

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.

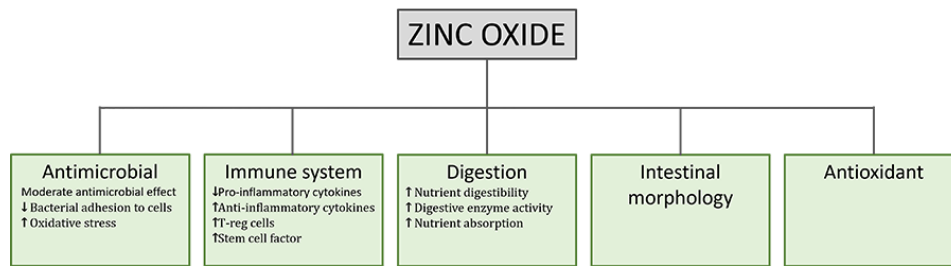


Figure 1. Beneficial effects and ZnO mode of action in postweaning piglets

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 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 + SiO₂ (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)-1 β 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

Feed hygiene protects animals and humans



By **Vaibhav Gawande**, Assistant Manager Technical Services, **Dr. Inge Heinzl**, Editor, and Technical Team, EW Nutrition

The utility value of feed consists of the nutritional value and the quality. The first covers all characteristics concerning the essential nutrients and is important for feed formulation and the adequate supply of the animals.

Feed quality comprises all characteristics of a feed influenced by treatment, storage, conservation, hygiene, and its content of specific substances. For many factors, guidance and threshold values are available which should be met to guarantee animal health and welfare, as well as to protect public health, since some undesirable substances can be transferred to animal products such as meat, eggs, and milk.

In this article, we will focus on feed hygiene. We will talk about the consequences of low feed quality, how to understand it, its causes, and possible solutions.

What are the effects of deficient feed hygiene?

The consequences of deficient feed hygiene can be divided into two parts, impurities and spoilage.

Impurities comprise:

- the presence of soil, sand, or dust
- contamination with or residues of heavy metals, PCB, dioxins, pesticides, fertilizers, disinfectants, toxic plants, or banned feed ingredients

In the case of spoilage, we see:

- degradation of organic components by the action of molds and bacteria
- growth of pathogens such as *E. coli*, salmonella, etc.
- accumulation of toxins such as mycotoxins or bacterial toxins ([Hoffmann, 2021](#))

Bad feed hygiene can also negatively impact the feed's nutritional value by leading to a loss of energy as well as decreasing the bioavailability of vitamins A, D3, E, K, and B1.

But, how can all signs of deficient feed hygiene be recognized? Soil, sand, and probably dust can be seen in well-taken samples and impurities can be analyzed. But is it possible to spot spoilage? In this case, agglutinated particles, rancid odor, moisture, and discoloration are indicators. Sometimes, also the temperature of the feed or ingredient increases. However, spoilage is not always obvious and an analysis of the feed can give more information about the spoilage-related organisms present. It also helps to decide if the feed is safe for the animals or not. In the case of obvious alterations, the feed should not be consumed by any animal.

Different organisms decrease feed quality and impact health

Several organisms can be responsible for a decrease in feed quality. Besides the visible pests such as rats, mice, or beetles, which can easily be noticed and combatted, there are organisms whose mastering is much more difficult. In the following part, the different harmful organisms and substances are described and solutions are presented.

Enteropathogens can cause diarrhea and production losses

In poultry, different bacteria responsible for high production losses can be transferred via the feed. The most relevant of them are *Clostridium perfringens*, *Escherichia coli*, and some strains of *Salmonella*.

Clostridium perfringens, the cause of necrotic enteritis

Clostridium perfringens is a Gram-positive, anaerobic bacterium that is extremely resistant to environmental influences and can survive in soil, feed, and litter for several years and even reproduce. *Clostridium perfringens* causes [necrotic enteritis](#) mainly in 2-16 weeks old chickens and turkeys, being more critical in 3-6 weeks old chicks.

There is a clinical and a subclinical form of necrotic enteritis. The clinical form can be detected very well due to clear symptoms and mortality rates up to 50%. The subclinical form, while harder to detect, also raises production costs due to a significant decrease in performance. The best prophylaxis against clostridia is the maintenance of gut health, including feed hygiene.

Clostridia can be found in animal by-products, as can be seen in table 1.

Sr. No.	Sample details	Clostridium perfringens contamination		Total number of samples	Positivity %
		Positive	Negative		
1	Meat and bone meal	39	52	91	42.86
2	Soya meal	0	3	3	0
3	Rape seed meal	0	1	1	0
4	Fish meal	21	17	38	55.26
5	Layer Feed	21	71	93	22.58
6	Dry fish	5	8	13	38.46
7	De-oiled rice bran	0	2	2	0
8	Maize	0	2	2	0
9	Bone meal	13	16	29	44.83

Table 1: Isolation of *Clostridium perfringens* from various poultry feed ingredients in Tamil Nadu, India ([Udhayavel et al., 2017](#))

Salmonella is harmful to animals and humans

Salmonella is a gram-negative enterobacterium and can occur in feed. There are only two species - *S. enterica* and *S. bongori* ([Lin-Hui and Cheng-Hsun, 2007](#)), but almost 2700 serotypes. The most known poultry-specific *Salmonella* serotypes are *S. pullorum* affecting chicks and *S. gallinarum* affecting adult birds. The other two well-known serotypes, *S. enteritidis* and *S. typhimurium* are the most economically important ones because they can also infect humans.

Salmonella enteritidis, in particular, can be transferred via table eggs to humans. The egg content can be infected vertically as a result of a colonization of the reproductive tract of the hen (De Reu, 2015). The other possibility is a horizontal infection, as some can penetrate through the eggshell from a contaminated environment or poor egg handling.

Salmonella can also be transferred through meat. However, as there are more production steps where contamination can happen (breeder and broiler farm, slaughterhouse, processing plants, food storage...), traceability is more complicated. As feed can be vector, feed hygiene is crucial.

Moreover, different studies have found that the same *Salmonella* types found in feed are also detected - weeks later - in poultry farms and even further in the food chain, as reviewed by Ricke and collaborators

(2019). Other researches even imply that Salmonella contamination of carcasses and eggs could be significantly reduced by minimizing the incidence of Salmonella in the feed (Shirota et al., 2000).

E. coli - some are pathogenic

E. coli is a gram-negative, not acid-resistant bacterium and most strains are inhabitants of the gut flora of birds, warm-blooded animals, and humans. Only some strains cause disease. To be infectious, the bacteria must have fimbriae to attach to the gut wall or the host must have an immune deficiency, perhaps due to stress. E. coli can be transmitted via contaminated feed or water as well as by fecal-contaminated dust.

Escherichia coli infections can be found in poultry of all ages and categories and nearly everywhere in the bird. E. coli affects the navel of chicks, the reproductive organs of hens, several parts of the gut, the respiratory tract, the bones and joints, and the skin and are part of the standard control.

The feed microbiome can contribute to a balanced gut microbial community. The origins of pathogenic E. coli in a flock can also be traced to feed contamination (Stanley & Bajagai, 2022). Especially in pre-starter/starter feeds, E. coli contamination can be critical as the day-old chick's gut is starting to be colonized. Especially in this phase, maintaining a low microbial count in feed is crucial.

Molds cause feed spoilage and reduce nutritional value

Molds contaminate grains, both in the field and during storage, and can also grow in stored feed and even in feed stored or accumulated in storage facilities in animal production farms.

The contamination of feed by molds and their rapid growth can cause heating of the feed. As molds also need nutrients, their growth results in a reduction of energy and the availability of vitamins A, D3, E, K, and B1, thus decreasing the feed's nutritional value. This heating occurs in most feeds with a moisture content higher than 15 /16%. Additionally, mold-contaminated feed tends to be dusty and has a bad taste impacting palatability and, as a consequence, feed intake and performance.

Molds produce spores that can, when inhaled, cause chronic respiratory disease or even death if the animals are exposed to contaminated feed for a longer time. Another consequence of mold contamination is the production of mycotoxins by several mold species. These mycotoxins can affect the animal in several ways, from decreasing performance to severe disease (Esmail, 2021; Government of Manitoba, 2023).

With effective feed hygiene management, we want to stop and prevent mold growth, as well as all its negative consequences.

Prevention is better than treatment

It is clear that when the feed is spoiled, it must be removed, and animal health supporting measures should take place. However, it is better to prevent the consequences of low feed hygiene on animals. Proper harvest and adequate storage of the feed are basic measures to stop mold growth. Additionally, different tools are available to protect the animals from feed bacterial load and other risk factors.

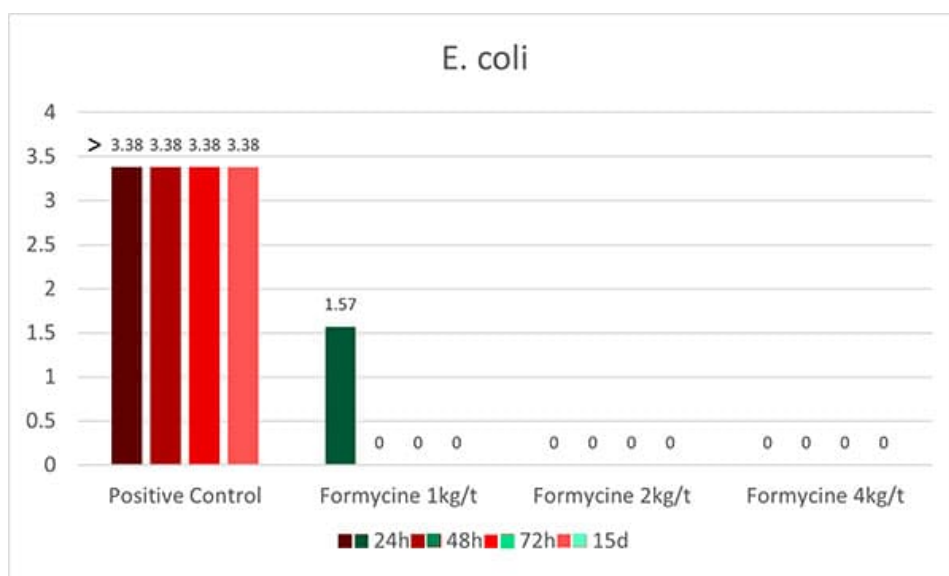
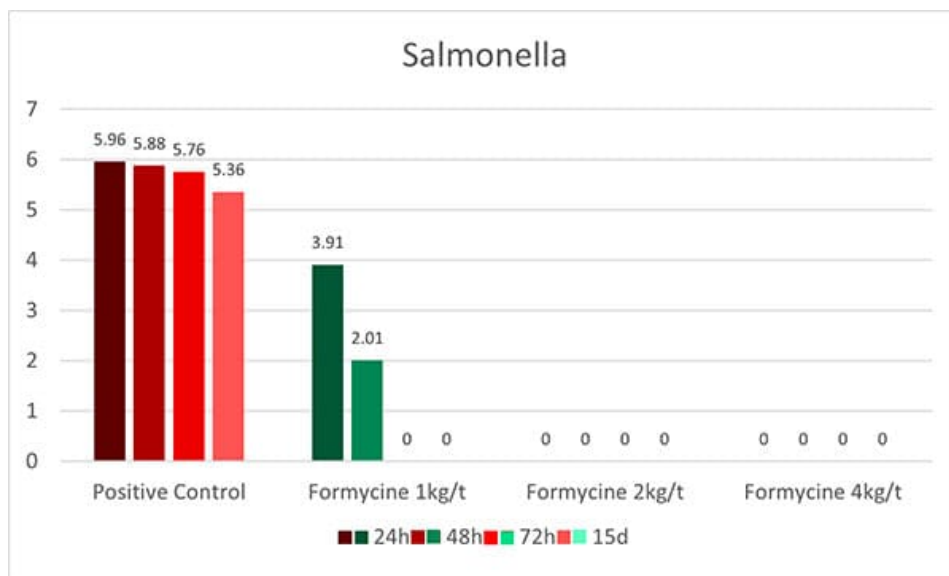
Solutions are available to support feed hygiene

