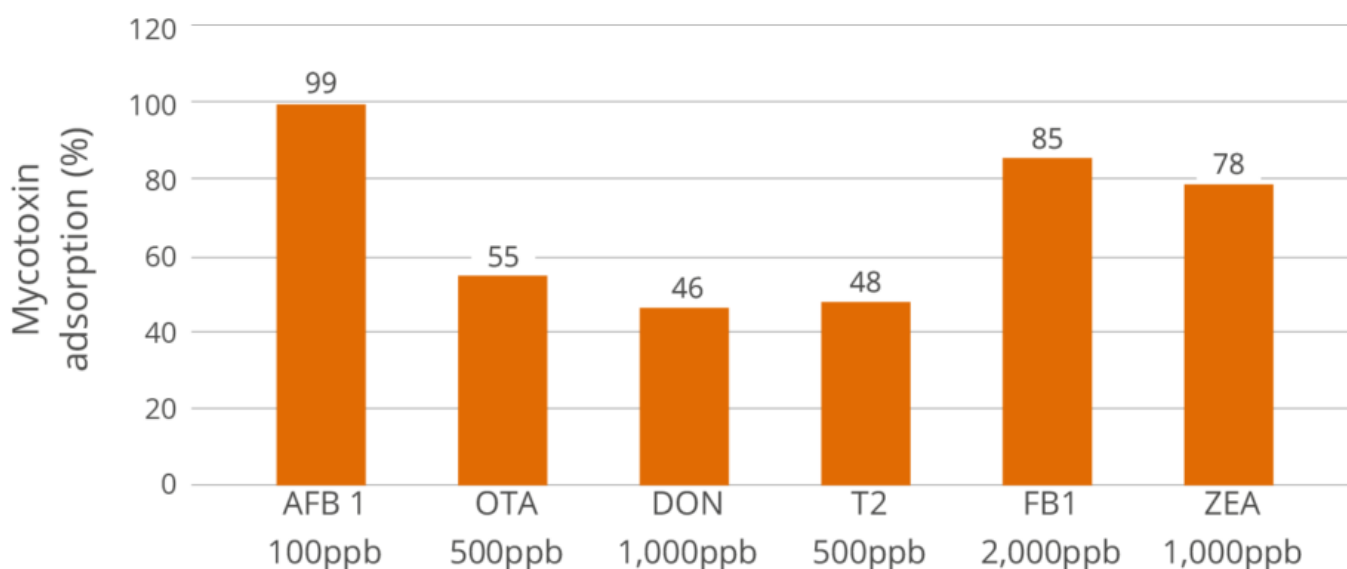


Figure 4: SOLIS MAX 2.0 (1 kg/t of feed) adsorption capacity against different mycotoxins (%)

The results (Figure 4) demonstrate that SOLIS MAX 2.0 is a highly effective solution against the most common mycotoxins in raw materials and animal feed.

Mycotoxin risk management for better animal feed

A healthy gastrointestinal tract is crucial to animals' overall health: it ensures that nutrients are optimally absorbed, provides adequate protection against pathogens through its immune function, and is key to maintaining a well-balanced microflora. Even at levels considered safe by the European Union, mycotoxins can compromise different intestinal functions, resulting in lower productivity and susceptibility to disease.

The globalized feed trade, which spreads mycotoxins beyond their geographical origin, climate change, and raw material market pressures additionally escalate the problem. On top of rigorous testing, producers should mitigate unavoidable mycotoxin exposures by using solutions such as SOLIS MAX 2.0 – for stronger animal health, welfare, and productivity.

