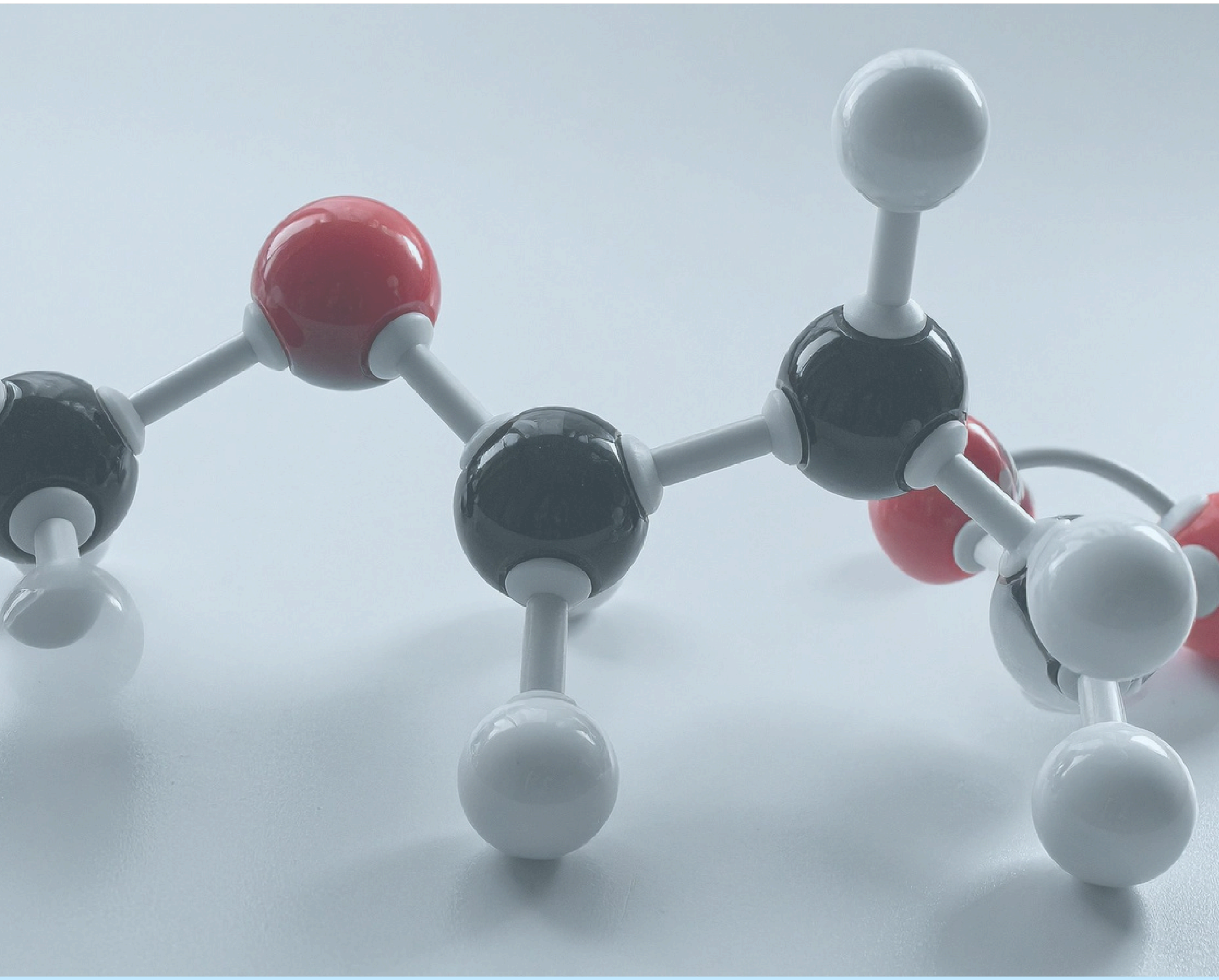


Organic Acids in Animal Nutrition

Second Edition

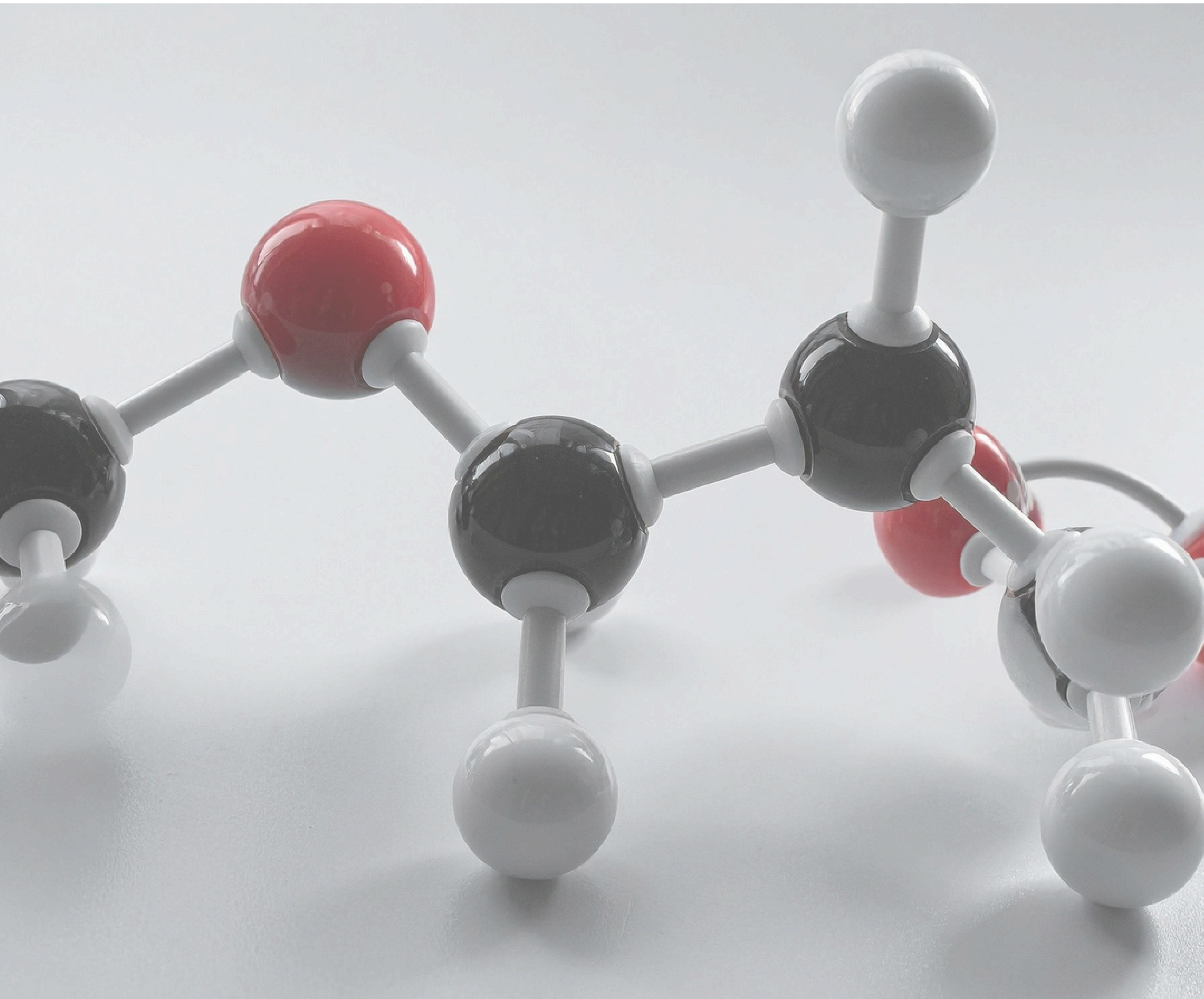
Organic Acids in Animal Nutrition

Second edition contributors:

Christian Lückstädt, ADDCON; Sébastien Oguey, ADM; Harri Otto Miettinen, BASF; Elke von Hemendahl, BASF; Julia Trautwein, BASF; Liza van den Eede, EASTMAN; Tania Soenens, EASTMAN; Maja Sakkers, KEMIN; Sabina Díaz Martinez, NOVUS; Hans van Dam, TROUW NUTRITION (NUTRECO) (editor).

Published September 2026

Table of Contents



Preamble

FEFANA, the EU Association of Specialty Feed Ingredients and their Mixtures, represents companies that manufacture, import and/or distribute specialty feed ingredients or mixtures for use in animal nutrition.

Since its establishment in 1963, FEFANA has been the recognised voice of the sector, representing and promoting the interests of the industry before national, European and international authorities, as well as partner organisations across the feed and food chain. Specialty feed ingredients and their mixtures are essential components of modern animal nutrition. They help ensure balanced diets, support animal health and welfare, and contribute to the production of safe and sustainable animal-derived food. Alongside quality and safety, innovation and sustainability remain at the heart of our industry's commitment to meeting current and future challenges.

Drawing on the extensive expertise and scientific knowledge of its membership, FEFANA provides a unique platform for collaboration and knowledge-sharing. This publication reflects the collective experience of our sector and aims to provide a comprehensive overview of the use of organic acids in animal nutrition.

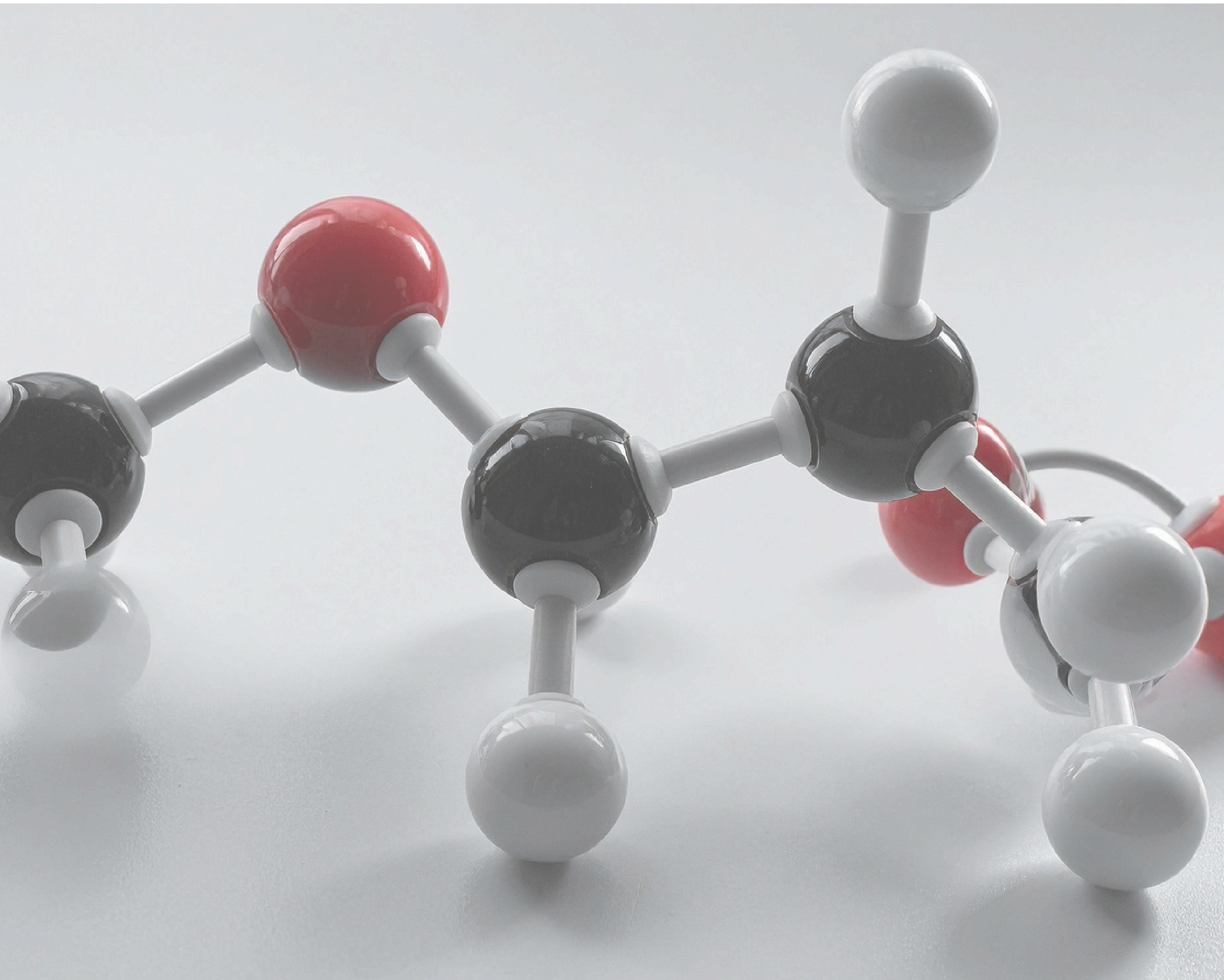
Organic acids have been used successfully in feed for several decades and play an important role in feed preservation, feed hygiene, animal health and livestock performance. Continuous innovation has led to the development of increasingly sophisticated products and solutions designed to control undesirable microorganisms, including bacteria and fungi, while supporting efficient and sustainable livestock production in a challenging global environment.