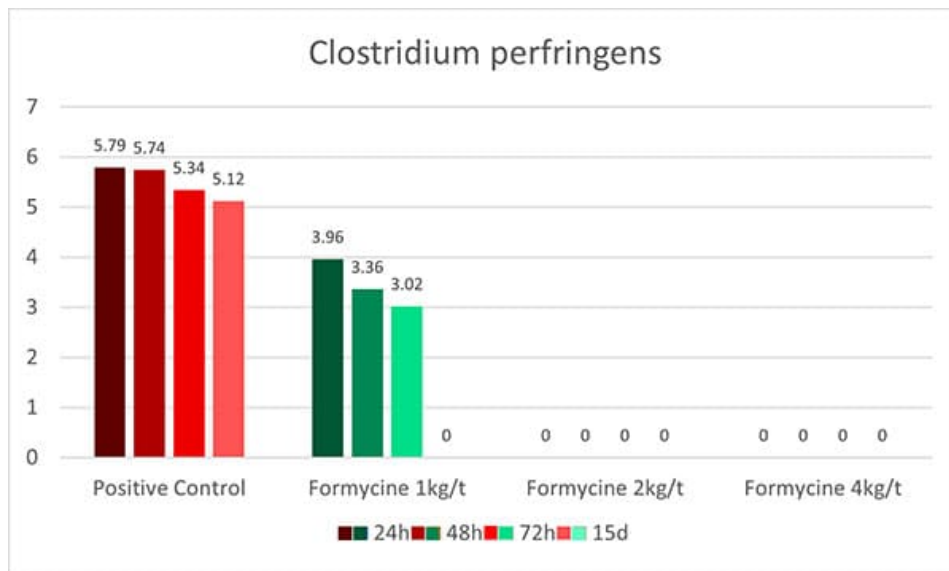
There are several solutions to fight the organisms which decrease feed quality. Some directly act against the harmful substances / pathogens, and others act indirectly, meaning that they change the environment to a non-comfortable one for the organism.

Formaldehyde and propionic acid – an unbeatable team against bacteria

A combination of formaldehyde and propionic acid is perfect to sanitize feed. Formaldehyde results in bacterial DNA and protein damage, and propionic acid is active against bacteria and molds. Together, they improve the microbiological quality of the feed and reduce the risk of secondary diseases such as necrotic enteritis or dysbiosis on the farm. In addition to the pure hygienic aspect, organic acids support digestion.

An in-vitro trial was conducted to evaluate the effect of such a combination (Formycine Gold Px) against common poultry pathogens. Poultry feed was spiked with three different bacteria, achieving very high initial contamination of 1,000,000 CFU/g per pathogen. One batch of the contaminated feed served as a control (no additive). To the other contaminated batches, 1, 2, or 4 kg of Formycine per ton of feed were added. The results (means of triplicates) are shown in figures 1 a-c.





Figures 1 a-c: Reduction of bacterial count due to the addition of Formycine

Formycine Gold Px significantly reduced the bacterial counts in all three cases. A clear dose-response-effect can be seen and by using 2 kg of Formycine / t of feed, pathogens could not be detected anymore in the feed.

A further trial showed the positive effects of feeding Formycine Gold Px treated feed to the animals. Also here, the feed for both groups was contaminated with 1,000,000 CFU of Clostridium/g. The feed of the control group was not treated and to the treatment group, 2 kg of Formycine per t was added.

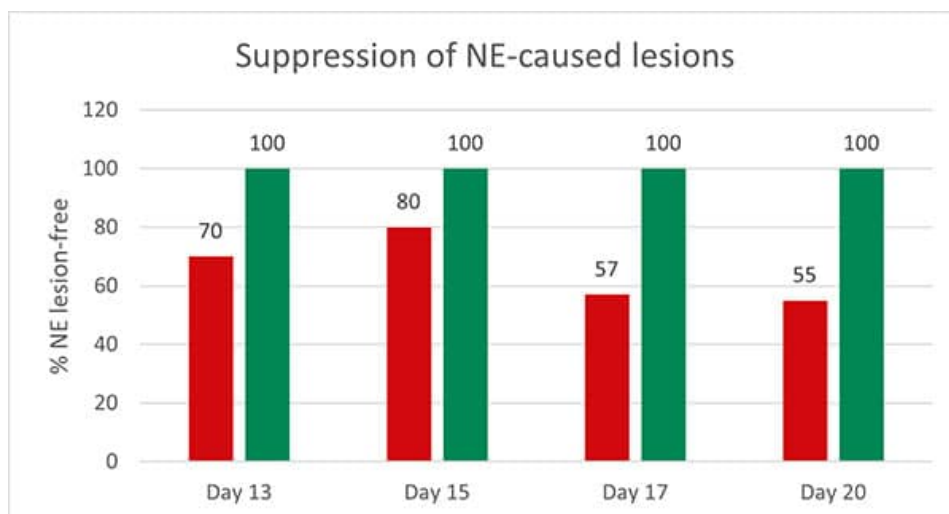


Figure 2: Preventive effect of Formycine Gold Px concerning necrotic enteritis gut lesions

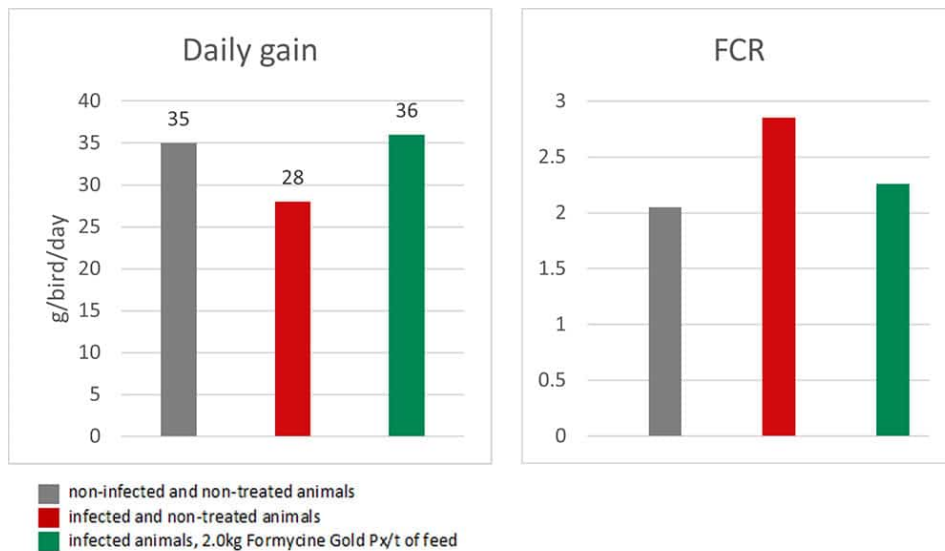


Figure 3a and 3b: Performance-maintaining effect of Formycine Gold Px

The trial showed that Formycine Gold Px reduced the ingestion of the pathogen, and thus could prevent the lesions caused by necrotic enteritis (Fig. 2). The consequence of this improved gut health is a better feed conversion and higher average daily gain (Fig.3a and 3b).

Products containing formaldehyde may represent a risk for humans, however, the adequate protection equipment helps to reduce/avoid exposure.

A combination of free acids and acid salts provides optimal hygienic effects

Additionally, another blend of organic acids (Acidomix AFG) shows the best effects against representatives of relevant feed-borne pathogens in poultry. In a test, 50 µl solution containing different microorganisms (reference strains of *S. enterica*, *E. coli*, *C. perfringens*, *C. albicans*, and *A. niger*; concentration 10^5 CFU/ml, respectively) were pipetted into microdilution plates together with 50 µl of increasing concentrations of a mixture of organic acids (Acidomix). After incubation, the MIC and MBC of each pathogen were calculated.

The test results show (figure 4, Minimal Bactericidal Concentration) that 0.5% of Acidomix AFG in the medium (\pm 5kg/t of feed) is sufficient to kill *S. enterica*, *C. albicans*, and *A. niger* and even only 2.5kg/t in the case of *E. coli*. If the pathogens should only be prevented to proliferate, even a lower amount of product is requested (figure 5, Minimal Inhibitory Concentration – MIC)

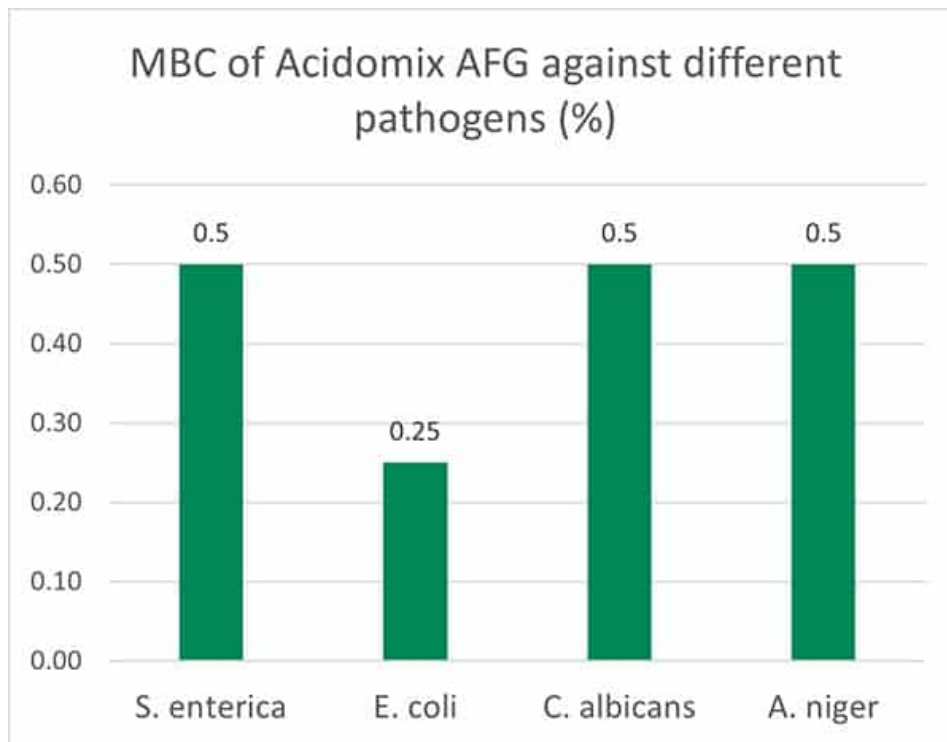


Figure 4: MBC of Acidomix AFG against different pathogens (%)

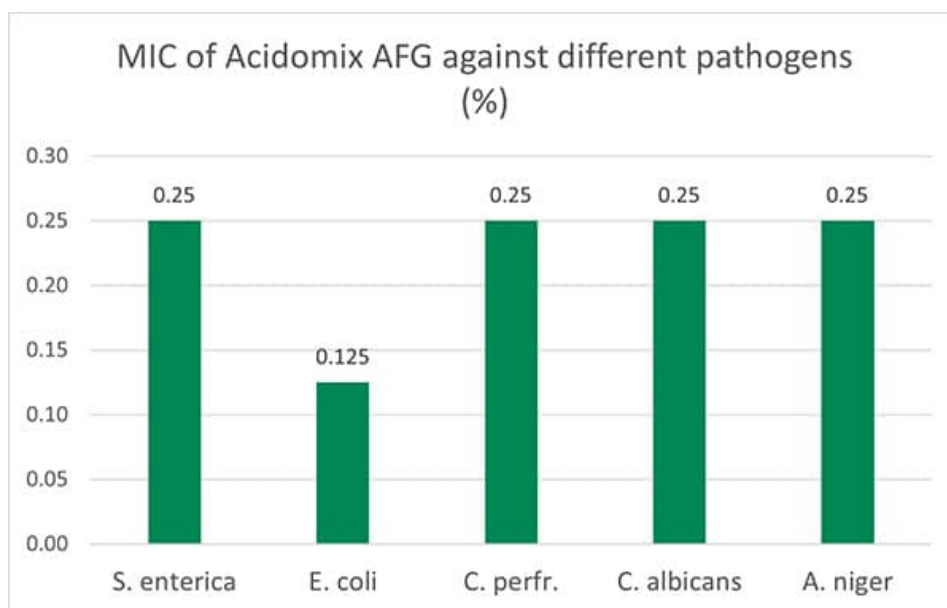


Figure 5: MIC of Acidomix AFG against different pathogens (%)

In addition to the direct antimicrobial effect, this product decreases the pH of the feed and reduces its buffering capacity. The combination of free acids and acid salts provides prompt and long-lasting effects.