References

- Antonissen, Gunther, An Martel, Frank Pasmans, Richard Ducatelle, Elin Verbrugghe, Virginie Vandenbroucke, Shaoji Li, Freddy Haesebrouck, Filip Van Immerseel, and Siska Croubels. "The Impact of Fusarium Mycotoxins on Human and Animal Host Susceptibility to Infectious Diseases." *Toxins* 6, no. 2 (January 28, 2014): 430–52. <https://doi.org/10.3390/toxins6020430>.
- Burel, Christine, Mael Tanguy, Philippe Guerre, Eric Boilletot, Roland Cariolet, Marilyne Queguiner, Gilbert Postollec, et al. "Effect of Low Dose of Fumonisin on Pig Health: Immune Status, Intestinal Microbiota and Sensitivity to Salmonella." *Toxins* 5, no. 4 (April 23, 2013): 841–64. <https://doi.org/10.3390/toxins5040841>.
- Burton, Emily J., Dawn V. Scholey, and Peter E. Williams. "Use of Cereal Crops for Food and Fuel – Characterization of a Novel Bioethanol Coproduct for Use in Meat Poultry Diets." *Food and Energy Security* 2, no. 3 (September 19, 2013): 197–206. <https://doi.org/10.1002/fes3.30>.
- Ghareeb, Khaled, Wageha A. Awad, Josef Böhm, and Qendrim Zebeli. "Impacts of the Feed Contaminant Deoxynivalenol on the Intestine of Monogastric Animals: Poultry and Swine." *Journal of Applied Toxicology* 35, no. 4 (October 28, 2014): 327–37. <https://doi.org/10.1002/jat.3083>.
- Mani, V., T. E. Weber, L. H. Baumgard, and N. K. Gabler. "Growth and Development Symposium: Endotoxin, Inflammation, and Intestinal Function in livestock1,2." *Journal of Animal Science* 90, no. 5 (May 1, 2012): 1452–65. <https://doi.org/10.2527/jas.2011-4627>.
- Obremski, K. "The Effect of in Vivo Exposure to Zearalenone on Cytokine Secretion by Th1 and Th2 Lymphocytes in Porcine Peyer's Patches after in Vitro Stimulation with LPS." *Polish Journal of Veterinary Sciences* 17, no. 4 (2014): 625–32. <https://doi.org/10.2478/pjvs-2014-0093>.
- Oswald, I. P., C. Desautels, J. Laffitte, S. Fournout, S. Y. Peres, M. Odin, P. Le Bars, J. Le Bars, and J. M. Fairbrother. "Mycotoxin Fumonisin B1 Increases Intestinal Colonization by Pathogenic Escherichia Coli in Pigs." *Applied and Environmental Microbiology* 69, no. 10 (2003): 5870–74. <https://doi.org/10.1128/aem.69.10.5870-5874.2003>.
- Pinotti, Luciano, Matteo Ottoboni, Carlotta Giromini, Vittorio Dell'Orto, and Federica Cheli. "Mycotoxin Contamination in the EU Feed Supply Chain: A Focus on Cereal Byproducts." *Toxins* 8, no. 2 (February 15, 2016): 45. <https://doi.org/10.3390/toxins8020045>.
- Pinton, Philippe, and Isabelle Oswald. "Effect of Deoxynivalenol and Other Type B Trichothecenes on the Intestine: A Review." *Toxins* 6, no. 5 (May 21, 2014): 1615–43. <https://doi.org/10.3390/toxins6051615>.
-

Successful weaning requires adequate pre-weaning preparation



Conference report

The abrupt transition from the sow's milk to solid feed, combined with environmental changes and social restructuring, creates a challenging situation for young piglets. Weaning is a critical phase that subjects piglets to multiple stressors, which can have cumulative effects on their health and development. Weaning stressors are inevitable in the piglets' development; however, effective pre-weaning management practices can significantly minimize their impact on health and performance.

Pre-weaning measures help improve weaner performance.

"Successful weaning of piglets is a multifaceted process that requires careful management and strategic planning well before the actual weaning event," says Dr. Merideth Parke, Global Application Manager, Swine, EW Nutrition. She emphasized the following key pre-weaning factors that can significantly influence success during this most critical time.

Genetics

Selecting the right genetics for your specific production system is crucial for ensuring successful weaning outcomes. The genetic traits of sows with a direct impact include sow resilience, litter size, piglet birth weights, and overall growth rates.

Furthermore, it is decisive for piglets' survival and performance that the sow shows strong maternal instincts, and, to ensure enhanced colostrum and milk uptake, an adequate number of functional teats and high milk production.

Gestation and farrowing influencers

Having an optimal body condition score at farrowing is essential for sows. Being overweight or underweight poses the risk of prolonged farrowing and reduced colostrum and milk production.

On the piglet side, prolonged farrowing negatively impacts their vitality at birth, which correlates with reduced colostrum uptake and increased pre-weaning mortality rates.