This booklet focuses on organic acids and their derivatives authorised as feed additives under Regulation (EC) No 1831/2003 within the technological and zootechnical categories. Amino acids and organic acids authorised as flavourings are outside the scope of this publication.

First developed by the FEFANA Organic Acids Working Group in 2014, this fully revised second edition reflects the latest scientific and technical knowledge on the manufacture, application and benefits of organic acids in animal nutrition. It also incorporates updated information on the regulatory framework and recent developments within the sector.

We trust that you will find this publication both informative and practical.

Joerg Seifert
FEFANA Secretary General



1. Introduction to organic acids: their nature, function and use in the EU market

The potential uses of organic acids as forage and grain preservatives and in livestock nutrition has been known for decades and is documented in many scientific publications. In nature, organic acids are produced by microbial fermentation, as well as occurring naturally in plants. Organic acids have been important for thousands of years as food and feed preservatives. This is why the industry has found them a natural choice for preservation, and they are generally accepted by the public to optimise animal production [1]. They also make a fundamental contribution to feed hygiene, as they suppress the growth or in some instances even reduce microbiological contamination of feed ingredients thus allowing more sustainable use of feed resources.

Organic acids are also the most cost-effective and eco-efficient performance-enhancing option available to the feed industry to date. The use of organic acids as acidifiers in feed is now a worldwide common practice for pigs, poultry and aquaculture, for feed preservation, for silage conservation and improving food and feed hygiene, thereby supporting the circular economy and sustainability in general.

Organic acids have gained worldwide acceptance in the animal nutrition industry. They have been used for more than 60 years, to reduce bacterial growth and mould in feedstuffs and thus preserve hygienic quality. In feed legislation they are mainly authorised as preservatives or silage additives. However, due to their antimicrobial activity, organic acids not only help to preserve feed, but they are also effective in reducing bacterial content in feed and maintaining the nutritional value of the feed to ensure animal performance, as well as improving nutrient digestibility – which in turn leads to stable animal health and increased performance [2].

Improved animal performance and wellbeing have been demonstrated for several organic acids. Besides improving feed hygiene, the corresponding reduction in pathogen intake, stabilises the gut microflora eubiosis, while positive effects on feed digestion and absorption have been demonstrated in several studies. In animal husbandry, better feed conversion rates, improved daily gain and a reduced incidence of diarrhoea all contribute to enhanced economic return, through lower feed costs and time to market weight [3]. As a group of chemicals, the organic acids are any organic carboxylic acid, including fatty acids, with the general structure R-COOH. The most common organic acids and their chemical structures and properties are shown in Table 1.

Organic acids are transparent and colourless liquids (lactic acid may also come in a brown colour, where the fermentation broth is still present) or white or off-white powders. Especially the highly concentrated liquid acids need to be handled with care, according to its hazard classification.

TABLE 1. PHYSICO-CHEMICAL PROPERTIES OF SOME ORGANIC ACIDS (AFTER [4])[1]

Acid	Structural formula	MM (g/mol)	Density (g/ml)	Physical form	pKa value	Solubility in water
Formic	HCOOH	46.03	1.22	liquid	3.75	∞
Acetic	CH3COOH	60.05	1.05	liquid	4.76	∞
Propionic	CH3CH2COOH	74.08	0.99	liquid	4.88	∞
Lactic	CH3CH(OH)COOH	90.08	1.21	liquid	3.83	v
Sorbic	CH3CH:CHCH:CHCOOH	112.14	1.2	Solid	4.76	s
Benzoic	C6H5-COOH (ring structure)	122.12	1.26	Solid	4.2	s
Fumaric	COOHCH:CHCOOH	116.07	1.64	Solid	3.02	s
					4.38	
Malic	COOHCH2CH(OH)COOH	134.09	1.61	Solid	3.4	∞
					5.2	
Citric	COOHCH2C(OH)(COOH)CH2COOH	192.14	1.67	solid	3.13	v
					4.76	
					6.4	

[1]*MM: molecular mass; ∞: soluble in all proportions; v: very soluble; s: sparingly soluble.*

Organic acids have been used for decades in commercial compound feeds, mostly in feed preservation, for which formic and propionic acids are particularly effective. In the European Union, these two organic acids and several others (e.g. lactic, benzoic, citric, fumaric and sorbic acids) and their salts (e.g. calcium formate, calcium propionate, ammonium propionate, potassium diformate and sodium formate) are widely used.

Blends of organic acids are used to broaden the spectrum of efficacy against different microbial species and serotypes (in terms of their Minimum Inhibitory Concentrations (MIC)).

Organic acids are available on the market in its simple acid form, but also certain derivatives are marketed for various reasons, notably:

- **Salts** - wholly or partially neutralised acids (Na, K, Ca, ammonium salts), e.g. ammonium propionate, sodium formate, or potassium sorbate; some partially neutralised acids come in liquid form. Salts are easier to handle in the feed mill (less corrosive, less odour);
- **Adsorbates** - liquid acids or mixtures of acids adsorbed onto a carrier, being an inert substrate, usually silica or vermiculite. Again, these may have advantages over liquid products for transport and administration in feed.
- **Glycerides** - mono-, di-, and triglycerides of organic acids formed from the reaction of glycerol with organic acids. Also available in solid form, being absorbed on a carrier (usually silica).

Organic acids are applied directly into feedstuffs and compound feed: liquid acids and blends are sprayed onto the feed whereas solid acids and derivatives are added directly or via special premixtures. Organic acids can also be applied to forage for silages and included in liquid feed supplied via water for drinking, depending on the regulations of the jurisdiction.

Our understanding of the modes of action of organic acids in the gastrointestinal tract (GIT) has grown significantly in recent decades. The demonstrated modes of action of organic acids are listed in Table 2.

The inclusion of organic acids and their derivatives in diets for pigs has been studied extensively. They have proved especially effective in maintaining growth performance since the ban on antibiotic growth promoters came into effect in Europe in 2006. Also, use of organic acids in poultry nutrition, and use of specific organic acids in ruminant nutrition has been studied and will be further explored in a next chapter.

More recently, the use of acids has been adopted by the aquaculture industry. The most recent developments also include synergistic combinations of straight organic acids with organic acid derivatives, and other bio-active ingredients such as plant extracts and enzymes.

TABLE 2. MODES OF ACTION OF ORGANIC ACIDS (AFTER [5])[1]

Area targeted	Mechanism
Feed	Decrease in pH
	Antimicrobial effect (bacteria, yeast, fungi)
	Reduced buffering capacity of feed
GIT1 Proton	Decrease in pH
	Increased efficiency of pepsin in the stomach (pH optima of 2.5 and 3.5) in free form Antimicrobial effect
Anion	Antimicrobial effect
	Complexing agent (Ca^{2+}, Mg^{2+}, Fe^{2+} etc.)
Intermediary metabolism	Energy source and gut integrity

[1] *GIT: Gastrointestinal tract.*

2. Manufacture of organic acids and blends

Organic acids (and their salts) are produced in a wide variety of ways. A generic distinction can be made between chemical synthesis and synthesis by fermentation.

Although the variety of production processes in both chemical synthesis and fermentation systems is very large, this chapter describes their main features in brief.

2.1 Production of organic acids by chemical synthesis

The production process of organic acids includes a reaction of the raw materials followed, if necessary, by separation of the acid from other fractions and a final distillation, to yield the acid at the required concentration.

Examples of organic acids produced by chemical synthesis are formic acid, acetic acid, propionic acid, sorbic acid and fumaric acid.

Production of the respective acid salts requires neutralisation of the acid with an appropriate base in the reactor. For solid forms of the product, this step will be followed by evaporation, crystallisation and drying.

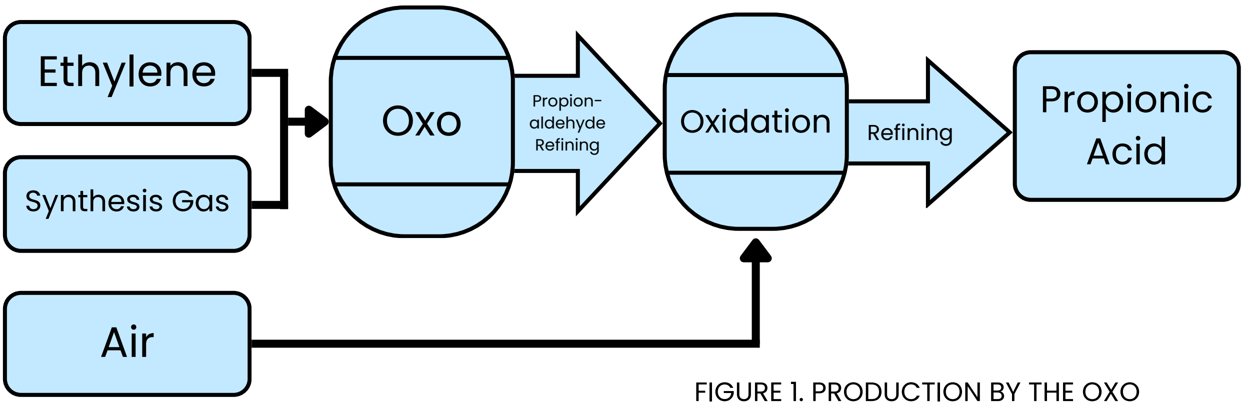


FIGURE 1. PRODUCTION BY THE OXO PROPIONIC ACID PROCESS

Neutralisation can be complete (e.g., calcium and sodium salts) but also partial, resulting in a buffered liquid (e.g. ammonium and sodium salts). Also partly buffered salts in crystalline (solid) form exist, like potassium diformate and sodium diacetate which still have one free proton per molecule.

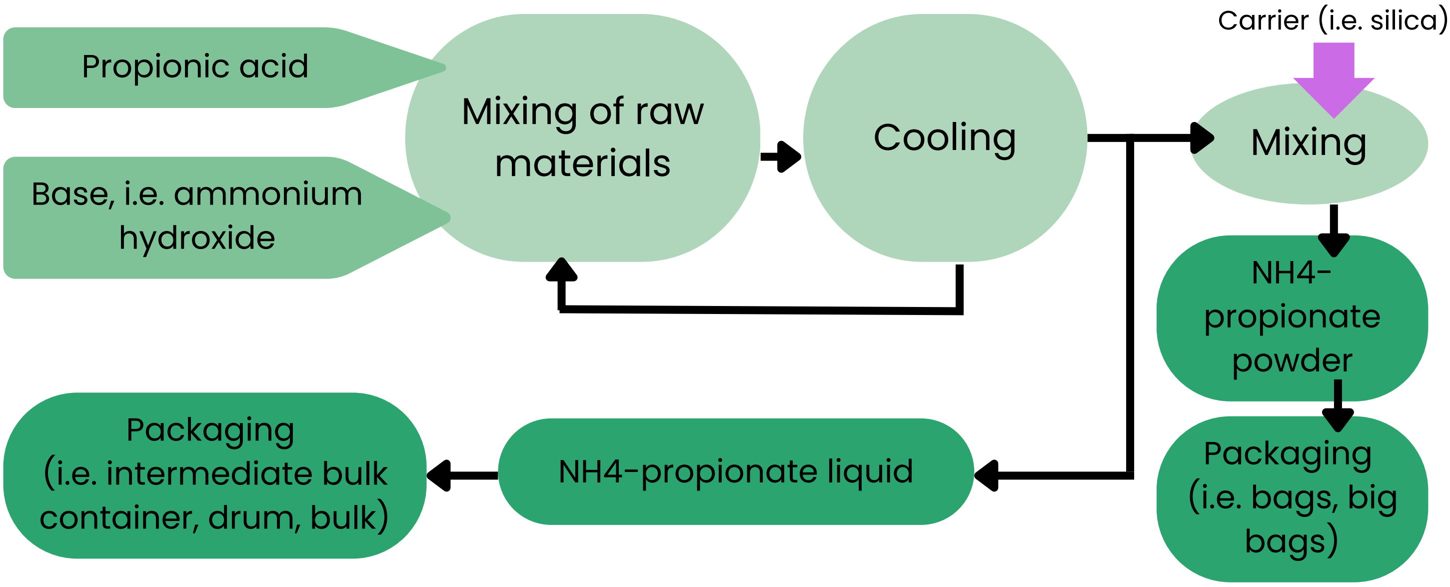


FIGURE 2. PRODUCTION OF THE AMMONIUM SALT OF PROPIONIC ACID ON CARRIER

2.2 Production of organic acids by fermentation

Fermentation is a metabolic process in which sugars are converted to acids, gases and/or alcohol using yeast or bacteria. It has been used by humans for thousands of years in the production of food and beverages. Fermentation is commonly used to produce wine and beer, but the process is also employed in preservation to create lactic acid in sour foods such as pickled cucumbers and yoghurt. Examples of organic acids resulting from large scale commercial fermentation are lactic and citric acids.