Feed hygiene: a critical path to animal performance

Feed accounts for 65-70% of broiler and 75-80% of layer production costs. Therefore, it is essential to use the available feed to the utmost. The quality of the feed is one decisive factor for the health and performance of the animals. Proper harvesting and storage are in the hands of the farmers and the feed millers. The industry offers products to control the pathogens causing diseases and the molds producing toxins and, therefore, helps farmers save feed AND protect the health and performance of their animals.

References:

Dinev, Ivan. *Diseases of Poultry: A Colour Atlas*. Stara Zagora: Ceva Sante Animal, 2007.

Esmail, Salah Hamed. "Moulds and Their Effect on Animal Health and Performance." *All About Feed*, June 17, 2021.

<https://www.allaboutfeed.net/all-about/mycotoxins/moulds-and-their-effect-on-animal-health-and-performance/>.

Government of Manitoba. "Spoiled Feeds, Molds, Mycotoxins and Animal Health." Province of Manitoba - Agriculture. Accessed March 16, 2023.

<https://www.gov.mb.ca/agriculture/livestock/production/beef/spoiled-feeds-molds-mycotoxins-and-animal-health.html>.

Hoffmann, M. "Tierwohl Und Fütterung." LKV Sachsen: Tierwohl und Fütterung. Sächsischer Landeskontrollverband e.V., January 25, 2021.

<https://www.lkvsachsen.de/fuetterungsberater/blogbeitrag/artikel/tierwohl-und-fuetterung/>.

Ricke, Steven C., Kurt Richardson, and Dana K. Dittoe. "Formaldehydes in Feed and Their Potential Interaction with the Poultry Gastrointestinal Tract Microbial Community-A Review." *Frontiers in Veterinary Science* 6 (2019). <https://doi.org/10.3389/fvets.2019.00188>.

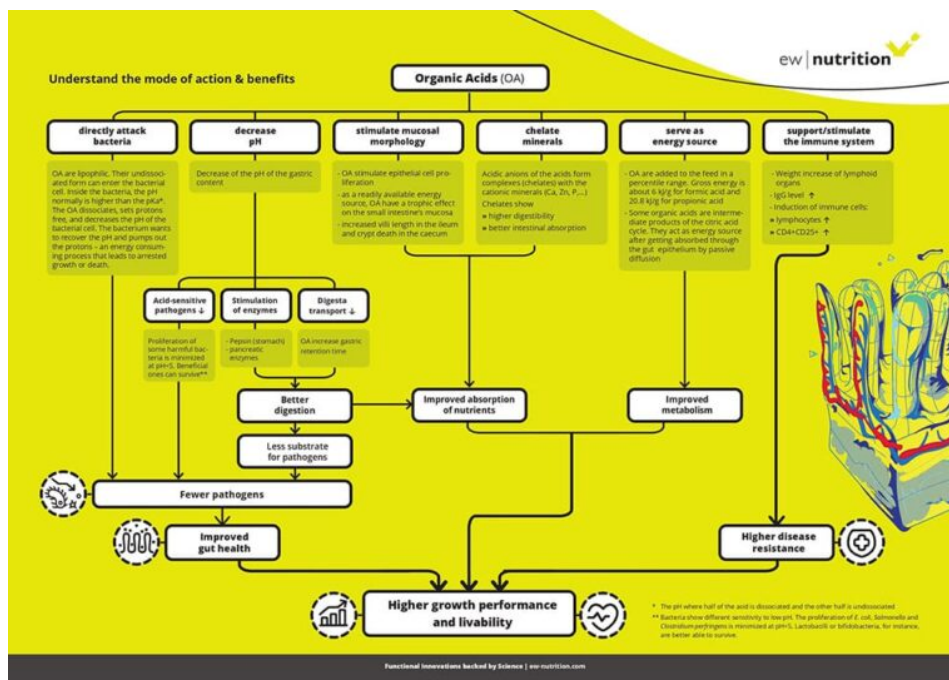
Shirota, Kazutoshi, Hiromitsu Katoh, Toshihiro Ito, and Koichi Otsuki. "Salmonella Contamination in Commercial Layer Feed in Japan." *Journal of Veterinary Medical Science* 62, no. 7 (2000): 789-91. <https://doi.org/10.1292/jvms.62.789>.

Stanley, Dragana, and Yadav Sharma Bajagai. "Feed Safety and the Development of Poultry Intestinal Microbiota." *Animals* 12, no. 20 (2022): 2890. <https://doi.org/10.3390/ani12202890>.

Su, Lin-Hui, and Cheng-Hsun Chiu. "Salmonella: Clinical Importance and Evolution of Nomenclature." *Chang Gung Med J* 30, no. 3 (2007): 210-19.

Udhayavel, Shanmugasundaram, Gopalakrishnamurthy Thippichettypalayam Ramasamy, Vasudevan Gowthaman, Shanmugasamy Malmarugan, and Kandasamy Senthilvel. "Occurrence of Clostridium Perfringens Contamination in Poultry Feed Ingredients: Isolation, Identification and Its Antibiotic Sensitivity Pattern." *Animal Nutrition* 3, no. 3 (2017): 309-12. <https://doi.org/10.1016/j.aninu.2017.05.006>.

Organic acids: How the mode of action delivers benefits | INFOGRAPHIC



Download this comprehensive A3 infographic to understand how organic acids can benefit your operation. For further questions or feedback, we'll be happy to hear from you.



CONTACT US

Salmonella in poultry: What are the most effective natural solutions?



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

Salmonella infection in poultry is a problem for the producer because of the performance losses of his flock. At the same time, products of salmonella-contaminated animals pose a severe risk to human health. In the USA, Salmonellosis in poultry is estimated to cost \$ 11.6 billion each year ([Wernicki et al., 2017](#)) and more than € 3 billion in the EU ([Ehuwa, 2021](#)). As the use of antibiotics needs to be reduced to keep them effective, Salmonella control in poultry requires new solutions. This article shows how organic acids and phytomolecules can help to fight this problematic disease.

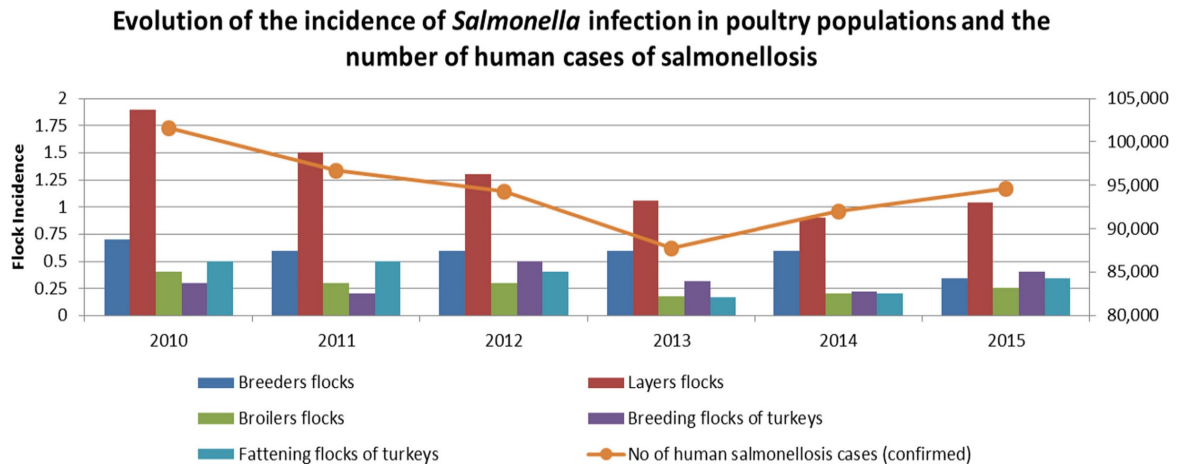
Salmonellosis: what it is, how it works, and why it's such a problem

Salmonellosis is a zoonosis, meaning that it can be easily transferred from animals to humans. The transfer can occur via different routes:

Direct contact with an infected animal

Handling or consuming contaminated animal products such as eggs or raw meat from pigs, turkeys, and chicken
Contact with infected vectors (insects or pets) or contaminated equipment

Frozen or raw chicken products, as well as the eggs of backyard hens, are the most frequent causes of animal-mediated *Salmonella* infections in humans. The following graphic shows a clear relationship between the occurrence of *Salmonella* in layer flocks and the event of disease in humans:



(Source: Koutsoumanis et al., 2019)

The impact of *Salmonella* on poultry depends on the bird's age

Within the poultry flock, there are two ways of spreading: the fecal-oral way (horizontal infection) or the infection of the progeny in the egg (vertical infection). The effects of the disease depend on the age of the birds: the younger the animals, the more severe the impact.

If the brood eggs already carry salmonellae, the hatchability dwindles. During their first month of life, infected chicks show ruffled downs and higher temperatures. Diarrhea leads to fluid losses and frequently to the chicks' death.

Adult animals usually do not die from Salmonellosis; often, the infection remains unnoticed. During a substantial acute salmonella outbreak, the animals show weakness and diarrhea. They lose weight, resulting in decreased egg production in layers and worse growth performance in broilers. The birds need more water to compensate for the fluid losses, and their crowns and jowls appear pale.

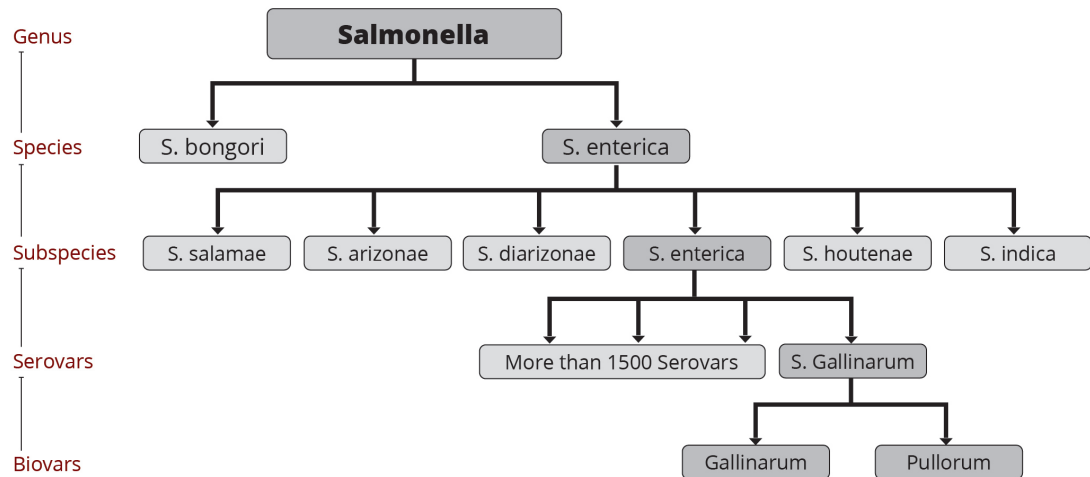
Salmonella protects itself through an intelligent infection style

Salmonellae have developed a clever way to protect themselves. After they arrive in the gut, they attach to the epithelial cells and form small molecular "syringes" to inject diverse substances into the gut cells (Type-3-injection system). These signaling substances make the gut cells bulge their membranes and enclose the bacterium. Finally, the manipulated gut cell absorbs the *Salmonella*, the host "allows" the bacterium to enter, and it can proliferate in the gut cells ([Fischer, 2018](#)).

When an antibiotic is attacking the bacterium, *Salmonellae* stop their cell division. Since many antibiotics are only effective against bacteria during cell division and growth, *Salmonellae* survive the attack by staying as dormant variants or persisters until the treatment stops ([Fischer, 2018](#)).