Environmental conditions

Newborn piglets are particularly vulnerable to hypothermia and have a minimal critical temperature of 33-35°C. Below this range, they struggle to maintain their body temperature, which can lead to increased mortality rates. Cold piglets are less likely to suckle, compromising their energy reserves and ability to maintain body temperature.

In contrast, lactating sows have an optimal temperature of 18-22°C to maximize feed intake and milk production. Therefore, to balance the temperature needs of sow and piglets, it is essential to create a controlled temperature, draft-free creep microenvironment for piglets.

Hygiene

The hygiene of farrowing crates plays an essential role in the successful weaning of piglets. Maintaining a clean environment significantly impacts the health and growth of piglets, ultimately influencing their survival and weight at weaning. "We must consider the time spent cleaning, disinfecting, and drying farrowing crates an investment, not a cost," emphasized Dr. Parke. "Doing these routine tasks really well will inevitably reduce the time spent treating sick pigs."

Lactation phase

The primary objective of pre-weaning measures is to ensure adequate colostrum and milk production throughout lactation while beginning the adjustment to solid feed. Efforts should be directed toward facilitating nursing access for all piglets, with particular attention to smaller or weaker ones probably facing difficulties accessing teats.

Split suckling can be the method of choice for improving their colostrum and milk intake, particularly in large litters. For that measure, larger, more robust piglets are separated, allowing smaller or weaker piglets to nurse first. Once the weaker piglets have had sufficient time, the groups are swapped.

However, according to Dr. Parke, fostering piglets is recommended to be undertaken cautiously. "While it can be beneficial, it can significantly disrupt pathogen stability and teat hierarchy, particularly when it occurs after the first 24-48 hours of birth when piglets have established their preference for specific teats. This can increase fighting among piglets as they establish a new hierarchy. This aggression can result in injuries, especially for weaker or smaller piglets. Fighting can also cause damage to the sow's udder, leading to infections or mastitis, compromising milk production and overall sow health," she stated.

Nurturing the gut

Providing creep feed for a minimum of 7 days before weaning significantly boosts litter weight at weaning and reduces the risk of post-weaning fallback. Early exposure to solid feed accelerates the development of digestive enzymes and acid production, both essential for breaking down carbohydrates and proteins.

Combining pre-weaning creep feeding with high-quality, palatable post-weaning diets has been shown to lead to piglets with increased post-weaning feed intake, health, and growth during the critical post-weaning transition.

As the swine sector evolves with larger litter sizes and, therefore, increased competition for sows' milk, using milk replacers is becoming common practice. Following a "little and often" approach by providing small amounts of fresh milk replacer multiple times a day is most effective. The hygienic preparation and feeding of milk replacers go without saying to prevent the growth of harmful bacteria and molds that can lead to diarrhea and other health issues in piglets.



Collaborative approach

The swine industry is grappling with mounting challenges associated with post-weaning stress and health, exacerbated by the prohibition of AGPs and the use of pharmacological levels of dietary zinc and copper in many regions. Addressing these issues requires a coordinated strategy to improve piglet welfare and optimize production outcomes. "By adopting a proactive approach emphasizing collaboration and comprehensive management strategies across the production system, piglet welfare and long-term productivity can be enhanced," concluded Dr. Parke.

EW Nutrition's Swine Academy took place in Ho Chi Minh City and Bangkok in October 2024. Dr. Merideth Parke, Global Application Manager, Swine, was one of the highly experienced speakers of EW Nutrition. She is a veterinarian who strongly focuses on swine health and preventive medicine.

Organic acids can play a crucial role in zinc oxide replacement



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

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

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

ZnO has a multifactorial mode of action

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

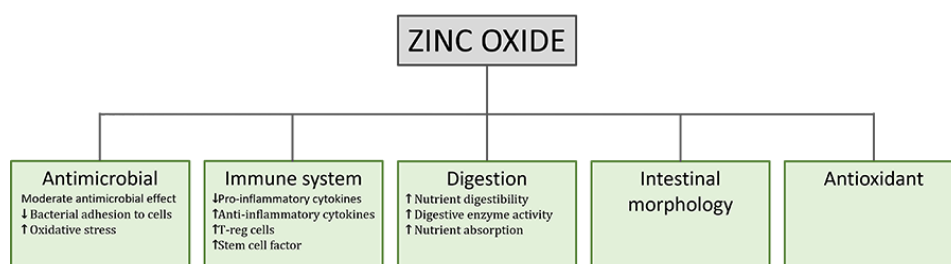


Figure 1. Beneficial effects and ZnO mode of action in postweaning piglets

1. ZnO acts as an antimicrobial

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

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

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

2. ZnO modulates the immune system

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

ZnO reduces the expression of several proinflammatory cytokines induced by ETEC ([Roselli et al., 2003](#)). Several studies have also shown a modulation effect on intestinal inflammation, decreasing levels of IFN- γ , TNF- α , IL-1 β 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 $\text{pH}<5$, 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 (II)- 1β 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