The following materials are generally required to produce organic acids by fermentation:
- Substrates/raw materials.
- Nutrients for the growth of bacteria.
- Neutralising agents.
- Micro-organisms.

2.2.1 Fermentation conditions

Depending on the organic acid required, not only does the selection of ingredients and micro-organisms differ, but also the fermentation conditions, such as pH and temperature.

2.2.2 Downstream process

After the fermentation process, the aqueous broth must be treated to obtain the final purified organic acid. Killing the microorganisms and removal of the biomass are mostly done by heating and increasing the pH, followed by decantation and filtration.

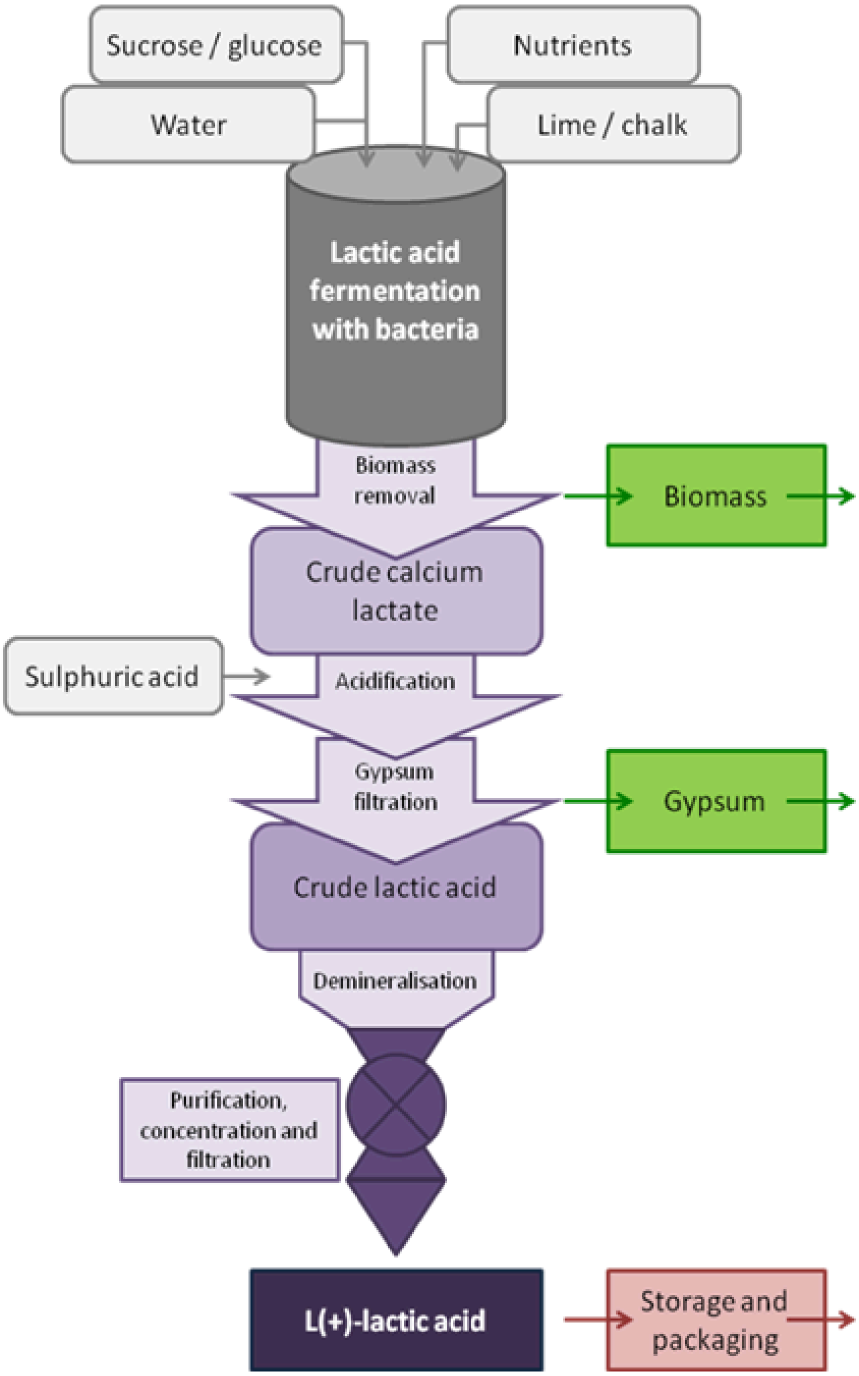


FIGURE 3. PRODUCTION OF LACTIC ACID

Fermentation: An aqueous solution of sugar/carbohydrates is combined with nutrients and lactic acid bacteria in the fermentation tank. The bacteria begin to transform the carbohydrates into lactic acid, which decreases the broth pH value. To stabilise the pH value, calcium hydroxide (lime) is added.

Biomass removal: When fermentation is finished, biomass is removed from the broth by filtration (it is then used as a fertiliser). At this stage, a solution of calcium lactate in water has been produced.

Acidification: To convert calcium lactate into lactic acid, sulphuric acid is added, producing gypsum (calcium sulphate) and lactic acid.

Gypsum filtration: Gypsum is then separated from the broth by filtration (gypsum is also used in the construction industry, among others).

Demineralisation: At this stage, the lactic acid is coloured: it contains impurities, which are removed by this pre-purification step.

Concentration, purification and filtration: The lactic acid is then concentrated and purified. Before packaging, a further filtration step allows removal of all potential physical contaminants.

Packaging: Lactic acid can be packaged in drums, totes or in bulk.

2.2.3 Purification process

The pure organic acid is obtained by:

- Filtration.
- Distillation.

A diluted solution of the crude organic acid, containing residual raw materials, must be purified by passing over a battery of columns filled with several ion exchange resins and activated carbon.

2.3 Production of blends

Organic acids blends are manufactured by several production processes:

- Blending and buffering.
- Adsorption on to a solid carrier.
- Granulation.

2.3.1 Blending acid premixtures with buffered solutions

Several liquid acids are dosed automatically into a tank and blended using a high-speed mixer, to ensure maximum homogeneity. The resulting liquid blends can be packed in cans, drums, Intermediate Bulk Containers (IBC) or delivered as bulk. The liquids may react with base ingredients like sodium hydroxide or ammonium hydroxide to produce (partially neutralised) acid blends. This is an exothermic reaction that requires extensive cooling.

2.3.2 Manufacturing acid adsorbates

To create a powder with good flowability, organic acids are sprayed onto special carrier materials in a blender, to ensure high acid load in the powder particles. The powder is packed in bags or Big-Bags.

2.3.3 Manufacturing of protected acids

There are several reasons why protection of organic acids is of interest for the animal nutrition industry. The right delivery/protection system for the right product is essential in adding value to the product (targeted release/delivery), not only in terms of its benefits for the animal (e.g. release in the hindgut), but also for ease of use for the user in the feed mill (preventing direct contact with the acid). To ensure that the functional ingredients reach the appropriate site of action, while ensuring safe and easy handling in the feed mill, a suitable carrier or coating needs to be chosen, and the correct type of encapsulation process applied. Protection of organic acids also reduces direct exposure to large quantities of the active ingredient, making the end product easier and safer to handle, protecting the active component(s) against the temperatures and pressures of processing, and preventing interaction with other ingredients in the premix or complete feed.

There are a variety of different methods to protect organic acids (e.g. coating), which lead to different manufacturing processes, but currently, many of these methods are closely guarded by the specific companies manufacturing these types of products. The most common coating material in the market consists of hydrogenated fat that is applied either as a true coating or where the active ingredient is dispersed into a fat matrix before spray cooling.

2.4 Quality standards for production of organic acids and their salts as feed ingredients

Independent of the type of production process, the production of organic acids is always done in the most accurate and responsible way.

Standard written operational procedures are available for the relevant production and process controls. The in-process controls are carried out by the operators and the laboratory and are documented. The test methods are described in an analytical procedures' manual.

Organic acids used for feed applications are only manufactured from controlled raw materials of specified purity. The manufacture is performed by highly controlled, certified processes (e.g. FAMI-QS, GMP+ISO 9001) in dedicated production units. HACCP systems are in place to ensure product quality according to the specifications.

The equipment in the production plant and that used for storage and transport should be made of appropriate materials to consider the inherent corrosiveness of the various organic acids and their salts (like stainless steel 316).

3. Organic acids and their salts in feed legislation

The manufacturing, marketing, transport and use of organic acids and their salts in animal nutrition are strictly regulated. Feed legislation is usually based on 3 main principles:

- Promote safety of feed and food, of human and animal health and the environment.
- Promote transparency, user right to information.
- Strengthen the effective functioning of the market.

The three principles above are reflected in the specific legislation of many countries and economic zones. Besides this, supranational bodies like the FAO promote a worldwide uniform approach, by creating standards such as the Codex Alimentarius.

The most relevant regulations and directives for organic acids as feed additives within the EU are described below.

3.1 Safety

The overarching regulation directed to assuring safety in the entire food and feed chain is the General Food Law (Reg. (EC) No 178/2002). It defines the general provisions for the safety and traceability of food and feed, based on scientific evaluation of risks. To this end, it describes the general obligations of feed business operators and establishes the roles of legislators and the risk assessment body EFSA (European Food Safety Authority).

For manufacturers to be allowed to manufacture organic acids or their salts, the Feed Hygiene Regulation (Reg. (EC) No 183/2005) provides an extensive set of safety criteria according to HACCP principles. Manufacturers must be registered by the competent authorities and comply with these safety criteria; in the case of production outside the EU, a representative importer is held responsible for adherence to EU legislation by the producer. Feed business operators and farmers may only source and use organic acids and salts from establishments which are registered in accordance with this Regulation.

The FAMI-QS Code of practice is an industry-derived system that addresses safety, quality and regulatory compliance of specialty feed ingredients and their mixtures, which enables an operator to implement the objectives of the Feed Hygiene Regulation.

In the European Union, organic acids and their salts are only allowed for use in feed when proven safe. Most organic acids are classified as 'feed additives' and therefore need to be evaluated for safety to the animals, the consumers, the users and the environment by EFSA. The additive can only be authorised by the Standing Committee on Plants, Animals, Feed and Food (SCoPAFF) of the European Commission after a positive evaluation by EFSA. The procedure for evaluation and registration of feed additives is regulated by the Feed Additive Regulation (Reg. (EC) No 1831/2003). The authorisation may contain specific provisions to ensure safety, e.g. a maximum content in feed per (group of-) target animal species, restrictions to the possible routes of application (feed, water), and possible other provisions like on labelling and user protection.

As will be further elaborated in Chapter 7, some organic acids are corrosive or irritant and are consequently classified by organic acid producers in harmony with the provisions of the Classification, Labelling and Packaging (CLP) Regulation (Reg. (EC) No 1272/2008). Labelling provisions in the CLP Regulation ensure the relevant information is provided to end users, to safeguard a high level of safety for themselves and their workers. In conjunction with this classification, other relevant provisions of legislation on transport and storage of hazardous goods are also relevant. As a matter of principle, the members of FEFANA active in organic acids and their derivatives commit to classifying products and mixtures according to internationally recognised procedures and communicating hazards in the chain via labelling and by provision of a safety data sheet.

3.2 Information to users

The users´ right to information on the feed additives and ingredients they purchase is covered by the Feed Additive Regulation for additives and premixtures and in the Marketing of Feed Regulation (Reg. (EC) No 767/2009) for feed materials and compound feed. This provision can be addressed from two different angles, firstly from the proof of efficacy of organic acids and their salts, and secondly from the information about product composition and use ('labelling').