Salmonellae - a big “family”

The genus of *Salmonella* consists of more than 2600 serovars (Ranieri et al., 2015), of which less than 100 are relevant for humans (CDC, 2020). More than 1500 serovars belong to the *Salmonella enterica* subspecies that colonize the intestinal tract of warm-blooded animals. These serovars are responsible for 99 % of salmonella infections (Mendes Maciel et al., 2017). The main serovars relevant for poultry are *S. Gallinarum* and *S. Pullorum*, but also *S. Enteritidis*, *Typhimurium*, and in recent years, *S. Kentucky*, *S. Heidelberg*, *S. Livingstone*, and *S. Mbandaka* (Guillén et al., 2020).



(Source: Mkangara et al., 2020)

The zoonosis Salmonellosis must be controlled

Several *Salmonella* serovars are critical for animals and humans. Since more than 91,000 salmonellosis cases are reported for Europe and more than 1.35 million for the USA every year (EFSA, 2022; FDA, 2020), their spread must be prevented by all means. Governments have enacted some laws to curtail this disease. The EU, for example, implemented extended control programs for zoonotic diseases, with *Salmonella* set as a priority. These programs include the provision of scientific advice, targets for reducing *Salmonella* in poultry flocks, and restrictions on the trade of products from infected flocks.

For farmers and vets, this means the obligation to notify the occurrence of the disease to the authorities. Depending on the country, it also entails compulsory vaccination and the documentation of hygienic measures. In the EU, due to the risk of developing resistances, the EFSA recommends limiting the use of antimicrobials to individual cases, e.g., to prevent inordinate suffering of animals.

Prevention of *Salmonella* infection is the key

The best strategy for salmonella control is prevention based on three key points (Visscher, 2014):

- Preventing the introduction of *Salmonella* into the farm/flock through effective hygiene measures
- Preventing the spread of the pathogens within a flock/farm
- Prophylactic measures to recover immune resistance of the animals against *Salmonella* infection

For this purpose, the following steps are requested/recommended:

1. Keeping the litter dry

The use of well-absorptive material such as wood shavings, straw pellets, or straw granulates and regular removal of the used litter is recommended. The animals must be controlled for diarrhea to avoid wet droppings. The water supply must be adequate; an excessive water supply wets the litter.

2. Providing a clean environment

To keep the poultry house clean, broken eggs and dead animals (potential sources of infection) must be removed. In general, the houses should be cleaned and disinfected before every restocking.

Clean feed and water are essential; therefore, feed should not be stored outside but be kept dry and protected from pests and rodents. The [feeding of the animals](#) should take place inside to avoid contamination by wild birds. Concerning the water for drinking, the flow rate must be high enough to provide the birds with sufficient water but not too high that the floor gets wet. The troughs must be clean from droppings.

3. Limiting contacts

To limit the spread of Salmonella, only a restricted number of persons can have access to the flocks. They must wear clothes, and instruments should be exclusively used for the respective poultry house.

Knowing the optimal growth conditions for Salmonella facilitates control

Salmonellae are a genus in the family of Enterobacteriaceae. They are gram-negative, rod-shaped (size: approx. 2 μm), glucose-fermenting facultative anaerobes that are motile due to peritrichous flagella. Since Salmonellae do not form spores, they can be easily destroyed by heating them to 60°C for 15-20 min ([Forsythe, 2001](#)), especially in food/feed with higher water content.



For the storage of food, [Bell and Kyriakis \(2002\)](#) found that most serovars of Salmonella will not grow at temperatures lower than 7°C and a pH lower than 4.5. [Wessels et al. \(2021\)](#) showed optimal growth conditions for Salmonella: temperatures between 5 and 46°C (optimum 38°C), a water activity of 0.94-0.99, and a pH of 3.8-9.5.

A high fat content in the feed or food increases the likelihood of infection with Salmonella because the fat protects the bacteria during the passage through the stomach. Doses of 10 to 100 Salmonella cells can already pose a severe risk ([University of Georgia, 2015](#)).

Natural alternatives to antibiotics: effective Salmonella control?

To reduce the incidence of Salmonella while simultaneously lowering the use of antibiotics in animal production, there are different possibilities. On the one hand, veterinary medicine offers vaccines. On the other hand, the feed industry provides additives that strengthen the immune system, improve gut health, or support the animals in another manner. Other than pro- and prebiotics, the main active ingredient categories for such additives are organic acids and phytomolecules.

Organic acids worsen the conditions for Salmonella

Already in ancient Egypt, the method of fermentation and the generated acids have been used for the conservation of food (Ohmomo et al., 2002). Nowadays, it is a standard tool to protect feed (silage) and food from spoilage. Also for animals, organic acids added to the feed or the water have proven helpful against pathogens. These modes of action can be combined against Salmonella: reducing the pathogen load in the feed to limit the intake of bacteria and fighting against these pathogens in the animal.

Organic acids reduce Salmonella in feed materials

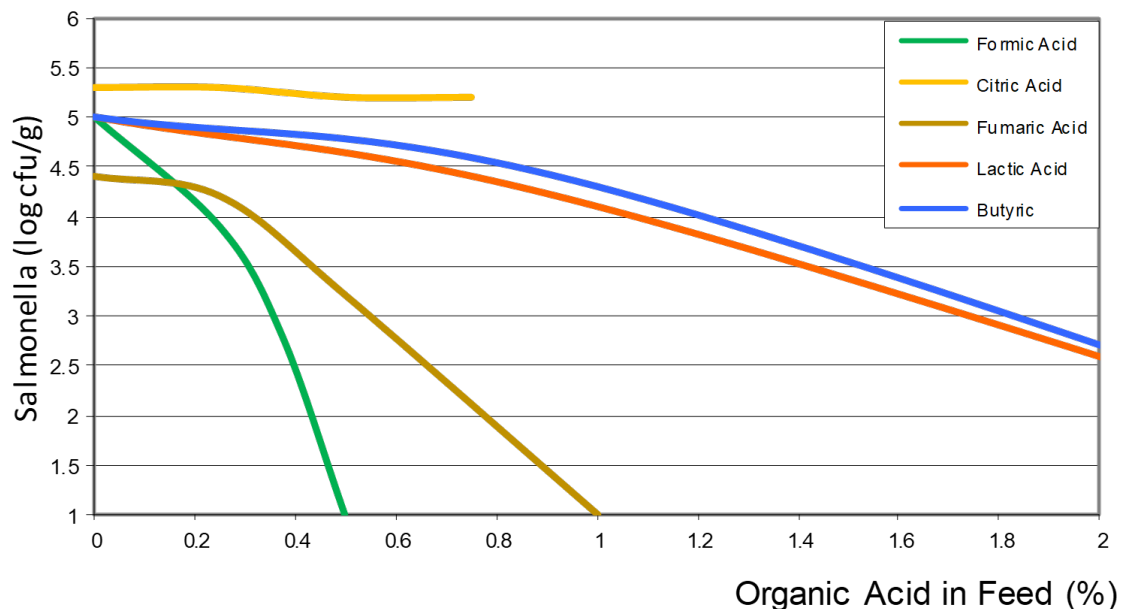
In general, the antimicrobial activity of organic acids **in feed** is based on lowering the pH (Pearlin et al., 2019). pH-sensitive bacteria such as Salmonella minimize their proliferation at a pH <5. Additionally, the organic acids attack bacteria directly. The acid's undissociated and more lipophilic form penetrates the bacterial cell membrane. At the neutral pH within the cell, the acid dissociates, releases protons, and lowers the pH, leading to the impediment of metabolic processes in the cell. The cell spends a lot of energy trying to get the pH back to neutral (Mroz et al., 2006). Additionally, the anions become toxic for the cell metabolites and disrupt the membrane (Russel, 1992).

What do organic acids do in the bird?

According to [Hernández and co-workers \(2006\)](#) and [Thompson and Hinton \(1997\)](#), the addition of organic acids to the feed does not change the pH in the various digestive tract segments. Still, literature shows a clear reduction of Salmonella in the gut or litter when using propionic or/and formic acid ([McHan and Emmett, 1992](#); [Hinton and Linton, 1988](#); [Humphrey & Lanning, 1988](#)). A likely mode of action is described by [Van Immerseel et al. \(2004\)](#). He asserts that SCFAs such as propionic and formic acid as well as MCFAs can inhibit Salmonella's penetration of the intestinal epithelium and, therefore, can control these invasive phenotypes of Salmonella (*S. Typhimurium* and *S. Enteritidis*).

Different acids show different efficacy

Depending on the acid, the efficacy against Salmonella varies (see figure 3). Formic acid shows the highest effect, followed by fumaric acid. Then, lactic, butyric, and citric acid follow, showing lower efficacy.



for 90 min, 37°C, pH 4

Figure 3: Efficacy of different organic acids against Salmonella in feed

Trials prove the efficacy of organic acids

An *in-vitro* trial was conducted at a commercial research facility in the US to test the efficacy of [Acidomix AFL](#), a liquid mixture of propionic and formic acid, against Salmonella. The bacterial strain used in these studies was nalidixic acid-resistant Salmonella typhimurium. The bacteria were maintained in broth cultures of tryptic soy broth.

They were added to 5 g of dry feed in a 50 ml tube to a final concentration of 40,000 CFU/g. Next, Acidomix AFL was added to the desired inclusion rate, and the samples were incubated at room temperature. After 18 to 72 hours of incubation, viable bacteria were counted using the plate count method.

Results: As shown in figure 4, the trial found that at an inclusion rate of 2.0 %, Salmonella inhibition was nearly 100 %. Already at a 0.4 % inclusion rate, Salmonella could be reduced by 45-60 %, showing a clear dose dependency.

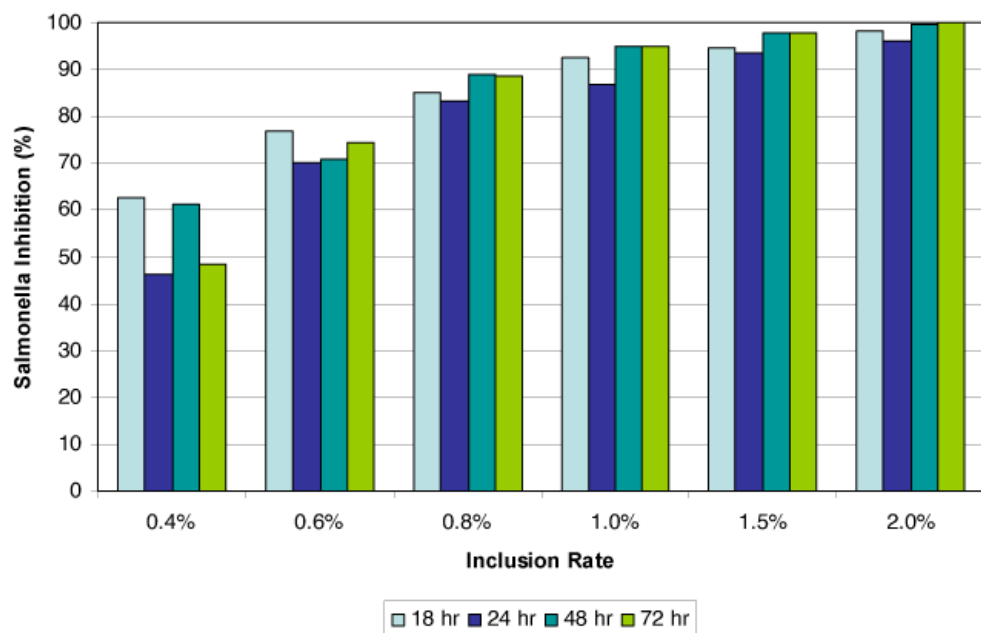


Figure 4: Efficacy of Acidomix AFL (liquid) on Salmonella Typhimurium in dry feed

Phytomolecules combat Salmonella through complex modes of action

Plants produce phytochemical substances to protect themselves from molds, yeasts, and bacteria, among others. After several purification steps, these phytomolecules can be used to fight Salmonella in poultry. They work through different modes of action, from attacking the cell wall (terpenoids and phenols) to influencing the genetic material of the pathogenic cells or changing the whole morphology of the cell.

Due to the different modes of action, it was long thought that there would be no resistance development. Still, [Khan et al. \(2009\)](#) found some microorganisms such as multidrug-resistant E. coli, Klebsiella pneumoniae, S. aureus, Enterococcus faecalis, Pseudomonas aeruginosa, and Salmonella typhimurium can show a certain – perhaps natural – resistance to some components of herbal medicines.