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

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

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

Which factors can contribute to meat quality?

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

drumsticks, breast), processing also affects the quality of meat and meat products.

Physical characteristics are objective determinants of meat quality

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

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

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

A further criterion is the composition of the fat, the fatty acids. Geese fat, e.g., is known for its high content of oleic, linoleic, linolenic, and arachidonic acid, all of them derivatives 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 ([Motttram, 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. OF04-11AC 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 *Lachnospirillum* and Christensenellaceae.

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 Tibetan 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 Christensenellaceae 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 vadinBE97 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 β -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, glycine, serine, 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 *Lachnospirillum*. 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 β -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 β -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:

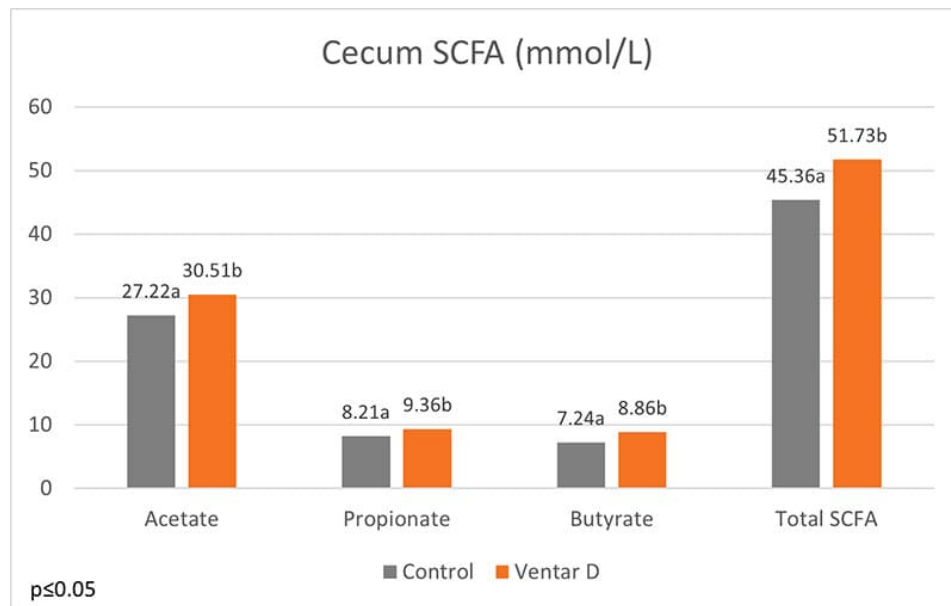


Figure 1: Short-chain fatty acids in the cecum of broilers

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 ($\pm$ 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.