3.3 Efficacy of organic acids and their salts

The proof of efficacy of organic acids and their salts is covered in the authorisation procedure set out within the Feed Additive Regulation and, where certain organic acids or its derivatives could be legally used in the EU as feed materials, through the provisions on claim substantiation in the Marketing of Feed Regulation. For feed additives, a list of functions has been established that describe the possible functionalities of feed additives (Annex I to Reg. (EC) 1831/2003). Organic acids are currently authorised under several 'Functional Groups':

- **Preservatives**: Protect feed against deterioration caused by micro-organisms or their metabolites
- **Hygiene condition enhancers**: Favourably affect the hygienic characteristics of feed by reducing a specific microbiological contamination
- **Acidity regulators**: Adjust the pH of feedingstuffs
- **Silage additives**: Improve the production of silage
- **Zootechnical additives**: Improve certain zootechnical parameters, when fed to the animals

An application for an authorisation under a functional group is evaluated by EFSA who, as with the safety criteria, assesses the available information in the dossier and draws a scientific conclusion on the efficacy of the organic acid for its claimed functionality. Only after evaluation by EFSA and authorisation by the European Commission may the product be placed on the market on the basis of this functionality. The EU Register of Feed Additives provides an up-to-date overview of authorised feed additives in the various functional groups.

The functional group of 'Zootechnical Additives' is the only group with a holder specific registration (next to coccidiostats) and can therefore only be placed on the market by the producer/registration holder or by companies that were licenced by the registration holder. A zootechnical additive can improve the digestibility of a diet or have effects on weight gain and feed efficiency in a target species or target age/stage of a certain species. Among these are benzoic acid, potassium diformate, and sodium benzoate as single acids. Also, various blends of acids/salts with other bioactive ingredients are listed as zootechnical additives, either as is or in an encapsulated/coated form. Most of these feed additives are authorised for use in pigs and/or poultry, usually in the growing/fattening phase.

As a general rule, the minimum amount of evidence that is needed for sufficient proof of efficacy is three studies showing statistically significant effects of the substance. Where animal criteria need to be assessed (e.g. for zootechnical additives), the tests should be done in the target species and age category. Although scientific research may help develop new, innovative applications for an organic acid, only those functions for which it is authorised can be claimed on the market.

Once authorised, feed additives including organic acids and their salts may be used in conventional farming in line with its authorisation (minimum or maximum dosage, animal species, labelling provisions). Under organic farming rules according to Reg. (EU) No 2018/848, only selected feed additives may be used that have received a position opinion by the expert group for technical advice on organic production (EGTOP) and are listed in implementing acts in accordance with art. 24(1) of the Organic farming regulation (latest version of this list at the moment of printing can be found in Reg. (EU) No 2021/1165).

3.4 Labelling

Labelling of feed is intended to protect the rights of customers, who need to be able to make informed choices on the purchase and use of products. It also enables competent authorities to make inspections on the compliance of feed products with relevant feed legislation.

The labelling provisions for additives and premixtures are given in the Feed Additive Regulation, whereas those of feed materials and compound feeds can be found in the Marketing of Feed Regulation. The main difference between these two regulation is that for premixtures all organic acids and salts that are used as feed additives need to be declared in full (authorisation code, official name and amount) whereas for labelling of feed materials and compound feeds only the feed additives that have a legal maximum limit in their authorisation need to be declared; for the other feed additives the person responsible for labelling may decide what to declare on a voluntary basis.

For organic acid derivatives classified as feed materials, the official name, as published in the feed catalogue should be declared on both labels for premixtures and compound feeds and all feed materials should be declared, in order of decreasing inclusion rate. At their wish, users of compound feed containing such feed materials shall be informed on the approximate amounts of each ingredient, with a tolerance of ±15%.

The relevant conditions of use (such as inclusion rate per target species and instructions required for safe application) should be detailed in the instructions for use of the feed additive, premixture, feed material and on the compound feed label. Labelling provisions of the CLP Regulation are being followed in the EU by FEFANA members for those additives and premixtures that are classified as hazardous.

3.5 Effective functioning of the internal market

According to the regulations that are involved in the manufacture, marketing and use of organic acids and their salts, a level playing field is created for all market players that are active with the same kind of products.

Effective functioning of the market, however, also requires a healthy balance between the interests of the producer/marketing company and the customer. If the producer had all the rights, the customer would probably pay a too high price due to lack of competition; in the reverse case, the producer will not be able to generate sufficient margin to survive, and/or innovation would cease. Neither situation is desirable for a healthy EU feed-to-food chain.

The provisions for proper information benefit the interests of the customer. Likewise, some provisions in the Feed Additive Regulation and the Marketing of Feed Regulation protect intellectual property rights and are intended to promote the interests of the producer, for example, the holder-specific authorisation of zootechnical feed additives. The required high level of safety of additive and premixture manufacturers also promotes the position of those companies that make a serious effort to provide their customers with high quality, effective products that can be applied safely.

4. Roles of organic acids in animal nutrition and their modes of action

<u>Disclaimer</u>
While in this booklet/chapter we give a full update on the scientifically proven functions of organic acids, it should be emphasised that the legal framework in each jurisdiction defines whether a certain function is approved for feed.

Organic acids have been investigated by universities, research institutes and feed companies for decades. In the early days, single organic acids were tested at sometimes very high doses. From this, it became clear that positive effects can be expected up to relatively high levels, indicating that organic acids have a low toxicity and that animals can process them very well during digestion and metabolism. Later research has focused more on blends of acids and special technologies for administration, such as esterification, encapsulation or adsorption onto carriers.

This research has yielded a wealth of data on the functionality of organic acids in feed and in animals. It gives insight into the modes of actions of organic acids and may even facilitate a quantitative estimation of the effects that can be expected, through meta- or holo-analysis using the data from multiple tests.

The main functions of organic acids have been elucidated, based on convincing scientific evidence. Depending on the jurisdiction, these same functions may also have been translated legally into functional additive groups, including the 'Preservatives', 'Hygiene Condition Enhancers', and 'Acidity Regulators', described in the EU Feed Additive Regulation.

Such extensive scientific research over recent decades has led to a better understanding of the modes of action of organic acids and their salts when used in feed, from the underlying modes of action in a variety of applications in animal nutrition, including improvements in feed hygiene and digestion (outlined in Section A), to their consequent effects on zootechnical performance (as detailed in Section B).

Section A - Effects of organic acids in animal nutrition - The functions of organic acids in the feed itself and the gastrointestinal tract of the animal. Improvements in feed hygiene and digestion consequently lead to support improved zootechnical performance (**4.2** to **4.7**).

Section B - Effect of organic acids on zootechnical performance - Extensive scientific research has led to several improvements in zootechnical performance (**4.8** to **4.11**).

4.1 Modes of action of organic acids and their derivatives

4.1.1. Modes of action of organic acids

For many applications, the efficacy of organic acids is determined by the same underlying modes of action. These are mainly based on the antimicrobial effects of organic acids on microorganisms and the beneficial effects that these triggers.

The mode of action of organic acids depends mainly on the chemical properties of the acid used and the microorganism affected. Organic acids are weak acids, which means that a certain proportion of the molecules remain undissociated, depending on the acid's pK_a value and the ambient pH value. These undissociated, uncharged molecules pass more easily across the cell membrane into the microorganism. Once inside the microbial cell, the acid releases its proton (H^+) in the more alkaline environment of the cytoplasm, resulting in a decrease of bacterial intracellular pH value. This influences microbial metabolism, inhibiting the action of important microbial enzymes. The bacterial cell is forced to expend energy to expel the protons, leading to an intracellular accumulation of acid anions, depending on the pH gradient across the membrane.

The anions within the microbial cell are thought to disrupt the metabolic processes in the cell, including RNA and DNA synthesis. This therefore affects cell multiplication and limits growth.

Several investigations have shown a strong bactericidal effect of organic acids without significantly decreasing the pH value in the GIT, providing evidence for the mode of action described above. Consequently, organic acids may still display efficacy where strong, inorganic acids (that only reduce the pH value and cannot enter the cell) do not. This process is depicted in Figure 4.

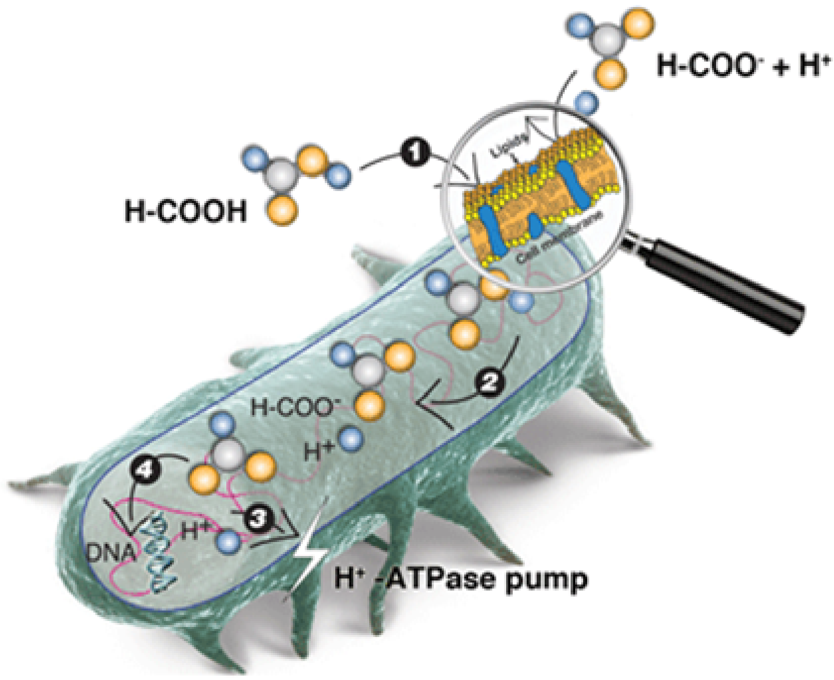


FIGURE 4. MODE OF ACTION OF ORGANIC ACIDS AGAINST GRAM-NEGATIVE BACTERIA

1. Undissociated organic acid entering bacterial cell.
2. Dissociation of proton, leading to pH reduction.
3. Expulsion of proton by energy-demanding process.
4. Inhibitory effect of acid anion on DNA.

Differences arise where certain acids are more effective against some organisms than others, due to the structure of their outer cell wall and/or membrane. Moulds are eukaryotic cells and hence have a more complex structure than bacteria, which are prokaryotes and therefore simpler in nature. This more complex structure is more easily crossed by propionic acid due to its lipophilic nature, making it the acid of choice for mould inhibition. Bacteria, in particular Gram-negative species, have a less complex cell membrane structure and allow a wider range of acids to easily pass across. This explains why formic acid is more effective as an antibacterial but less so as a mould inhibitor.

Because of the varying sensitivity of the different classes of microorganism toward different organic acids, blends of organic acids and their salts are effective against a wider range of microbial species than single acids.

The efficacy of organic acids, their salts and their glycerides against various microbial species is typically evaluated by assessment of the Minimum Inhibitory Concentration (MIC). The MIC is the lowest concentration of a substance that can inhibit growth of a specified microorganism after overnight incubation. Originally, small paper discs were soaked in a standardised amount of the antimicrobial substance and put on top of an agar plate containing a monoculture of the microbial species. Alternative methods have since been developed, e.g. where the substance and the microorganism are diluted in liquid agar in flasks or tubes (esp. for anaerobic species), or where the optical density of microbial cultures is measured in liquid media incubated with the antimicrobial substance, giving a measure of microbial growth.

4.1.2 Modes of action of organic acid derivatives

Salts of organic acids have lost part or all their acidifying power. While this could facilitate safe manual handling, it takes away the pH-reducing effect that organic acids exhibit. Consequently, also knock-on effects of reduced pH value (e.g. regarding microbial control) are diminished or absent. Depending on the solubility of the organic acid salt (in case of solids) and the relative moisture level in the matrix (feed or gut) anions may enter into solution. Dissolved anions of organic acids may display antimicrobial effects, provided that the ambient pH facilitates the partial re-association of the anion and protons present. As a rule of thumb, the ambient pH value of the matrix shall not be more than 2 units higher than the pK_a value of the acid in order to be effective.