Gram-negative bacteria such as Salmonella are usually less attackable by phytomolecules because the cell

wall only allows small hydrophilic solutes to pass; however, phytomolecules are hydrophobic. However, mixing the phytomolecules with an emulsifier facilitates the invasion into the cell. Their efficacy depends on their chemical composition. It is also decisive if single substances or blends (possible positive or negative synergies) are used.

The best-clarified mode of action is the one of thymol and carvacrol, the major components of the oils of thyme and oregano. They can get into the bacterial membrane and disrupt its integrity. The permeability of the cell membrane for ions and other small molecules such as ATP increases, decreasing the electrochemical gradient above the cell membrane and the loss of energy equivalents.

Trials show the efficacy of phytomolecules against Salmonella

Two different phyto-genic compositions were tested for their efficacy against Salmonella.

Trial 1: Blend of phytomolecules and organic acids shows best results in an in-vitro assay

To evaluate its potential as a tool for antibiotic reduction, a trial was conducted to test the antimicrobial properties of [Activo Liquid](#), a mixture of selected phytomolecules and an organic acid designed for application in water. The laboratory test was carried out at the Veterinary Diagnosis Department of Kasetsart University in Thailand. Standardized suspensions [1×10^4 CFU/ml] of three poultry-relevant Salmonella strains were incubated in LB medium, either without or with Activo Liquid. The tests were run at concentrations of 0.05%; 0.1%; 0.2% and 0.4%. After incubation at 37°C for 6-7 hours, serial dilutions of the cell suspensions were transferred onto LB agar plates and incubated for 18-22h at 37°C. Subsequently, colonies (CFU/ml) were determined.

Results: Activo Liquid was found to be growth-inhibiting to all Salmonella strains from a concentration of 0.1% onwards. At 0.2%, Activo Liquid already exhibited bactericidal efficacy against all tested Salmonella isolates, which was confirmed at a concentration of 0.4%.

Bacteria		Activo Liquid (%)				
	CFU	negative control 0	0.05	0.1	0.2	0.4
Salmonella Typhimurium	1.9×10^9	–	–	+-	++	++
Salmonella Enteritidis	1.3×10^9	–	–	+-	++	++
Salmonella Corvalis	6.4×10^9	–	–	+-	++	++

– No effect +- Growth-inhibiting ++ Bactericidal

Table 1: Inhibiting effect of Activo Liquid against three different Salmonella serovars

Trial 2: Blend of nature-identical phytomolecules inhibits Salmonella

On Mueller Hinton agar plates where Salmonella enterica were spread uniformly, small disks containing 0 (control, only methanol), 1, 5, and 10 µl of Ventar D were placed and incubated at 37 °C for 16 hours. The presence of clearing zones indicates antimicrobial activity.

Additionally, a motility test was performed in tubes with a motility test medium containing 0 (control) and 750 µL Ventar D. For this purpose, one colony of Salmonella enterica grown on the agar was stuck in the

middle of the medium and incubated at 37 °C for 12-16 hours. Growth can be visualized through the formation of red color.

Result: Ventar D inhibited *S. enterica* in a dose-dependent manner. Clearing zones were visible within the lowest tested concentration. At its inhibitory concentration, Ventar D suppressed *S. enterica* motility (figures 5 and 6).

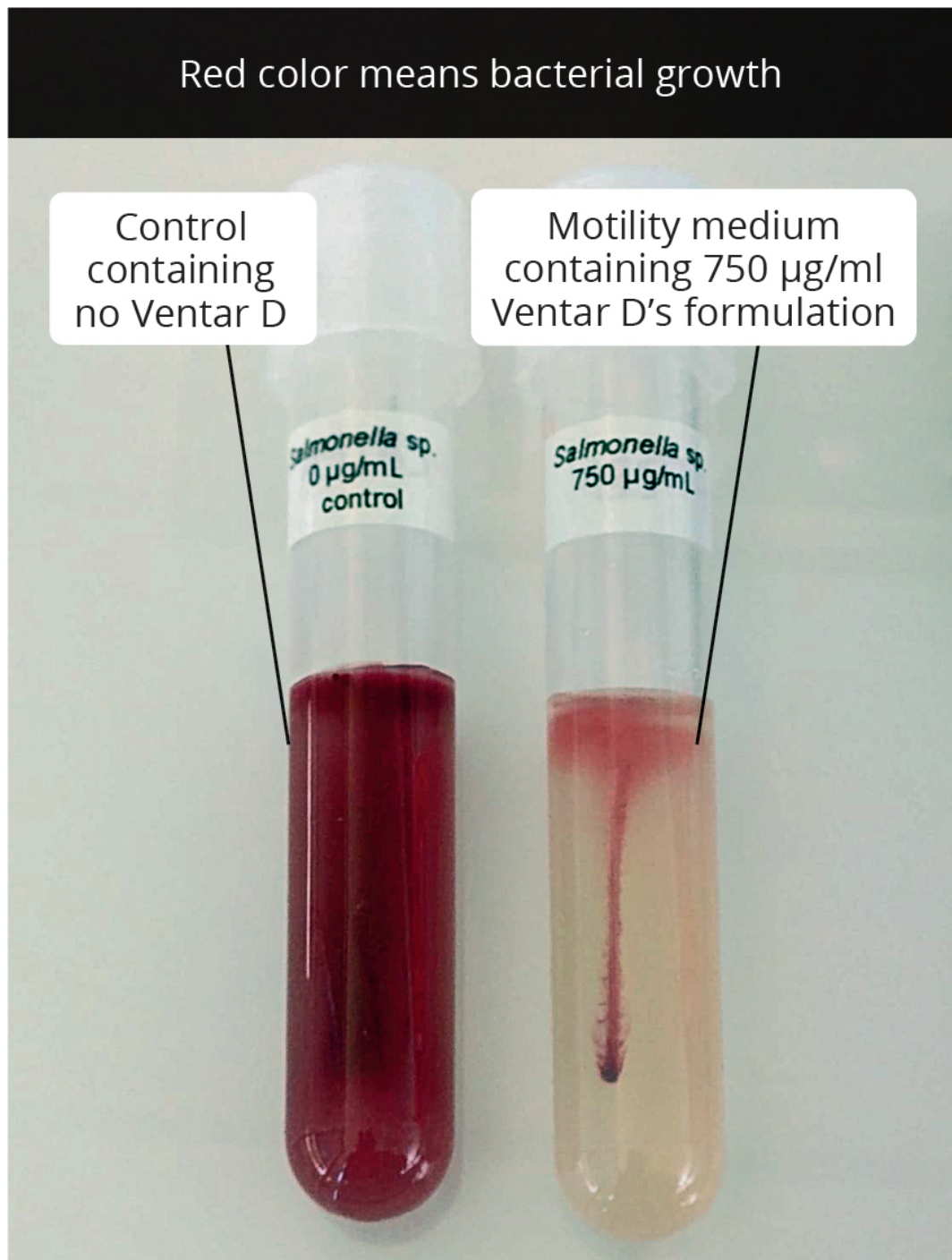


Figure 5: *S. enterica* motility test

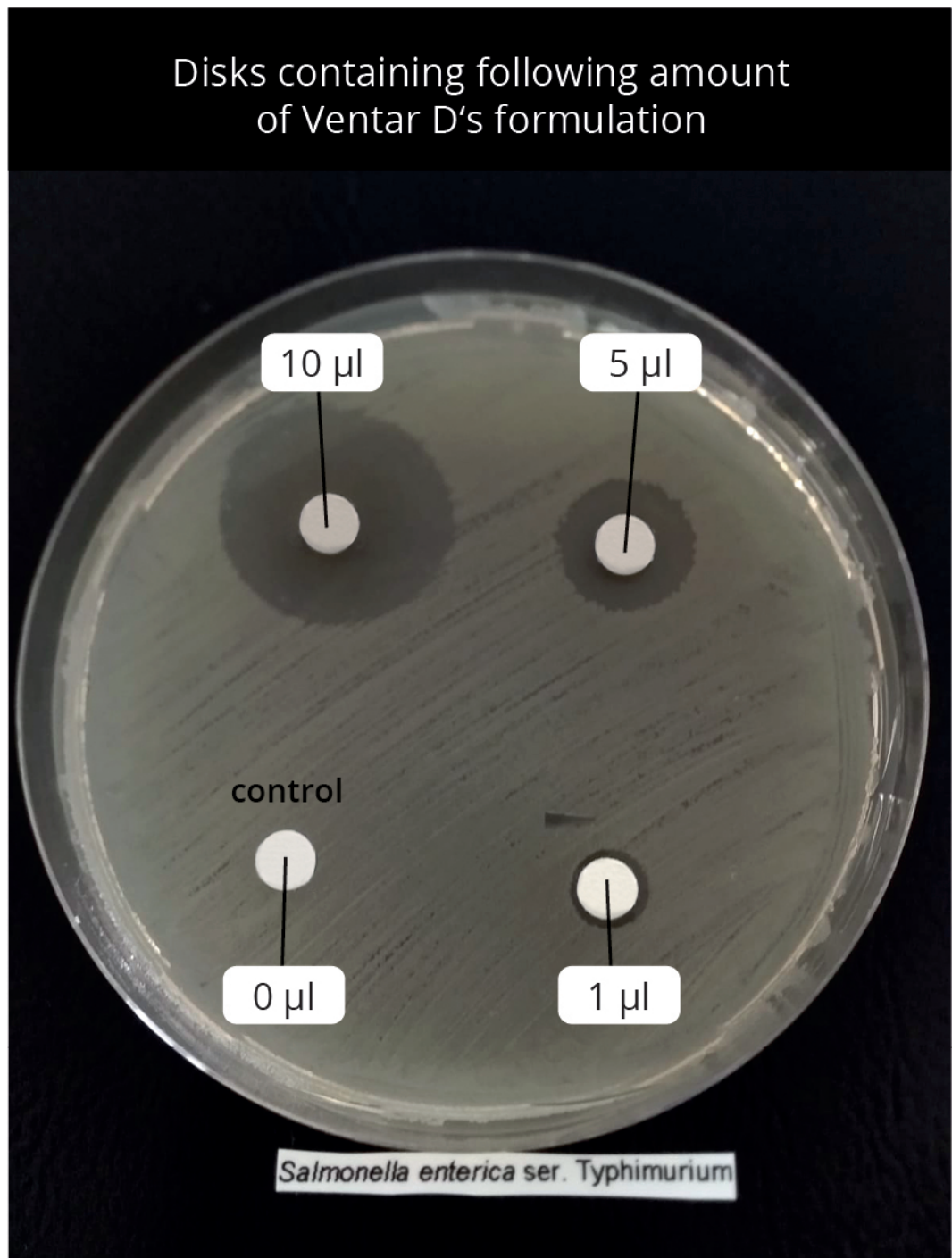


Figure 6: Disk diffusion assay employing *S. enterica*

Let's fight *Salmonella* through effective and sustainable natural tools

The zoonosis *Salmonella* generates high costs in the poultry industry. As *Salmonellosis* can be transferred to humans, it must be kept under control by all means. Antibiotics are one tool to fight *Salmonella*, but they have their "side effects": they are no longer well respected by the consumer, and, even more critically, they create resistance. To help keep antibiotics effective, poultry producers seek to use effective but not resistance-creating natural solutions against *Salmonella*.

As shown with the reviewed trials, organic acids and phytochemicals are highly active against diverse *Salmonella* serovars. Accordingly, feed additives based on these active ingredients offer effective tools for controlling *Salmonella* in poultry while also contributing to the overarching aim of reducing antibiotic use in poultry production.