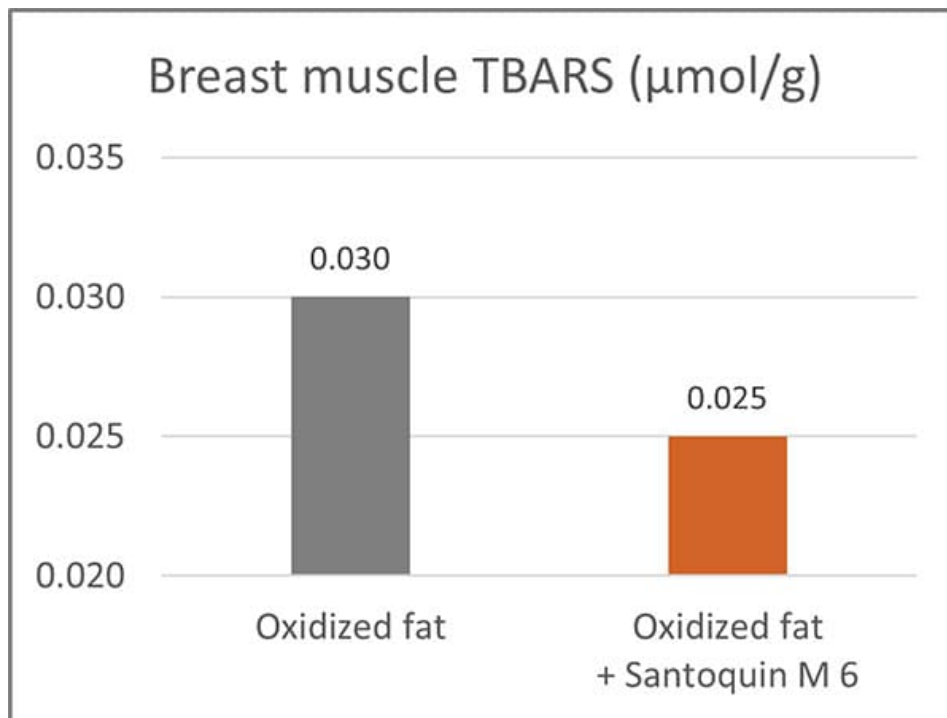


Figure 2: Thiobarbituric acid reactive substances (TBARS) in broiler breast muscles. TBARS are formed as a by-product of lipid peroxidation.

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.

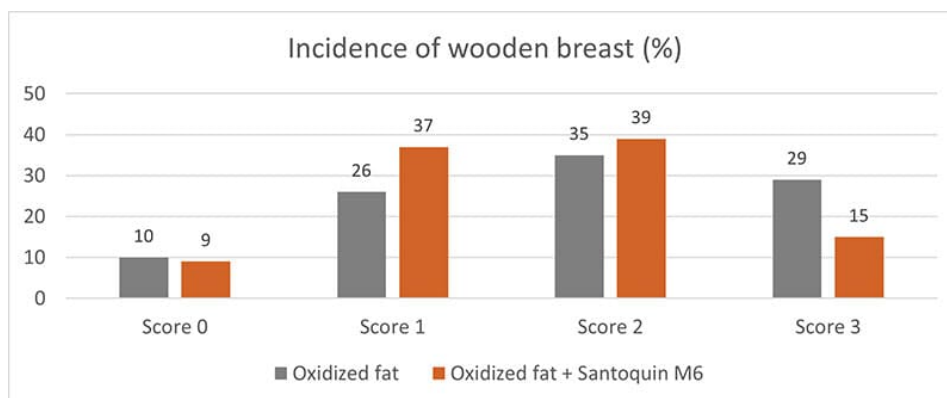


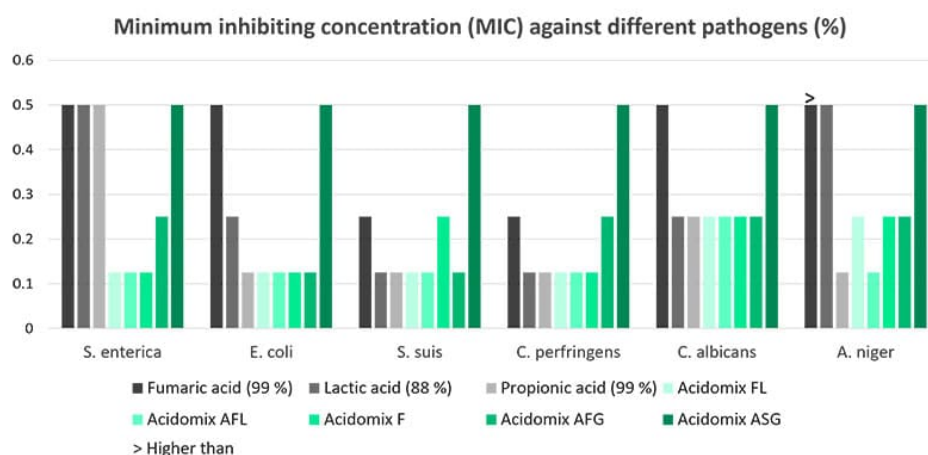
Figure 3: Incidence of wooden breast in broilers

*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. "EFFECT 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)." *PeerJ*; 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://ew-nutrition.com/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. Zygodlo, 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](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>.