Glycerides (esters of glycerol and fatty acids) of organic acids differ from straight organic acids in their antimicrobial effect mainly because of how they interact with microbes. Straight organic acids act by releasing free acid molecules that can easily penetrate the microbial cell membrane in their undissociated form. Once inside the cell, they lower the internal pH and disrupt vital functions, leading to the death or inhibition of the microorganism. Glycerides, on the other hand, directly interact with microbial membranes due to their lipid or amphiphilic nature, potentially disrupting membrane integrity. Since glycerides work pH independently, they can also have a more gradual effect along the whole gastrointestinal tract, being also effective in the intestinal part, where pH is higher than in the stomach. Glycerides can also have an antimicrobial action through enzymatic release of the fatty acid. Blends of glycerides have the strongest antibacterial effect.

Guanidinoacetic acid is an organic acid derivative that is authorised as a zootechnical additive. The mode of action toward microorganisms is not confirmed but it has been shown to improve performance of animals.

SECTION A – EFFECTS OF ORGANIC ACIDS IN ANIMAL NUTRITION

In this section, the effects of organic acids are described on the feed in which it is incorporated, in water in which it is dissolved as well as its direct influences in the gastrointestinal tract before absorption. Zootechnical effects (viz. on the performance of animals) will be discussed in section B.

4.2 Preservation

Preservation is defined as 'the act of maintaining something in its unaltered condition or to prevent it from decaying or spoiling'. Feed Preservatives, according to Regulation (EC) No. 1831/2003, are "Substances or, when applicable, micro-organisms which protect feed against deterioration caused by micro-organisms or their metabolites".

When considering this in relation to feed and feed ingredients, moulds are typically the primary concern as they cause visible changes, but other organisms such as yeast and enterobacteria can be equally detrimental.

4.2.1 Efficacy of feed preservatives

Moulds are fungi which grow in the form of multicellular filaments known as hyphae, the visible parts we see as fine coloured threads on the surface of mouldy food and feed. These hyphae produce spores, which allow moulds to reproduce and give a dusty nature to mouldy products.

Moulds are present in all feeds and feed ingredients at varying levels and will grow when environmental conditions allow. Generally, rapid mould growth will occur at higher moisture levels and temperatures between 20 to 30 °C. However, some species have adapted to grow at lower or higher temperatures to survive the conditions in which they are typically found.

Moulds can be a problem in most environmental conditions, and it is important to ensure they are controlled, to avoid deterioration of feeds and feed ingredients. The most common moulds are species of Fusarium, which grow on crops in the field, and Aspergillus, which are classed as storage moulds. Moulds cannot be controlled unless they are growing and the spores from which the mould grows are very difficult to kill as they are resistant to acids and only affected by very high temperatures. Once mould growth begins, it accelerates rapidly, increasing the moisture content and temperature of the surroundings, leading to proliferation of other species and further deterioration of the feed or feed ingredient. This typically occurs in pockets, where moisture has gained access through holes in storage bins or during rapid changes in temperature, which cause condensation build-up. It is therefore important to have a mould controlling agent already present in the feed to prevent mould growth should these favourable environmental conditions for growth arise. Moulds use nutrients from the feed or feed ingredient to grow thus reducing its feed value, as shown in Table 3.

TABLE 3. EFFECT OF MOULD CONTAMINATION ON NUTRIENT CONTENT OF GRAIN (IN [6])

Grain type	Moisture content (in %)	Mould contamination	Fat content (%)
Maize	13	No	3.8
	15.1	Yes	2.4
Sorghum	12.1	No	2.5
	15	Yes	1.3

Here, a clear relationship between moisture content and mould contamination was demonstrated. More importantly, where there was mould contamination, there was a significant reduction in the fat content of the grain. This would have important nutritional consequences due to a much-reduced energy content of the grain (Table 4).

TABLE 4. EFFECT OF MAIZE QUALITY ON PERFORMANCE OF YOUNG CHICKS [7]

Maize quality	Weight gain (g/3 weeks)	Feed Conversion Ratio	Metabolisable Energy (MJ/kg)
Good	738	1.8	11.5
Mouldy	612	2.15	10.7

Tables 3 and 4 clearly show the need for mould control in feeds and feed ingredients. The products typically used are classed as mould inhibitors, as they inhibit mould growth but do not kill the spores.

An additional problem with moulds is the production of mycotoxins, which are metabolites of their growth. These are extremely toxic chemicals at low concentrations and cause many problems in livestock production. Prevention of mould growth will thus reduce the potential for these compounds to be formed.

An important consideration must be made about the type of moisture present and whether it is free or bound. Water activity (a_w) (a measure of the energy status of the water in a system) is the most important factor to determine the mould growth as it is freely available for use, as opposed to that bound within cells.

Some moulds can grow at relatively low a_w levels (0.6). Total moisture content is therefore not always the best indicator of whether mould growth will occur. This is why mould inhibitors should be used routinely as an 'insurance policy' to prevent mould growth should the optimal environmental conditions arise.

The practice of adding moisture to cereals for rolling or crimping, or to compound feed in the feed mill requires the addition of mould inhibitors to prolong shelf life. The added water creates ideal conditions for the growth of mould but is necessary for the optimal processing of the grains. Care must be taken to ensure sufficient mould inhibitor is added to provide effective control. The most used organic acids for mould inhibition are propionic acid, acetic acid, and their salts and sorbic acid.

Yeasts are another source of feed spoilage, particularly in liquid feeds, where they can rapidly reduce nutritive value while producing CO_2 and ethanol. Liquid feeds are typically by-products from the human food industry and are ideal substrates for yeast growth. Controlling yeast, for example by sorbic or benzoic acid, will protect the feed value, thus allowing effective use of these economical and circular feed ingredients.

4.2.2 Industry practice

There are a huge number of mould inhibitors available on the market, currently ranging from single acids in liquid form to complex blends of acids and their salts on specific carriers. All these products have a place in the market, as some are more suitable for certain applications than others. For example, liquid propionic acid is very suitable for application onto high moisture whole cereal grain where mould growth is very likely and a rapid control measure is needed. Combining different short chain fatty acids (e.g. propionic acid in combination with acetic or formic acid) is known to enhance the mould-reducing effect of single acids. The use of a powder mould inhibitor product applied to whole grains as explained above, would be ineffective due to product segregation (due to large particle size differences) and insufficient adhesion to grain particles, resulting in lower efficacy under this application scenario, whereas application via mixing into ground feed materials in the mixer would be a suitable and effective application for powdered mould inhibitors.

Buffered products are often favoured over non-buffered acids as they are safer and easier to handle, having a more neutral pH and reduced corrosiveness. Propionic acid is corrosive to both carbon steel and human skin and requires special and careful handling by operators.

Mould inhibitors are routinely used in certain situations where high moisture levels are found or where long-term storage or climatic conditions dictate. Not all feeds are treated with mould inhibitors, and it is down to individual choice as to whether they are included on a continual basis as an insurance policy against the detrimental effects of moulds, bacteria and yeasts.

4.3 Feed hygiene enhancement

The measuring of the level of Enterobacteriaceae in feed is often used for monitoring the risk of occurence of *Salmonella*. Following EU legislation on animal byproducts and member states' *Salmonella* Control Plans, levels between 2 Log_{10} and 3 Log_{10} colony forming units per gram (cfu/g) are set maximum limits to take effective control measures against *Salmonella*, especially for high impact feeds, such as breeder feeds and layer feeds (Reg. (EU) No 142/2011) [8]. Water activity (a_w) is a measure of the energy status of the water in a system and along with pH and temperature, is a key factor in determining the risk of bacterial growth, as it relates to freely available water that is available to microbes for their own metabolism and growth, whereas bound water resides within cells. Water activity is variable, depending on environmental conditions, as moisture fluctuates to maintain equilibrium, thus a_w levels can change. Some bacteria can grow at a_w levels starting from 0.9. This is why bacterial growth inhibitors should be used routinely as an 'insurance policy' to prevent bacterial growth, should the optimal environmental conditions arise.

As *Salmonella* remains the 2nd most important zoonoses in Europe, there remains a clear need for authorised and effective solutions for use in feed, enabling management of risk from the start of the feed-food chain by reducing *Salmonella* shedding and spread. Treatment of feedstuffs with organic acids has been shown to contribute to feed hygiene, protecting feed from zoonotic agents like *Salmonella*. Moreover, routine inclusion of organic acids has a residual protective effect in feed, which helps to reduce recontamination risks from the environment. In poultry production, organic acids have mainly been used to sanitise the feed, considering problems with *Salmonella* infections [9, 10, 11, 12]. This function – Hygiene Condition Enhancer - has been added to the list of functions applicable for technological feed additives in 2015 by Reg. (EU) No 2015/2294 and is described as follows: "*substances or, when applicable, microorganisms which favourably affect the hygienic characteristics of feed by reducing a specific microbiological contamination*".

4.3.1 Proposed use in animal feed

Interest in the use of organic acids in animal nutrition is based on their potential to provide a mechanism for feed hygiene and/or a (subsequent) function in the animal, such as positively influencing gastric function, the gastric barrier or the possibility of affecting the gut microflora when administered via feed. Single acids and acid blends have been used in poultry diets to improve feed efficiency and reduce *Salmonella* contamination in feed Selection of a single acid or an acid blend, however, requires an understanding of the mode of action of the acids to target a particular production situation.

Animal feed, due to its composition, provides a favourable environment for the growth of various microorganisms. The efficacy of the acid treatment varies between different feedstuffs. The types of feedstuffs which might be contaminated are:

- **Feed materials** (raw material – single commodity): raw material treatment (with varying moisture levels).

- **Compound feedingstuffs** (complementary feed and complete feed): treatment of mash feed; pelleted feeds, liquid feeds.

The efficacy of the acid treatments (formic acid and different blends of formic acid, propionic acid and their salts) varies significantly between different feed materials [13]. The strongest reduction was seen in pelleted and compound mash feed (2.5 $\log_{10}$ reduction) followed by rapeseed meal (1 $\log_{10}$ reduction) after five days of exposure. However, in soybean meal, the acid effects were limited (less than 0.5 $\log_{10}$ reduction) even after several weeks' exposure.

4.3.2 Organic acids use to combat Salmonella in feed

The EFSA Panel on Biological Hazards has identified *Salmonella* spp. as the major hazard for microbial contamination of animal feed [14]. *Salmonella* is frequently found in feed ingredients, especially protein-rich feed sources, but may also be found in compound feed and even in heat-treated and pelleted feed, due to environmental contamination of feed mills and the high likelihood for cross contamination in the feed mill and during transport to and storage at the farm [15, 16].

Organic acids have been found to be effective against *Salmonella* in feed [17]. It has also been suggested that blends of acids may be more efficacious than single acids for reduction of *Salmonella*. Commonly used acids are formic acid and mixtures of formic acid and propionic acid, often in a ratio of 80%:20% [9].

Koyuncu et al. [13] reported a study on the efficacy of formic acid (FA) and different blends of FA, propionic acid (PA) and sodium formate (SF). No difference in *Salmonella* reduction was observed between FA and a blend of FA and PA, whereas a commercial blend of FA and SF was slightly more efficacious (0.5-1 $\log_{10}$ reduction) than a blend of FA and PA in compound mash feed. The *Salmonella* Infantis strain was found to be the most acid tolerant strain followed by *S.* Putten, *S.* Senftenberg and *S. Typhimurium*. The tolerance of the *S. Infantis* strain compared with the *S. Typhimurium* strain was statistically significant ($p<0.05$). The lethal effect of FA on the *S. Typhimurium* and *S. Infantis* strains was lower at 5 and 15°C than at room temperature.

When Iba and Berchieri [10] inoculated feed with high doses of a *Salmonella Typhimurium* strain, a commercial mixture of formic and propionic acid decreased the viability more than 1000-fold over seven days.

In a large-scale study [18], the number of *Salmonella*-positive breeder feed samples decreased from 4.1 to 1.1% after the feed was supplemented with 0.5% formic acid.

4.3.3 Use of organic acids to combat other bacteria and micro-organisms in feed

Animal feed is also subject to contamination by bacteria other than *Salmonella*; EFSA reports that *Listeria monocytogenes, Escherichia coli* O157: H7 and *Clostridium* spp. are other hazards for which feed is regarded a far less important source [14].