References

- Bell, Chris, and Alec Kyriakides. *Salmonella: A Practical Approach to the Organism and Its Control in Foods*. Oxford: Blackwell Science, 2002.
- Castro-Vargas, Rafael Enrique, María Paula Herrera-Sánchez, Roy Rodríguez-Hernández, and Ilang Schronilgen Rondón-Barragán. "Antibiotic Resistance in *Salmonella* Spp. Isolated from Poultry: A Global Overview." *October-2020* 13, no. 10 (October 3, 2020): 2070–84. <https://doi.org/10.14202/vetworld.2020.2070-2084>.
- CDC. "Serotypes and the Importance of Serotyping *Salmonella*." Centers for Disease Control and Prevention, February 21, 2020. <https://www.cdc.gov/salmonella/reportspubs/salmonella-atlas/serotyping-importance.html>.
- EFSA. "Salmonella." European Food Safety Authority. Accessed February 1, 2022. <https://www.efsa.europa.eu/en/topics/topic/salmonella>.
- Ehuwa, Olugbenga, Amit K. Jaiswal, and Swarna Jaiswal. "Salmonella, Food Safety and Food Handling Practices." *Foods* 10, no. 5 (2021): 907. <https://doi.org/10.3390/foods10050907>.
- FDA. "Get the Facts about Salmonella." U.S. Food and Drug Administration, July 28, 2020. <https://www.fda.gov/animal-veterinary/animal-health-literacy/get-facts-about-salmonella>.
- Fischer, Andreas. "Clever Infiziert – Die Tricks Der Bakterien." HZI – Helmholtz Zentrum für Infektionsforschung, August 19, 2021. <https://www.helmholtz-hzi.de/de/aktuelles/thema/clever-infiziert-die-tricks-der-bakterien/>.
- Forsythe, Steve J. *The Microbiology of Safe Food*. Hoboken, NJ: Wiley-Blackwell, 2020.
- Gheisari, A.A., M. Heidari, R.K. Kermanshahi, M. Togani, and S. Saraeian. "Effect of Dietary Supplementation of Protected Organic ..." WPSA, 2007. <https://www.cabi.org/Uploads/animal-science/worlds-poultry-science-association/WPSA-france-2007/74.pdf>.
- Guillén, Silvia, María Marcén, Ignacio Álvarez, Pilar Mañas, and Guillermo Cebrián. "Stress Resistance of Emerging Poultry-Associated *Salmonella* Serovars." *International Journal of Food Microbiology* 335 (2020): 108884. <https://doi.org/10.1016/j.ijfoodmicro.2020.108884>.
- Hernández, F., V. García, J. Madrid, J. Orengo, P. Catalá, and M.D. Megías. "Effect of Formic Acid on Performance, Digestibility, Intestinal Histomorphology and Plasma Metabolite Levels of Broiler Chickens." *British Poultry Science* 47, no. 1 (2006): 50–56. <https://doi.org/10.1080/00071660500475574>.
- Hinton, M. "Antibacterial Activity of Short-Chain Fatty Acids." *The Veterinary Record* 126 (n.d.): 416–21.
- Hinton, M., and A. Linton. "Control of *Salmonella* Infections in Broiler Chickens by the Acid Treatment of Their Feed." *Veterinary Record* 123, no. 16 (1988): 416–21. <https://doi.org/10.1136/vr.123.16.416>.
- Humphrey, T. J., and D. G. Lanning. "The Vertical Transmission of *Salmonellas* and Formic Acid Treatment of Chicken Feed: A Possible Strategy for Control." *Epidemiology and Infection* 100, no. 1 (1988): 43–49. <https://doi.org/10.1017/s0950268800065547>.
- Khan, Rosina, Barira Islam, Mohd Akram, Shazi Shakil, Anis Ahmad Ahmad, S. Manazir Ali, Mashiatullah Siddiqui, and Asad Khan. "Antimicrobial Activity of Five Herbal Extracts against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin." *Molecules* 14, no. 2 (2009): 586–97. <https://doi.org/10.3390/molecules14020586>.
- Koutsoumanis, Kostas, Ana Allende, Avelino Alvarez-Ordóñez, Declan Bolton, Sara Bover-Cid, Marianne Chemaly, Alessandra De Cesare, et al. "Salmonella Control in Poultry Flocks and Its Public Health Impact." *EFSA Journal* 17, no. 2 (2019). <https://doi.org/10.2903/j.efsa.2019.5596>.

Maciel, Bianca Mendes, Rachel Passos Rezende, and Nammalwar Sriranganathan. "Salmonella Enterica: Latency." *Current Topics in Salmonella and Salmonellosis*, 2017. <https://doi.org/10.5772/67173>.

McHan, Frank, and Emmett B. Shotts. "Effect of Feeding Selected Short-Chain Fatty Acids on the in Vivo Attachment of Salmonella Typhimurium in Chick Ceca." *Avian Diseases* 36, no. 1 (1992): 139. <https://doi.org/10.2307/1591728>.

Mkangara, M. and M., R. Mwakapuja, J. Chilongola, P. Ndakidemi, E. Mbega, and M. Chacha. "Mechanisms for Salmonella Infection and Potential Management Options in Chicken." *The Journal of Animal & Plant Sciences* 30, no. 2 (April 2, 2020): 259-79. <https://doi.org/10.36899/japs.2020.2.0050>.

Mroz, Z., S.-J. Koopmans, A. Bannink, K. Partanen, W. Krasucki, M. Øverland, and S. Radcliffe. "Chapter 4 Carboxylic Acids as Bioregulators and Gut Growth Promoters in Nonruminants." *Biology of Growing Animals*, 2006, 81-133. [https://doi.org/10.1016/s1877-1823\(09\)70091-8](https://doi.org/10.1016/s1877-1823(09)70091-8).

OHMOMO, Sadahiro, Osamu TANAKA, Hiroko K. KITAMOTO, and Yimin CAI. "Silage and Microbial Performance, Old Story but New Problems." *Japan Agricultural Research Quarterly: JARQ* 36, no. 2 (2002): 59-71. <https://doi.org/10.6090/jarq.36.59>.

Ranieri, Matthew L., Chunlei Shi, Andrea I. Moreno Switt, Henk C. den Bakker, and Martin Wiedmann. "Comparison of Typing Methods with a New Procedure Based on Sequence Characterization for Salmonella Serovar Prediction." *Journal of Clinical Microbiology* 51, no. 6 (2013): 1786-97. <https://doi.org/10.1128/jcm.03201-12>.

Russell, J.B. "Another Explanation for the Toxicity of Fermentation Acids at Low Ph: Anion Accumulation versus Uncoupling." *Journal of Applied Bacteriology* 73, no. 5 (1992): 363-70. <https://doi.org/10.1111/j.1365-2672.1992.tb04990.x>.

Thompson, J. L., and M. Hinton. "Antibacterial Activity of Formic and Propionic Acids in the Diet of Hens on Salmonellas in the Crop." *British Poultry Science* 38, no. 1 (1997): 59-65. <https://doi.org/10.1080/00071669708417941>.

USDA – United States Department of Agriculture – Research, Education & Economics Information System. University of Georgia, 2015. <https://portal.nifa.usda.gov/web/crisprojectpages/0228031-effect-of-fat-content-on-the-survival-of-salmonella-in-food.html>.

"USDA Launches New Effort to Reduce Salmonella Illnesses Linked to Poultry." USDA, October 19, 2021. <https://www.usda.gov/media/press-releases/2021/10/19/usda-launches-new-effort-reduce-salmonella-illnesses-linked-poultry>.

Van Immerseel, F., J. B. Russell, M. D. Flythe, I. Gantois, L. Timbermont, F. Pasmans, F. Haesebrouck, and R. Ducatelle. "The Use of Organic Acids to Combatsalmonellain Poultry: A Mechanistic Explanation of the Efficacy." *Avian Pathology* 35, no. 3 (2006): 182-88. <https://doi.org/10.1080/03079450600711045>.

Van Immerseel, Filip, Jeroen De Buck, Isabel De Smet, Frank Pasmans, Freddy Haesebrouck, and Richard Ducatelle. "Interactions of Butyric Acid- and Acetic Acid-Treated Salmonella with Chicken Primary Cecal Epithelial Cells in Vitro." *Avian Diseases* 48, no. 2 (2004): 384-91. <https://doi.org/10.1637/7094>.

Visscher, C. "Über Das Futter Helfen – Den Salmonellen Das Leben Schwer Machen." *Bauernblatt Schleswig-Holstein + Hamburg* 68/164, no. 51 (December 20, 2014): 66-68.

Wernicki, Andrzej, Anna Nowaczek, and Renata Urban-Chmiel. "Bacteriophage Therapy to Combat Bacterial Infections in Poultry." *Virology Journal* 14, no. 1 (September 16, 2017). <https://doi.org/10.1186/s12985-017-0849-7>.

Wessels, Kirsten, Diane Rip, and Pieter Gouws. "Salmonella in Chicken Meat: Consumption, Outbreaks, Characteristics, Current Control Methods and the Potential of Bacteriophage Use." *Foods* 10, no. 8 (2021): 1742. <https://doi.org/10.3390/foods10081742>.

Stop feed spoilage: How organic acids can preserve feed quality



By Technical Team, EW Nutrition

Feed spoilage is a significant issue for the feed industry, leading to loss of nutrients, feed waste, and substantial economic issues for feed and animal producers worldwide ([Leyva Salas et al., 2017](#)). Fungal growth is one of the main causes of feed spoilage; it can occur at any stage of the feed production chain, including grain pre- and post-harvest processes, during feed production or storage. Organic acids and their salts are globally used in animal nutrition for microbial preservation and supporting animal health.



Organic acids help preserve animal feed and prevent spoilage through molds, yeasts, and mycotoxins

The threat of molds and yeasts in animal feed

Yeasts and molds can have both positive and negative effects on products consumed by animals and humans. On the one hand, yeasts are used to produce fermented products, such as bread, wine, and beer. On the other hand, yeasts and molds promote the spoilage of raw materials, food, and feeds ([Lowes et al., 2000](#)). Molds are among the most potent food and feed spoilers. They can be very resilient to environmental stress, which is a concern in climate change scenarios ([Perrone et al., 2020](#)) and enables them to withstand feed preservation measures ([Punt et al., 2020](#)).

Several hundred species of molds and yeasts can invade a large variety of raw materials and feeds. They show an easy adaptation to different environments; for instance, they can grow and reproduce in media with pH levels ranging from 2 to above 9 ([Tournas et al., 2001](#)). However, the majority of yeasts and molds require free oxygen to grow and thrive.

Excess moisture, high water activity, and high temperatures in feedstuffs are the main mold growth factors that concern the feed industry ([Mohapatra et al., 2017](#)). At storage, grains' moisture content should not exceed 13%, and the water activity of raw materials, feedstuffs, and finished feed should be maintained below 0.8 ([Dijksterhuis et al., 2019](#)). Controlling these points contributes to preventing the growth of most pathogens and undesirable microorganisms.



Mold growth reduces the nutritional value of feed, which affects animal health and performance

The microbiology of molds and how they affect the feed

The microbial growth dynamic of grain storage depends on several factors, including [the harvest season](#), grain temperature and moisture content, as well as the type of facility and its environment. For instance, in some areas, grains are harvested at the beginning of the cold season and stored through the following warm season. Storage molds constitute a significant threat to the quality of these raw materials, especially during the warm months, when the stored grains may become hotter than the surrounding environment. This leads to condensation, which increases moisture and water activity. Molds easily thrive in these conditions.

Storage molds reduce the nutritional and commercial value of grains and feeds. For grains, their commercial value decreases when the appearance of kernels changes in a manner recognized by the grain industry as kernel damage. The chemical composition of feeds may deteriorate due to enzymatic actions, resulting in a loss of nutrients (energy, vitamins) and the production of free fatty acids and other unwanted by-products ([Reed et al., 2007](#)).

Extensive research has established the factors that influence mold-induced deterioration during grain storage and which management strategies are required:

- **Moisture content and water activity** (a function of the temperature, moisture content, and substrate) – Microorganisms have a limiting water activity below which they cannot grow; therefore, drying the grains below that critical level is part of an effective mold control strategy ([Mannaa & Kim, 2017](#)).
- **Temperature** – Grain-contaminating molds thrive in tropical regions, where high temperature and humidity conditions predominate. In general, molds are inactive if the grains are stored below 20 °C ([Mousa et al., 2013](#)). However, the temperature of stored grains increases as molds begin to grow in the warmer and/or wetter parts of the grain/feed mass and feed, and heat is generated due to respiration, accelerating the deterioration rate. Moreover, the presence of a temperature gradient in the feedstuffs causes air to move, accelerating the transfer of moisture to cooler grain ([Mannaa & Kim, 2017](#)).
- **Grain quality**, including previous storage conditions, insect infestation, presence of broken kernels, and impurities – When grain is too warm, the rate of insects' breeding is higher (they respond to higher temperatures), the grain contains more humidity and may carry fungal spores. Broken kernels are an easier target for mold and insect infestations than whole ones, increasing the possibility of spoilage ([Marcos Valle et al., 2021](#)).
- **Duration of storage, management, and aeration** influence the oxygen and carbon dioxide concentration in the grain mass, which plays a role in mold growth ([Marcos Valle et al., 2021](#)).