The rate of the reduction of *E. coli* in fish meal is proportional to the amount of organic acid mixture added. Cherrington et al. [19, 20], treated cultures of a test strain of E. coli with different concentrations of propionic or formic acid. Addition of 5 mmol/l propionic acid resulted in 30 minutes' bacteriostasis, whereas 0.5-0.7 mol/l of the acid caused the death of 90% of the experimental population. Application of 10 mmol/l formic acid caused a bacteriostatic effect lasting for 120 minutes, while the death of 90% of the microorganisms was achieved after the 3-hour incubation with 0.5-0.7 mol/l of the acid. In another experiment, propionic acid at concentrations of 25-40 mmol/l significantly inhibited E. coli strain O157:H7 [21]. Malicki et al. [22] added propionic and formic acids to fish meal at concentrations of 8-24 mmol/kg and 30-90 mmol/kg, respectively. The resulting reduction of the test strain was more pronounced than previously described. Consequently, if applied in a mixture, propionic and formic acid may work synergistically against *E. coli*.

4.4 Silage Making

The basic reason for making silage is to produce and store forage for a longer period, without major losses of dry matter and nutrients. Silage making is a crucial management tool for dairy farmers to optimise crop utilisation and dairy cow feeding programs [23].

The principle of silage production dates to Egyptian times but has evolved rapidly in the last 50 years [24]. A Finnish researcher, Artturi Ilmari Virtanen, received a Nobel prize in 1945 for the innovation of using acids to ensile fresh forage. The use of formic acid in ensiling fresh or wilted forage subsequently became common in the 1960's, but other acids have also been introduced, often in blends.

4.4.1 The silage preservation process

A proper forage ensiling process follows four phases:

Phase 1 - Respiration phase. During this phase, aerobic respiration of plant tissues continues, as cellular metabolism within the plant only stops from a lack of moisture, nutrients and/or oxygen [25, 26]. The objective is to produce silage with minimal loss of nutrients and dry matter. The most important management tool to minimise losses is to exclude oxygen in the silage, by packing the chopped forage either in bales or packing the forage in bunker silos [27]. The latter is improved by employing sufficient weight on the clamp, to optimise dry matter content of the ensiled material and by optimised forage particle length [28].

Phase 2 - Lag phase or *Enterobacteria* fermentation phase. This starts when, after ensiling, the concentration of oxygen decreases and the growth of facultative aerobic bacteria is stimulated, as these have the capability of living either with or without oxygen. The most dominant group of facultative aerobic bacteria is the *Enterobacteriaceae*, which can ferment sugars and convert them into short chain fatty acids [29]. However, when organic acids are produced, the pH of the silage drops [30, 31]. A further drop in oxygen levels combined with the drop in pH levels of the silage favour the growth of lactobacilli, strictly anaerobic bacteria.

Phase 3 - Lactic acid fermentation phase. In the third phase, lacticacid fermentation overtakes *Enterobacteria* fermentation. The most dominant species are now the *Lactobacilli*, of which some are homo-fermentative and others are hetero-fermentative. From the perspective of silage preservation, the first category is preferred, as they only produce lactic acid as a fermentation end-product, whilst the latter produces a whole range of organic acids, including lactic acid. The total production of lactic acid depends predominantly on the number of bacteria present, the concentration of easily fermentable sugars and the absence of oxygen in the environment [26]. Wilting grass prior to ensiling seems to increase the number of lactic acid producing bacteria [32].

Phase 4 - Stable phase. After approximately 2 weeks' the pH of the silage drops between 3.5 and 4.2. At these relatively low pH levels the growth of most bacteria, including lactic acid producing bacteria, is stopped and the fourth phase starts. This can last for years, if oxygen is prevented from entering the silage [24]. Unstable silages are characterised as those that maintain elevated temperatures for a prolonged period after ensiling and usually have a low bunk life after opening the silage.

The four phases of the silage preservation process are depicted in Figure 5.

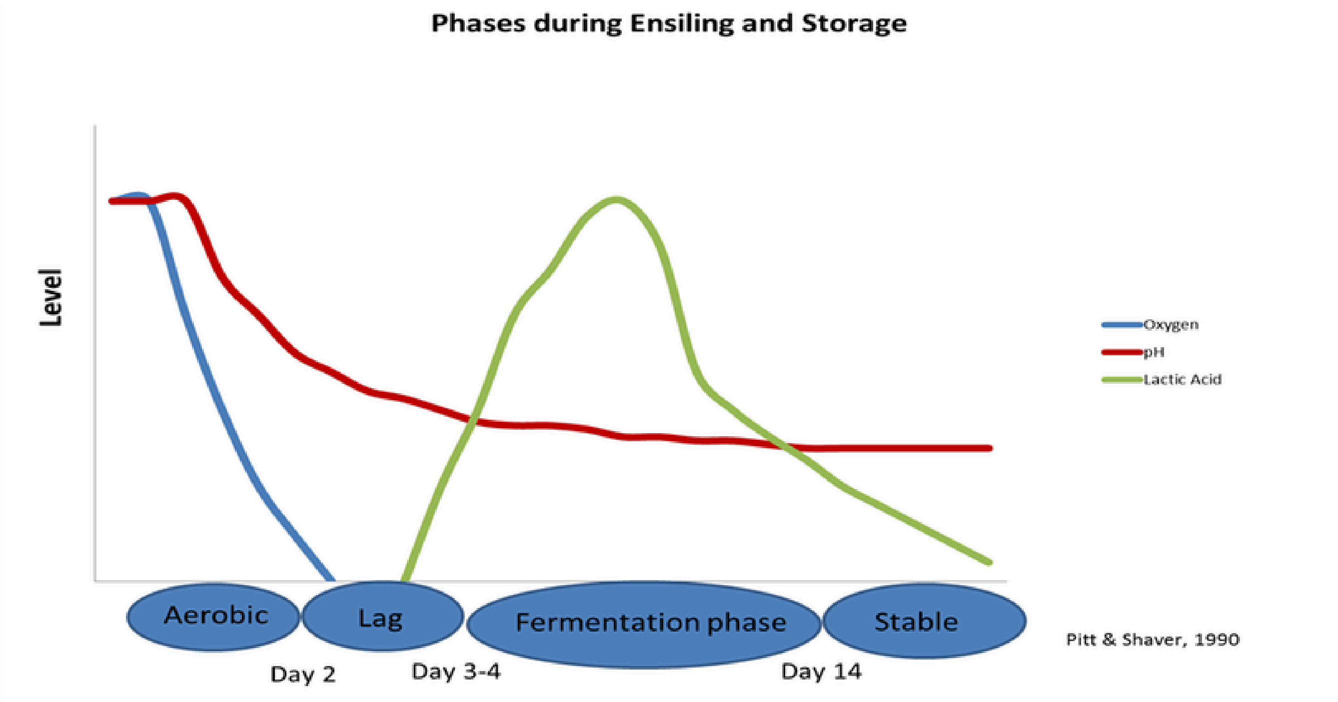


FIGURE 5. STAGES IN THE SILAGE PRESERVATION PROCESS [33]

4.4.2 Silage quality

Ensiling fresh forage with organic acids is based on low pH and anaerobic conditions. The rapid decrease in pH in the silage supports lactic acid fermentation, which effectively inhibits suboptimal fermentation and the growth of harmful microbes such as *enterobacteria*, clostridia and yeasts. These microbes can produce unwanted substances, such as acetic acid and butyric acid, ammonia and ethanol in the silage [34]. Elevated levels of such unwanted substances highlight a poor silage process and reduced nutritional quality of the silage; feed intake of such silages is also often reduced.

Another quality criterion is the aerobic stability of the silage, which determines the shelf life/ bunk life of silage after opening and the consequent exposure to oxygen. A low aerobic stability means that silage should be consumed immediately by the animals and determines a high speed by which the silo should be emptied to maintain nutritional quality of the silage. This is usually caused by elevated microbial levels in the silage, e.g. yeasts and moulds.

The amount of effluent coming from silage is another quality criteria; the presence of effluent implies nutritional losses and a burden to the environment. High amounts of effluent can be caused by high moisture levels of the forage at the time of ensiling, while other factors, such as additive use in the silage, can also influence effluent production [35].

4.4.3 Organic acids in silage making

A major aim of cattle farmers is to maintain the nutritive value of ensiled forage, by minimising the energy and protein losses during ensiling. In practice, proteolysis can only be restricted by a very rapid decrease in silage pH [36] or by increasing the dry matter content of the silage. A combination of extensive wilting and the addition of organic acids may result in preservation of 80 % of the original protein present, whilst only 40 % could be preserved when no wilting or acidification were employed [37, 38]. Preserving silage crude protein and readily available carbohydrates may stimulate microbial protein synthesis and, as a result, microbial fermentation in the rumen [39]. In practical circumstances, this implies that less rumen undegradable protein is needed to achieve the same milk yield. This may save the farmer a significant amount of money, as rumen undegradable protein is relatively expensive.

Adding organic acids is much more effective at inhibiting proteolysis than natural fermentation, as the pH of the silage is rapidly reduced [40]. Several studies have shown that formic acid application is a very good tool to quickly reduce the pH of the silage. A rapid drop in silage pH values results in an inhibition of plant protease activity and a significant reduction of silage fermentation, thus preventing the breakdown of sugars and heating of the silage. Adding organic acids results in a significantly higher nutritional value of silage and reduces leakage of ammonia-nitrogen into the environment [41].

Indeed, it was found that an increase in the amount of formic acid used at ensiling leads to a higher utilisation of energy and/or protein-yielding substrates for rumen microbes [42]. In most experiments there was no significant difference between buffered and non-buffered organic acids in silage preservation [40]. This offers the opportunity to supply organic acids to the farm in a buffered, and thereby less corrosive form (e.g. liquid sodium or ammonium salts).

Formic acid works especially well as a silage preservative when the dry matter and sugar content of the forage are low, and the level of nitrogen is high. Formic acid can be used in preservatives alone or with other organic acids, such as propionic acid. Propionic, sorbic and benzoic acids in particular inhibit the activity of moulds and yeasts and are therefore ideal candidates for promoting aerobic stability (bunk life) of silages.

4.4.4 Grain preservation (crimping)

Crimping is the technique used to ensile rolled, acid treated, high moisture grains, such as wheat and maize in silage clamps or plastic silo bags [43]. Harvest windows are widened and the costs relating to feed supplementation are reduced. Besides the economic benefits, crimped grain has many other advantages over dried grain. These include good palatability, dust free grain, less dependence on the weather at harvesting and high nutritive value. Several studies have confirmed that dairy cows produce as much milk with crimped grain as with dried grain [44]. Crimped grain can also be used for feeding pigs and poultry. Ensiling crimped grain requires lactic acid fermentation by lactic acid bacteria. Formic acid is also used in crimping to rapidly lower the pH value of crimped grain to 4. Propionic acid can also be used, as this is considered the best preservative against mould growth [45, 46].

4.5 Acidity regulation

Commercial farming conditions place various stressors on the gastrointestinal tracts of livestock. With the ban on antibiotic growth promoters in animal feed in Europe in 2006, the need for alternatives to reduce harmful microorganisms in the feed and intestinal tract of animals has increased significantly. Studies have shown that dietary acidifiers could reduce the number of harmful microorganisms in both the feed and gastrointestinal tract of animals.

4.5.1 What is an acidity regulator?

By definition, the acidity regulators are substances which adjust the pH value of feedingstuffs. Mostly this means lowering the pH value and buffering capacity of the feed. The ability of organic acids to reduce the activity of harmful bacteria is, among others, related to the ambient pH value and the pK_a value of the respective acid [47]. The pH value reflects the acidity of a solution ($pH = -\log [H^+]$), which means that the higher the hydrogen ion (H^+) concentration, the lower the pH value and therefore the more acidic the environment. The pK_a value is the pH value at which 50% of the acid has released its hydrogen ions. Therefore, the ambient pH value plays a very important role in the ability of the acid to dissociate and therefore, to reduce the pH value. If the ambient pH is higher than the pK_a value, more acid molecules will be dissociated, which results in quicker pH reduction. Therefore, acids with a low pK_a value will be more effective in reducing the pH value.

Acids with low molecular weight have the highest number of molecules per kg, resulting in the highest number of available hydrogen ions to release. This makes these acids very effective in reducing the pH value, even at low doses, both in the feed and in the stomach of livestock/ animals. Acids with more than one proton can also be very efficient acidifiers, depending on the pK_a value of the second (and if present, third) proton.

Animal feed ingredients differ in their acid binding capacity (ABC) when brought into an acidic environment, like the monogastric stomach [48]. ABC is particularly relevant to digestion, as ABC is measured in relation to the amount of hydrochloric acid (HCl) required to reduce pH of a material to a specific level. HCl is the acid secreted by the stomach and required for pepsin activation, which is critical for protein digestion. Ingredients high a high ABC will thus require more HCl secretion in the stomach for the proteins to be fully digested.

4.5.2 Effect of acidification in the feed and gastrointestinal tract

Dietary organic acids act as antimicrobials in feed and thus improve its hygienic quality [49]. It is well known that groups of microorganisms have optimum pH ranges for growth. If the environmental pH value is outside this range, growth is disturbed. Therefore, organic acids can be applied to reduce the pH value in feed. In addition, organic acids can cross the cell membranes of bacteria. Once inside the cell they will dissociate and disturb metabolism. As a result, proliferation of the bacteria is reduced, resulting in lower pathogen counts in the intestinal tract. With the combination of these two modes of action of organic acids, growth of pathogenic bacteria in the feed can be reduced. This results in lower uptake of harmful microbes by the animal and therefore reduces digestive disturbances in the intestinal tract. The efficacy of acids in reducing microbial counts in feed may depend on the type of acid, the type of raw material, temperature, buffering capacity and water activity (a_w).

The pH value in the stomach of monogastric animals is low (2.5 – 4.0), because hydrochloric acid (HCl) is produced in the stomach. The low pH value inhibits the growth of harmful microorganisms and therefore reduces the number of microbes that can colonise the intestinal tract. The gastric pH value is typically elevated in newly weaned piglets, compared to older pigs, due to lower HCl production (digestive function is still developing) and a switch from milk to feed. Animal feed typically has a pH value increasing effect in the stomach, due to the acid binding capacity (ABC) of the ingredients [48]. Therefore, these animals have a higher need for support, in decreasing stomach pH by including organic acids in the diet [47], as organic acids have a negative ABC [48]. Because of the low pH value in the stomach, organic acids with a low pK_a value such as formic, fumaric or lactic acids, are effective in reducing the pH value in this environment. Also, organic acid salts, although not directly acidifying the gut content, may replace certain mineral ingredients in the feed that have a higher ABC, thereby reducing the buffering capacity of the whole feed. Furthermore, a low pH value (e.g. in the stomach), results in more undissociated molecules will be present, resulting in more acid molecules entering the bacterial cell and disrupting proliferation. Studies have also shown that an acidic environment promotes the growth of lactobacilli in the stomach. This might inhibit colonisation and proliferation of *E. coli*, by blocking adhesion sites. Production of lactic acid by lactobacilli also lowers the pH and inhibits *E. coli* proliferation [50]. A low pH in the stomach is also required for the conversion of pepsinogen to pepsin, the active form of the most important gastric proteolytic enzyme. Pepsin is most active at a low pH value. Therefore, acidification of the diet may also promote protein digestion, especially in young animals where HCl production is still developing [51]. The level of pancreatic juice secreted by the pancreas is based on the pH value of the stomach contents. When stomach content pH value is lowered by the addition of organic acids in the diet, more pancreatic juice is produced. Since pancreatic juice contains digestive enzymes, this can result in improved breakdown of carbohydrates, proteins and fats.

There is no scientific evidence/data demonstrating that dietary supplementation of unprotected organic acids reduces the intestinal pH value. However, studies with encapsulated/ bypass organic acids have shown a reduction in intestinal pH via targeted release of the organic acids in the intestinal tract. Additionally, many studies have shown positive effects of dietary supplementation with organic acids on dry matter and crude protein digestibility in the small intestine and on growth performance. The greatest effects on growth performance were found in young animals.

4.5.3 Inorganic acids

Inorganic acids have a very low pK_a value, which means that they are mainly present in dissociated form. Therefore, they will not be able to enter the bacterial cell and disturb proliferation via the same route. They also have a large chloride, phosphate or sulphate component in the molecule. From several studies it has be concluded that inorganic acids in the diet results in reduced growth in pigs, probably due to an unfavourable electrolyte balance in the feed [50]. If the electrolyte balance in the animal's body is disturbed because of imbalanced electrolyte uptake through the feed, feed intake and growth of animals will be negatively impacted [52]. One test with phosphoric acid added to the feed did not result in growth depression, but neither did it show any improvement. Supplementation with hydrochloric acid in piglet diets resulted in improved daily gain, probably due to the increased availability of chloride for pepsinogen activation, improving protein digestion. Phosphoric acid is sometimes added to organic acid blends at low doses, to make use of its strong acidifying ability.

4.6 Applications in water for drinking

Water is the most crucial nutrient for livestock. Animals typically ingest at least twice as much water as dry feed. At higher environmental temperatures, water intake may be further increased. High-performance animals in modern animal production are increasingly less tolerant of stressors, such as poor water quality. The application of organic acids to water for drinking can provide a solution for negative effects of low water quality on health and performance of farm animals.

4.6.1 Microbial inhibition

The use of organic acids in water for drinking controls undesirable microorganisms by reducing the pH value, as well as through direct activity on the microorganism. The antimicrobial effect in water for drinking is considered a biocidal effect under EU legislation, meaning that the products marketed for this aspect need to be authorised under the Biocide Regulation (EU) 528/2012 (Product Type 5) and not under the Feed Additive Regulation. For this product type 5, the only organic acid being currently approved in the EU as active substance is formic acid. The pH is reduced by the acid releasing protons (H^+) into the water. Each organic acid has its own physical and chemical characteristics, leading to a specific antimicrobial activity. When applying single acids to water, the pH value will be decreased rapidly, because of the quick release of protons. Buffered acids have a weaker effect on pH value reduction. Buffered acids are acids mixed with a conjugate base. The conjugate base in this mixture neutralises released protons, therefore the pH value reduction will be less compared with single acids. Internal data have shown that buffering acids leads to a higher final pH value of a solution than when unbuffered acids are used. This is of importance in the acidification of water for drinking, because when the pH value of the water is too low, water intake might be reduced. Besides this, a sufficient dosage of organic acids can be added to the water for preservation, while limiting the reduction of the water pH value.

Therefore, using a synergistic blend of free and buffered acids is the most favourable strategy for product efficacy.

Acetic, lactic, propionic and formic acids are all effective pH value reducers in water for drinking [53]. Açikgöz et al. [54] found that water pH was reduced from 7.4 to 4.5 when formic acid was added. The optimal pH value of water for drinking to aim for is 3.8 – 4.2, since from within this range, *Enterobacteria* will be inhibited. Field results showed that a commercial product based on propionic acid eliminated Salmonella from water for drinking, while over 80% of samples in the control group were positive [53]. A combination of organic acids with different pK_a values is usually more effective in inhibiting microbial growth in water. Formic acid, for example, has a very low pK_a value (3.75) which means that it will release more protons into the water and reduce the pH value more than acetic or propionic acids, which have a higher pK_a value. More molecules of the acids with a higher pK_a value will remain undissociated and are therefore able to pass through the cell wall of the bacteria, inhibiting proliferation and reducing uptake of bacteria by the animal through water.

4.6.2 Biofilm reduction

Both organic and inorganic material will deposit on the surfaces inside water lines. Microbes adhere to this material, forming a so-called 'biofilm'. The build-up of biofilms is promoted when vitamins and medications are supplied via water, since their carriers are ideal substrates for microbes to proliferate. As a result, the drinking water becomes a source of infection for the animals in the stall. Biofilms may also cause drinking nipples to become blocked, restricting water intake by the animals and therefore negatively affecting growth and production performance as well as animal welfare.

Products based on organic acids can be applied through drinking water, to reduce biofilm formation and therefore to reduce microbial uptake by the animal through this route [55]. As for direct microbial effects in water for drinking, also biofilm reduction is considered part of the water sanitation and thereby a biocidal function (Product Type 4, currently approved active substances in the EU are benzoic acid, lactic acid and formic acid).

4.6.3 Effect in the digestive tract

The addition of organic acids to water for drinking may aid in an antimicrobial strategy and support digestion in the digestive tract of the animal (Figure 6). A lower pH value in the stomach, because of organic acid addition, could also induce increased activity of proteolytic enzymes [56], which will enhance protein digestion. Furthermore, it is generally known that undissociated organic acid molecules are lipophilic and are therefore able to pass through the cell wall of Gram-negative bacteria such as S*almone -lla* or *E. coli*. Once inside the cell they dissociate, lowering the internal pH of the bacterial cell and ultimately destroying it. This way, organic acids have an effect in reducing the number of harmful microbes passing into the small intestine, as described earlier.

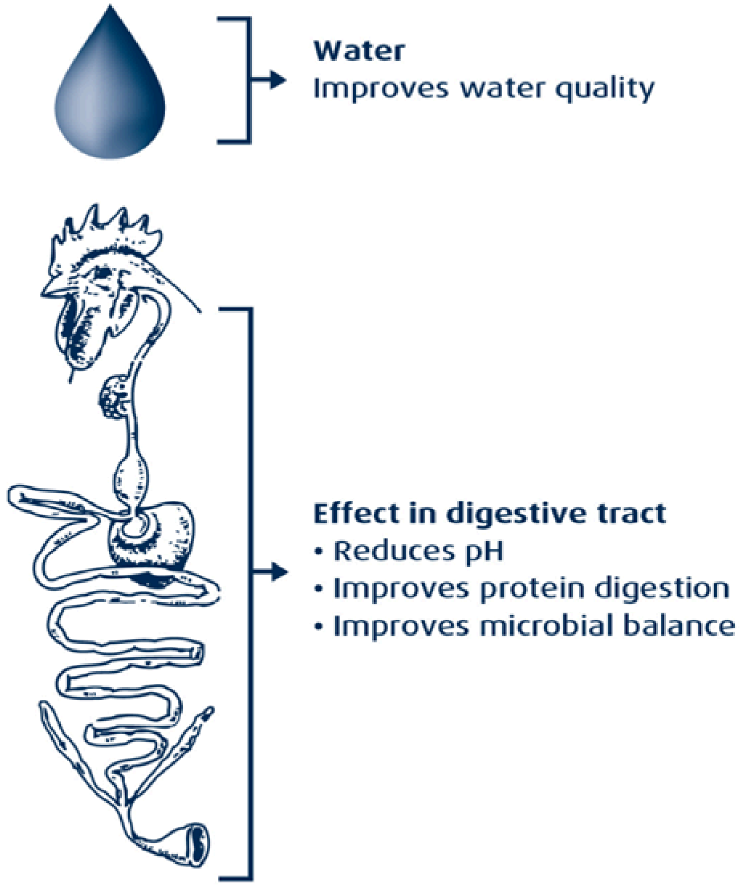


FIGURE 6. ADDITION OF ORGANIC ACIDS TO WATER FOR DRINKING MAY AID IN ANTIMICROBIAL ACTION AND SUPPORT OF DIGESTION IN THE DIGESTIVE TRACT OF THE ANIMAL

In piglets, the stress associated with weaning is known to disturb the balance of intestinal microflora and adversely affect gastrointestinal function. Increased pH in the stomach and undigested feed due to an immature digestive system could also accelerate the proliferation of pathogenic bacteria. Several studies have shown that better faeces consistency and improved gut health and growth were found in piglets when organic acids were applied. In animals dry feed intake will drop during a period of disease. They will, however, maintain water intake or would be more comfortable receiving feed administered via water for drinking. The same may happen in piglets during stress. Therefore, supplementing organic acids as liquid feed through water for drinking is a very useful strategy to control microbial uptake and to support gut health during periods of lower dry feed intake.

In poultry, dry feed is usually withdrawn for several hours before slaughter to reduce the potential for carcass contamination from the crop and intestinal contents. The incidence of *Salmonella* contamination in the crop might be increased up to five times during feed withdrawal. This is probably caused by coprophagy [57]. Studies have shown that addition of organic acids through liquid feed administered via water for drinking during the pre-slaughter feed withdrawal period significantly reduced *Salmonella* and *Campylobacter* contamination of crops and broiler carcasses at processing.

The supply of organic acids via liquid feed administered via water for drinking makes dosing very flexible. The dosage may be adjusted at any given moment. With a well-designed dosing system, aqueous acidifiers can be applied directly to the water lines.

With a regular intake of water throughout the day, organic acids may also support the animals' system in reducing Salmonella that may enter the animal via other routes.

4.7 Potency of organic acids as feed to food chain hygiene agent

The presence of zoonotic agents in the food chain poses a serious threat to human health. When animal feed is contaminated, it becomes a potential route of disease transmission to animals and consequently, to humans. Micro-organisms found in feed materials originate from a variety of sources, including soil and manure. Interventions are needed to reduce the prevalence of microorganisms in feed, and therefore to increase feed hygiene. This function was added to the list of functional groups in Regulation (EC) 1831/2003 in 2015 as 'Hygiene condition enhancer'.