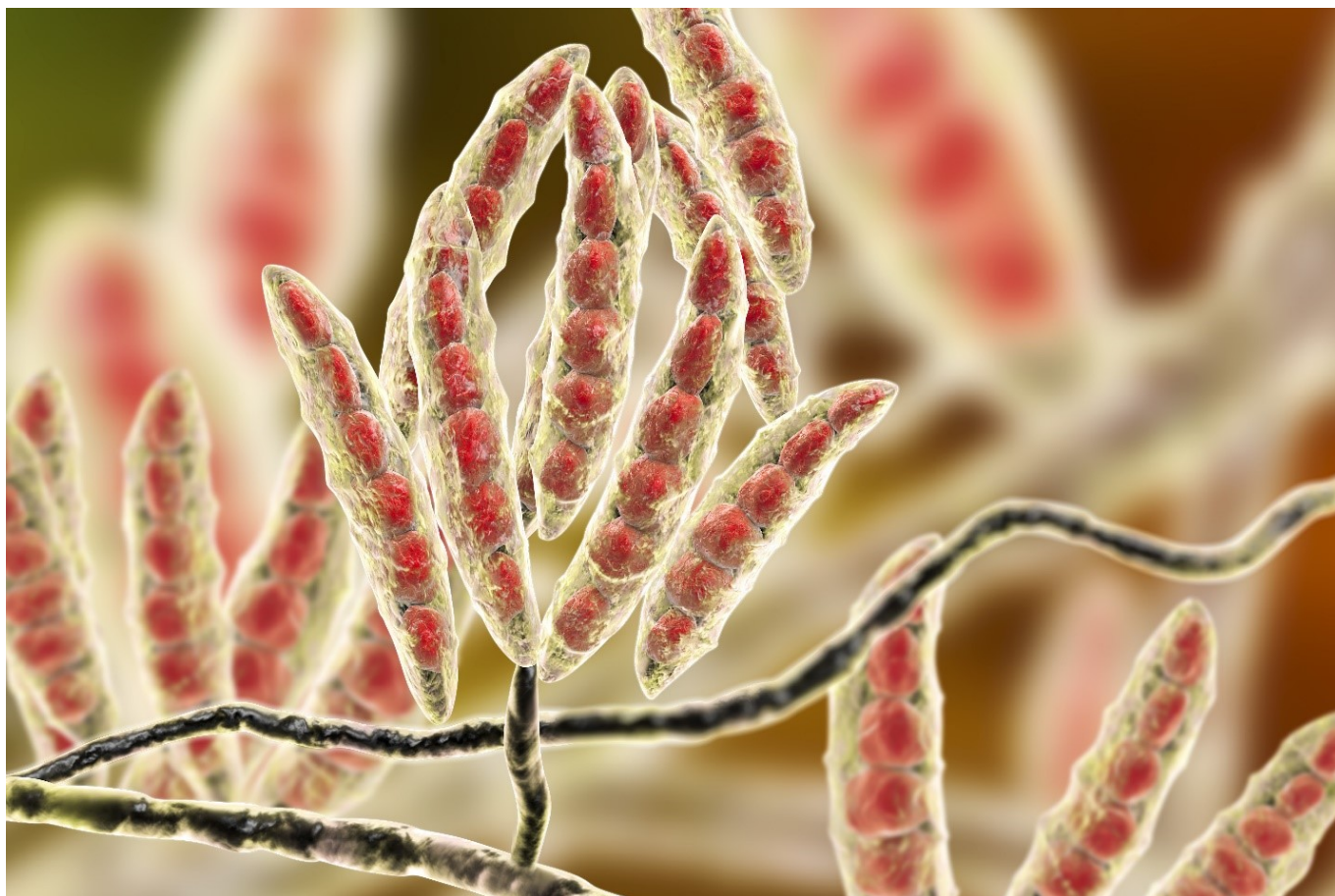
The consequences of storage deterioration include:

- worse organoleptic properties (aspect, texture, taste, and aroma) of grains and feeds
- more kernel damage,
- higher fat acidity,
- slight increase in protein content as non-protein constituents are consumed by mold respiration, causing
- lower energy value of the grain/feed ([Reed et al., 2007](#)), and
- lower content of vitamins A, B1, D3, E, and K.

Molds and mycotoxins: a toxic relationship for animal health

Beyond their negative impact on feed quality, some fungal genera such as *Aspergillus*, *Penicillium*, *Alternaria*, and *Fusarium* can produce [mycotoxins, secondary metabolites that have toxic effects on humans and animals](#) ([Greco et al., 2015](#)). Roughly 60% of raw materials produced for agriculture purposes worldwide are estimated to be contaminated by fungi and mycotoxins ([Eskola et al., 2020](#)). Mycotoxins can

induce toxic, carcinogenic, and mutagenic reactions even at low concentrations. Their presence in the final feed is a sign of alert as, usually, these metabolites are resistant to technological treatments. Thus, it is important to stop them from entering the feed production chain ([Leyva Salas et al., 2017](#)).



Feed-contaminating Fusarium species produce mycotoxins such as trichothecenes, zearalenone, and Fumonisin.

Organic acids: Unrivaled in preventing feed spoilage

It is crucial to reduce the feed losses and improve animal health by controlling fungal contamination at all stages of the feed production chain: from pre-harvest strategies on the field to post-harvest management during storage and even at feed processing. Throughout these processes, producers can apply different management practices. For instance, in field crops, fungal growth can be prevented through crop rotation and tillage; the use of fungicides is a later measure when mold presence exceeds critical levels.

Post-harvest management of grains and their by-products includes drying and storage management through moisture and temperature monitoring and aeration programs. Other spoilage-prevention measures include good hygiene practices and thermal treatments in feed production. However, feed producers and farmers face limitations in applying and linking such measures to tackle the occurrence of these undesirable pathogens ([Dijksterhuis et al., 2019](#)).

Certain organic acids, such as propionic, sorbic, benzoic, and acetic acids, have [proven effective in preventing mold growth and feed spoilage](#). These organic acids are used globally now, not only for improving animal nutrition but also for supporting animal health ([Dijksterhuis et al., 2019](#)).

Pro-Stabil BSL is a product that harnesses the feed preservation effects of organic acids and combines them with surfactants. This means that it can offer a strong yeast and mold inhibition while maintaining the moisture in feed, thus reducing the risk of microbial challenges while prolonging the shelf life of

feedstuffs and compound feeds.

Trial results: Pro-Stabil BSL is a great tool to reduce mold growth and manage moisture

Pro-Stabil BSL contains a synergistic blend of organic acids and a surfactant that leads to

- » Improved moisture dispersion in the feed
- » Increased water retention (reduced water activity)
- » Improved anti-mold agent dispersion in the feed and grain

Trial results show a significant decrease in mold growth when ProStabil BSL was added to compound feed. In addition, when moisture was added at 2%, moisture from the environment was also observed, but the mold counts still decreased (Figure 1).

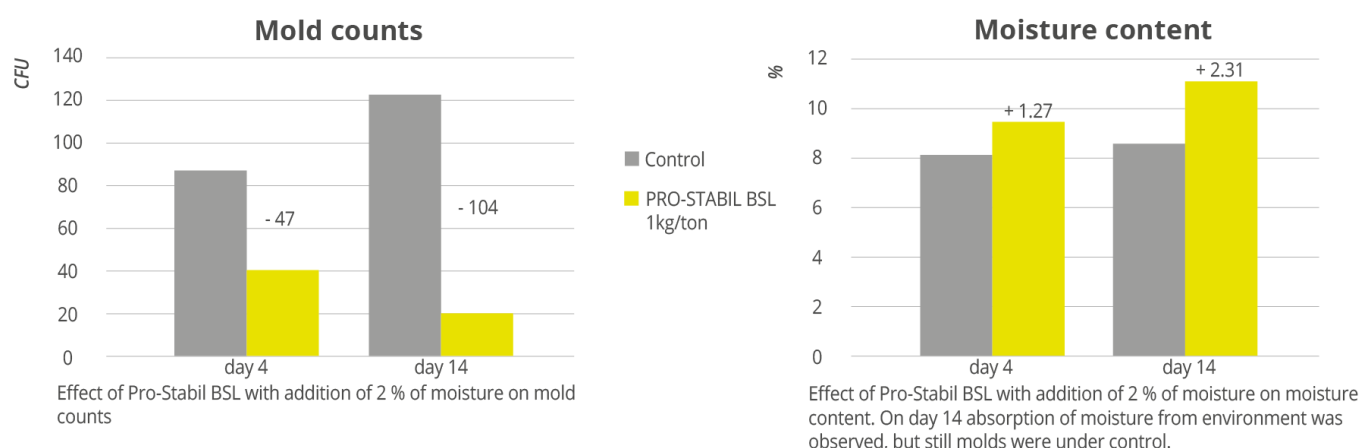


Figure 1: Effects of Pro-Stabil BSL with addition of 2 % moisture on feed quality indicators

When adding Pro-Stabil BSL to animal feed, the following benefits can be expected:

- Reduction and prevention of mold growth and recontamination
- Improved moisture management
- Improved feed mill efficiency production
- Improved microbiological quality of grains and feed
- Shrinkage management by increasing moisture in feed with no risk of mold development
- Reduced water dissipation

Mold growth can lead to sensory defects in feed and reduce its nutritional value. It can also harm animals through the production of mycotoxins. [Pro-Stabil BSL](#) offers a safe solution that is also easy to handle. Using the preservative properties of organic acids, Pro-Stabil BSL helps to reduce feed spoilage and its associated effects on animal health and performance.

References

Dijksterhuis, Jan, Martin Meijer, Tineke van Doorn, Jos Houbraken, and Paul Bruinenberg. "The Preservative Propionic Acid Differentially Affects Survival of Conidia and Germ Tubes of Feed Spoilage Fungi." *International Journal of Food Microbiology* 306 (2019): 108258. <https://doi.org/10.1016/j.ijfoodmicro.2019.108258>.

Eskola, Mari, Gregor Kos, Christopher T. Elliott, Jana Hajšlová, Sultan Mayar, and Rudolf Krska. "Worldwide

contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25%." *Critical Reviews in Food Science and Nutrition* 60, no. 16 (2020): 2773-2789.

<https://doi.org/10.1080/10408398.2019.1658570>

Greco, Mariana, Minna Kemppainen, Graciela Pose, and Alejandro Pardo. "Taxonomic Characterization and Secondary Metabolite Profiling Of Aspergillus Section Aspergillus Contaminating Feeds And Feedstuffs." *Toxins* 7, no. 9 (2015): 3512–37. <https://doi.org/10.3390/toxins7093512>.

Harein, P., & Meronuck, R. (1995). Stored grain losses due to insects and molds and the importance of proper grain management. In V. Krischik, G. W. Cuperus, & D. Galliard (Eds.), *Stored product management* (pp. 29e31). Oklahoma Cooperative Extension Service Publication. E-912.

Leyva Salas, Marcia, Jérôme Mounier, Florence Valence, Monika Coton, Anne Thierry, and Emmanuel Coton. "Antifungal Microbial Agents for Food Biopreservation—a Review." *Microorganisms* 5, no. 3 (2017): 37. <https://doi.org/10.3390/microorganisms5030037>.

Lowes, K. F., C. A. Shearman, J. Payne, D. MacKenzie, D. B. Archer, R. J. Merry, and M. J. Gasson. "Prevention of Yeast Spoilage in Feed and Food by the Yeast Mycocin Hmk." *Applied and Environmental Microbiology* 66, no. 3 (2000): 1066–76. <https://doi.org/10.1128/aem.66.3.1066-1076.2000>.

Mannaa, Mohammed, and Ki Deok Kim. "Influence of temperature and Water activity on Deleterious fungi AND Mycotoxin production during grain storage." *Mycobiology* 45, no. 4 (2017): 240–254. <https://doi.org/10.5941/myco.2017.45.4.240>.

Marcos Valle, F. J., Castellari, C., Yommi, A., Pereyra, M. A., & R. Bartosik. "Evolution of grain microbiota during hermetic storage of corn (zea mays l.)." *Journal of Stored Products Research* 92 (2021): 101788. <https://doi.org/10.1016/j.jspr.2021.101788>.

Mohapatra, D., Kumar, S., Kotwaliwale, N., and K. K. Singh. "Critical factors responsible for fungi growth in stored food grains and non-Chemical approaches for their control." *Industrial Crops and Products* 108 (2017): 162–182. <https://doi.org/10.1016/j.indcrop.2017.06.039>.

Mousa, W., Ghazali, F. M., Jinap, S., Ghazali, H. M., and S. Radu. "Modeling growth rate and assessing AFLATOXINS production by Aspergillus flavus as a function of Water activity and temperature on polished and brown rice." *Journal of Food Science* 78, no. 1 (2013). <https://doi.org/10.1111/j.1750-3841.2012.02986.x>.

Perrone G, Ferrara M, Medina A, Pascale M, and N. Magan. "Toxigenic Fungi and Mycotoxins in a Climate Change Scenario: Ecology, Genomics, Distribution, Prediction and Prevention of the Risk." *Microorganisms* 8, no. 10 (2020): 1496. <https://doi.org/10.3390/microorganisms8101496>.

Punt, Maarten, Tom van den Brule, Wieke R. Teertstra, Jan Dijksterhuis, Heidy M.W. den Besten, Robin A. Ohm, and Han A.B. Wösten. "Impact of Maturation and Growth Temperature on Cell-size Distribution, Heat-Resistance, Compatible Solute Composition and Transcription Profiles of Penicillium Roqueforti Conidia." *Food Research International* 136 (2020): 109287. <https://doi.org/10.1016/j.foodres.2020.109287>.

Reed, Carl, Stella Doyungan, Brian Ioerger, and Anna Getchell. "Response of Storage Molds to Different Initial Moisture Contents of Maize (Corn) Stored AT 25°C, and Effect on Respiration Rate and Nutrient Composition." *Journal of Stored Products Research* 43, no. 4 (2007): 443–58. <https://doi.org/10.1016/j.jspr.2006.12.006>.

Tournas, Valerie, Michael E. Stack, Phillip B. Mislivec, Herbert A. Koch, and Ruth Bandler. "Bacteriological Analytical Manual Chapter 18: Yeasts, Molds and Mycotoxins." U.S. Food and Drug Administration. April 2001. <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-18-yeasts-molds-and-mycotoxins>.

Feed hygiene in animal nutrition is vital - and organic acids help achieve it



by *Technical Team*, EW Nutrition

Feed safety is essential for animal health and performance - and food safety. Inadequate feed sanitization is still a problem across the globe. It impacts not only the feed industry and animal producers but also puts workers and consumers at risk of being exposed to harmful substances.

Developing a hygiene program for the whole feed chain needs to include proper monitoring of microbial growth, as well as feed processing methods that prevent feed contamination and enable decontamination. This article outlines the importance of feed hygiene and focuses on how organic acids help reduce contamination from “farm to fork”.



*Corn is often contaminated with *Aspergillus* fungi that can produce poisonous [mycotoxins](#)*

How to achieve feed hygiene

[Feed hygiene](#) requires the control of microorganisms throughout the feed production chain. However, producers or retailers can rarely certify or verify feedstuffs' safety due to the wide range of potential microbial contamination agents and hazards encountered in different feed environments ([den Hartog, 2003](#)). The relationship between feed and microorganisms varies, depending on the conditions: feed can transport pathogenic microorganisms and thus directly transmit disease; likewise, microorganisms can also be responsible for feed spoilage and thereby indirectly cause issues ([Baer, Miller, and Dilger, 2013](#)).

Since its foundation, the [World Organization for Animal Health](#) (OIE) has established standards, guidelines, and recommendations for toxin risk management, including for microorganisms that are transmissible via feed. Recurring outbreaks of *Salmonella*, *Escherichia coli*, and other familiar *Enterobacteriaceae* are a key concern for animal health professionals and the feed industry ([Elsayed et al., 2021](#)). However, as factors ranging from climate change to genetic mutations come into play, feed producers are working with moving targets; some of the most significant issues they might face tomorrow are unknown today. There are no easy solutions to these multifactorial problems – but in any case, corrective measures need to include quality control and quality assurance for assessing and managing the pathogenic and microbial risk situation.

To improve animal productivity sustainably, producers regularly experiment with modifying production techniques, innovating feed formulations, but also exploring new ingredients. The inclusion of new ingredients such as animal proteins, oils, and fermented products, among others, heightens the need for strict feed quality monitoring ([Truelock et al., 2020](#)). New ingredients come with causative agents of feedborne illnesses, some of which might be unknown ([Goodarzi Boroojeni et al., 2016](#)). Therefore, feed and animal producers need to consider how feed changes impact feed safety and include these hazards in their planning and risk assessments.

Better feed hygiene is crucial

For any animal production, feed processing constitutes the most crucial part of feed hygiene management, as it covers all treatments of the feed before ingestion. It is referred to as “hydrothermal processing” due to the use of heat that is required to kill most of the pathogens in raw materials, feedstuffs, and compound feed ([Jones, 2011](#)). However, whether or not hydrothermal processing will effectively eliminate a given pathogen depends on its heat resistance. Moreover, factors such as the type of feed components involved and water activity levels also need to be considered to reduce microbial pressure ([Doyle and Mazzotta, 2000](#)).

The new generation of feed milling equipment – besides elevating feed costs – can also improve feed quality ([Truelock et al., 2020](#)). These technologies tend to enhance feed stability and hygiene by modifying the physicochemical properties of the ingredients. This improves the absorption of nutrients, thereby enabling a higher feed intake efficiency with positive results for animal performance ([Abdollahi, Svihus, and Ravindran, 2013](#)). However, while increasing processing time at a given temperature can lead to a better decontamination process, it can also negatively affect some nutrients’ dynamics. This includes enzymes, proteins, minerals, vitamins, fiber and starch, and especially non-starch polysaccharides ([Goodarzi Boroojeni et al., 2014](#)).

Organic acids as a solution of feed hygiene risk management

Hence, while significant progress in feed science and feed production technology has already been made, researchers and the industry are still searching for alternative approaches to supporting feed hygiene ([Goodarzi Boroojeni et al., 2016](#)). Organic acids are a central research field as they offer promising antimicrobial properties. In combination with feed mill techniques, they already play an essential role in feed preservation ([Brul et al., 2002](#)). Despite their efficacy in inhibiting microbial growth, weak organic acids are safe to handle (especially when they are buffered) compared to inorganic acids.

In addition to their preservative effect in feed, it has been shown that organic acids can support gut health. They are not just antimicrobial agents but also acidifiers that display their impact in the stomachs of monogastric animals ([Tugnoli et al., 2020](#)).

A combined solution for microbial contamination challenges

To support the feed industry and animal production in light of feed safety challenges in AGP-free production, EW Nutrition focuses research efforts on maximizing the beneficial effect of organic acids. The [ACIDOMIX range of products](#) supports the stabilization of the gastrointestinal microflora, inhibiting pathogenic bacterial growth in feed and water. Acidomix is an efficient acidifier specially formulated to have strong antimicrobial effects applicable in feed hygiene programs. Various powder and liquid solutions offer a wide range of benefits:

- Strong antimicrobial effect, supporting the prevention of bacterial infections
- Reducing the incidence of dysbiosis
- Acidifying the feed and digestive tract
- Supporting the improvement of production performance
- Preventing feed re-contamination
- Flexible application

Feedstuffs and compound feed are at risk of contamination and re-contamination throughout the feed production chain: processing, transportation, delivery, storage, and on-farm. Thus, a holistic and

integrated approach that includes optimized feed mill processing and customized organic acids is required to improve the feed's hygiene status. The positive effects are clear: feed producers benefit economically, animal producers reap the effects of improved animal health and performance, and people get to enjoy producing and consuming safe and nutritious food.

References

- Abdollahi, M R, B Svihus, and V Ravindran. 2013. "Pelleting of Broiler Diets: An Overview with Emphasis on Pellet Quality and Nutritional Value." *Animal Feed Science and Technology* 179 (1-4): 1-23. <https://doi.org/10.1016/j.anifeedsci.2012.10.011>.
- Baer, Arica A, Michael J Miller, and Anna C Dilger. 2013. "Pathogens of Interest to the Pork Industry: A Review of Research on Interventions to Assure Food Safety." *Comprehensive Reviews in Food Science and Food Safety* 12 (2): 183-217. <https://doi.org/10.1111/1541-4337.12001>.
- Brul, Stanley, Peter Coote, Suus Oomes, Femke Mensonides, Klaas Hellingwerf, and Frans Klis. 2002. "Physiological Actions of Preservative Agents: Prospective of Use of Modern Microbiological Techniques in Assessing Microbial Behaviour in Food Preservation." *International Journal of Food Microbiology* 79 (1-2): 55-64. [https://doi.org/10.1016/s0168-1605\(02\)00179-4](https://doi.org/10.1016/s0168-1605(02)00179-4).
- Doyle, M Ellin, and Alejandro S Mazzotta. 2000. "Review of Studies on the Thermal Resistance of Salmonellae." *Journal of Food Protection* 63 (6): 779-95. <https://doi.org/10.4315/0362-028x-63.6.779>.
- Elsayed, Mohamed Sabry Abd Elraheam, Awad A Shehata, Ahmed Mohamed Ammar, Tamer S Allam, and Reda Tarabees. 2021. "The Beneficial Effects of a Multistrain Potential Probiotic, Formic, and Lactic Acids with Different Vaccination Regimens on Broiler Chickens Challenged with Multidrug-Resistant Escherichia Coli and Salmonella." *Saudi Journal of Biological Sciences*. <https://doi.org/10.1016/j.sjbs.2021.02.017>.
- Goodarzi Borojeni, Farshad, Birger Svihus, Heinrich Graf von Reichenbach, and Jürgen Zentek. 2016. "The Effects of Hydrothermal Processing on Feed Hygiene, Nutrient Availability, Intestinal Microbiota and Morphology in Poultry—A Review." *Animal Feed Science and Technology* 220: 187-215. <https://doi.org/10.1016/j.anifeedsci.2016.07.010>.
- Den Hartog, Johan den. 2003. "Feed for Food: HACCP in the Animal Feed Industry." *Food Control* 14 (2): 95-99. [https://doi.org/10.1016/S0956-7135\(02\)00111-1](https://doi.org/10.1016/S0956-7135(02)00111-1).
- Jones, Frank T. 2011. "A Review of Practical Salmonella Control Measures in Animal Feed." *Journal of Applied Poultry Research* 20 (1): 102-13. <https://doi.org/10.3382/japr.2010-00281>.
- Truelock, Courtney N, Mike D Tokach, Charles R Stark, and Chad B Paulk. 2020. "Pelleting and Starch Characteristics of Diets Containing Different Corn Varieties." *Translational Animal Science* 4 (4): txaa189. <https://doi.org/10.1093/tas/txaa189>.
- Tugnoli, Benedetta, Giulia Giovagnoni, Andrea Piva, and Ester Grilli. 2020. "From Acidifiers to Intestinal Health Enhancers: How Organic Acids Can Improve Growth Efficiency of Pigs." *Animals* 10 (1): 134. <https://doi.org/10.3390/ani10010134>.
- Goodarzi Borojeni, F., W. Vahjen, A. Mader, F. Knorr, I. Ruhnke, I. Röhe, A. Hafeez, C. Villodre, K. Männer, and J. Zentek. "The Effects of Different Thermal Treatments and Organic Acid Levels in Feed on Microbial Composition and Activity in [Gastrointestinal Tract](#) of Broilers." *Poultry Science* 93, no. 6 (June 1, 2014): 1440-52. <https://doi.org/10.3382/ps.2013-03763>.