4.7.1 Food-borne diseases

Salmonellosis is one of the most frequently reported food-borne diseases in humans in the European Union. The available literature shows that several pathogens can be spread by feed, in particular *Salmonella* spp. and Listeria monocytogenes. Evidence for transmission of other pathogens via feed is scarce [58]. Although not all *Salmonella* serotypes isolated from feed are found to cause clinical disorders in animals, they may all be pathogenic to humans [58]. Contamination of feed with these serotypes usually results in their colonisation in the animal's intestine. Consequently, long-term shedding will occur in the intestinal tract, which will cause continuous re-infection on the farm and the risk of further spreading the infection to neighbouring farms and the environment. Humans can be exposed to the bacteria when consuming improperly prepared eggs, meat or milk from infected animals.

Factors known to influence the risk of contamination of animal feed include contaminated ingredients, contaminated feed mill equipment and recontamination of feed during storage. Raw materials can be contaminated with microorganisms at any time during growing, harvesting, processing and storage. The extent of contamination depends on the type of material, location of origin or climatic conditions. Organic acids can be effective in reducing microbial pressure, from harvest to slaughterhouse.

4.7.2 Use of organic acids in feed to food chain hygiene

Originally, organic acids were only used in feed for decontamination purposes, to prevent microbial uptake by the animal. Later, it became clear that organic acids in the feed are also effective in the upper part of the gastrointestinal tract. Their mode of action in the intestinal tract is mainly explained by two mechanisms:

Lowering gastric pH: reducing survival of ingested pathogens in the stomach resulting in lower numbers of pathogens reaching the small intestine.

Acids can cross the bacterial cell membrane: where they dissociate and disturb metabolism. Proliferation of pathogens is reduced, resulting in lower counts in the intestinal tract.

The literature shows that adding organic acids to the feed has a positive effect on acid concentrations in the crop in poultry. In one study, when hens were given feed supplemented with a commercial mixture of formic and propionic acids, pH values of the crop, gizzard, jejunum, caecum and colon did not change compared to the control group, but formic acid and propionic acid concentrations in the crop and gizzard were increased significantly [59]. This higher antimicrobial action aids in controlling horizontal transmission of infection. In pigs, the strongest effects of organic acids with respect to digesta pH and antimicrobial activity are found in the stomach and small intestine [60]. It was found that lactic acid delayed the multiplication of an enterotoxigenic *E. coli* and reduced the mortality rate of animals [61]. Studies have also shown that various organic acids can reduce colonisation by *Salmonella* spp. in the gut. When formic acid-supplemented feed is fed to chicks from the day of hatching, the number of *Salmonella*-positive faecal samples is reduced dramatically compared to control animals [59]. Different studies have shown that caecal colonisation by *Salmonella* spp. is reduced when butyric acid is added to the feed [62, 63]. Preventing initial *Salmonella* spp. colonisation is very important. As soon as an infection is established, it is very difficult to reduce this with acid-supplemented feed, at least in the same production round.

It is known that short chain fatty acids are only active in the upper part of the small intestine. Therefore, no action of these acids will be shown in the lower intestine. In recent years, attempts have been made to extend the antibacterial activity of organic acids to the lower part of the gut. For example, organic acids can be coated or micro-encapsulated, which should prevent absorption in the upper tract and ensure release further down the gastrointestinal tract [59, 64].

Acids can modulate the microbiota by favouring the beneficial flora: A positive effect of benzoic acid (BA) enclosed in an oil matrix, used at a level of 2 g/kg BA, on broiler performance and gastrointestinal microbiota was demonstrated [64]. Total lactate in the crop and D-lactate in the jejunum was higher in the BA treated group. *Lactobacilli* populations were significantly altered by BA supplementation. A trend for increased lactobacilli was observed in the crop, while it became significant in the jejunum and ileum. Correlation analysis showed that BA modified the intestinal microbiota in multiple compartments of the intestinal tract for various microbial species clusters. Encapsulated BA modified the intestinal microbiota which can lead to the conclusion that the main beneficial mode of action of BA in the gut appears to be the enhancement of lactic acid bacteria, which in turn may act as a vanguard against pathogens.

Organic acids can also play a role as immunomodulator: It was shown how benzoic acid strengthens intestinal anti-infection defences via immunomodulatory pathways, with an impact on macrophage polarization and Immunoglobulin A mediated immune responses in young broiler chickens challenged *Salmonella enterica* Serovar *Typhimurium* infection [65].

The addition of organic acids to feed is an efficient way of increasing feed chain hygiene, by reducing microbial uptake through feed, as well as acting as a vehicle for transport of the acids into the intestinal tract to support the digestion process and ensure gut health (Figure 7).

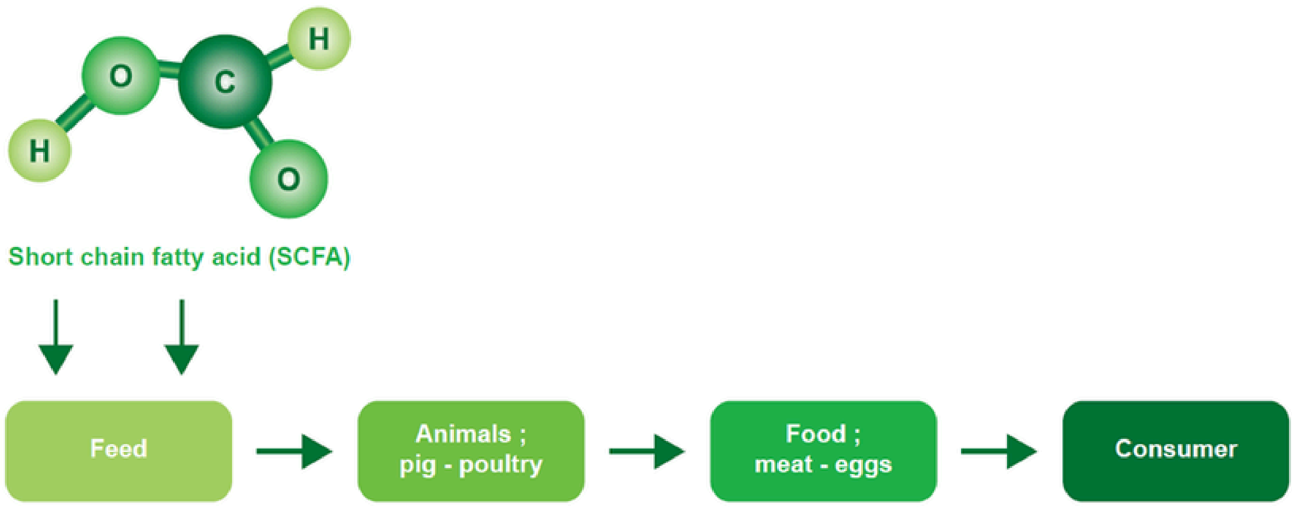


FIGURE 7. ORGANIC ACIDS SUPPORT THE CONTROL OF BACTERIA IN THE TOTAL FEED TO FOOD CHAIN

SECTION B - EFFECT OF ORGANIC ACIDS AND THEIR DERIVATIVES ON ZOOTECHNICAL PERFORMANCE OF FOOD-PRODUCING ANIMALS

General mode of action

In animal nutrition, organic acids and their salts exert their performance-promoting effects via three different mechanisms: through the feed, in the gastro-intestinal tract (GIT) and on metabolism [66]. The effects in the GIT and on metabolism are especially noteworthy, for the increased weight gain and especially the improved feed efficiency which result. Kirchgessner and Roth [67] attribute the following benefits: pH-effect; improved pepsin activity and thus an improved protein digestibility; effects on mineral absorption, e.g. calcium and the effect on intermediary metabolism. Improved utilisation and availability of metabolisable energy will also favour protein retention and may prevent amino acid catabolism. Furthermore, these additives have been shown to be effective against Gram-negative pathogenic bacteria, such as *Salmonella* and *E. coli* [68], which may also have an impact on animal performance.

4.8 Use in pig nutrition

One of the best ways to demonstrate the beneficial effects of adding organic acids and their salts to diets for growing pigs is to compare the results of several studies.

Classically, this is achieved through meta-analysis, a statistical technique commonly used in clinical medical reports that compares the outcomes of similarly designed studies.

Partanen [69] evaluated the effects of dietary organic acids on performance (average daily gain, feed intake and feed to gain ratio) in weaned piglets and fattening pigs, through a meta-analysis of data collected from the literature. The findings are summarised in Table 5.

TABLE 5. MULTIFACTORIAL ANALYSIS OF THE EFFECT OF ORGANIC ACIDS AND SALTS IN PIGLETS (ADAPTED FROM [69])

	Formic acid	**Fumaric acid**	**Citric acid**	**Potassium diformate**
Experiments/ Observations	6 / 10	18 / 27	9 / 19	3 / 13
Acid levels, g/kg feed	3 - 18	5 - 25	5 - 25	4 – 24
Feed intake, g/d Control	667±87	613±148	534±276	764±9
Experimental	719±75	614±152	528±302	823±38
Weight gain, g/d Control	387±65	358±99	382±121	479±4
Experimental	428±62	374±101	396±127	536±26
Feed to gain, kg/kg Control	1.64±0.13	1.59±0.16	1.67±0.25	1.60±0.02
Experimental	1.60±0.14	1.55±0.14	1.60±0.24	1.54±0.04
Unbiased effect size, d				
Feed intake	0.46a±0.16	-0.08b±0.10	-0.20b±0.13	0.59a±0.14
P≤*	0.01	0.42	0.14	0.001
Weight gain	0.77a±0.16	0.25b±0.10	0.32b±0.11	0.89a±0.15
P≤	0.001	0.01	0.01	0.001
Feed to gain	-0.91±0.36	-0.71±0.27	-0.81±0.30	-1.04±0.42
P≤	0.02	0.01	0.01	0.02

**Probability values for a comparison with the control diet.*
a,b Means in the same row without common superscripts differ significantly (P<0.05)

This multifactorial analysis demonstrated that organic acids improved all performance parameters in weaned and fattening pigs compared to non-acidified control diets.

More recent statistical approaches aim to develop more accurate, predictive models that can potentially be applied to any production scenario. Such models require the input of considerably larger datasets, but these have the advantage of being more diverse in their source data. One such model, termed 'holo-analysis', was developed for pig feed additives in the 1990's. Holo-analysis makes use of as much of the literature as possible and so is less restricted by the experimental parameters, e.g. housing, feed components, acidifier dose, etc. [70]. In 2008, it was applied to a dataset collected from all available published material on the use of acidifiers in pigs [71], using nearly 500 studies that had been published on the use of 158 acid or derivative products. The dataset used for the 'Acipig' model covers the results of all the published, negative control studies conducted on their effects, used singly or in admixtures and reflects the wide range of applications of these products. The more data collected from peer-reviewed, trade and corporate publications, the more accurate and useful the model.

Thus, the data from 484 publications were used, comprising 658 negative control studies of acids (184 publications), on 37,924 pigs (an average of 57.6 pigs per test). Most of these tests included fumaric, citric, formic or propionic acids, calcium formate, potassium diformate and propionic acid salts. The outputs included in models were the effect of the additive on feed intake, liveweight gain and feed conversion compared to negative controls. Most tests were performed with weaners.

The holo-analytical models derived demonstrate that using organic acids in pig diets improves the productivity parameters of greatest importance to economic success. The magnitudes of the improvements were on average: 1.2% on feed intake, 5.5% on weight gain and 3.7% on feed conversion ratio.

This model shows that not only is there a benefit of using organic acids or their salts in diets for pigs, supporting their long-standing use in the industry over almost 50 years [72], but that the discrepancy between the increase in feed intake and the magnitude of the growth and feed conversion responses to acidifier inclusion in the diet, indicates beneficial physiological effects within the pig that improve feed component digestibility and availability.

Also, more recent meta-analyses of organic acids in pig nutrition indicate that beneficial effects are to be expected [73, 74]. From a pig study it was concluded that in comparison with antibiotic supplementation, the acidifier-supplemented diets had similar Gain to Feed ratio, but lower average daily gain (ADG) and average daily feed intake (ADFI). Furthermore, blends of acids were found to be superior to individual organic acids or salts of acids in improving growth performance in pigs [73]. Positive effects of organic acid (salts) were found in another study not only on growth and feed conversion but also on villi height: crypt depth ratio (a measure of the proper functioning of the intestinal gut wall involved in nutrient uptake and immune response), as well as on faeces consistency [74]. Next to the acids presented in table 1 of this booklet, both meta-analyses also included butyric acid (salts), not listed as feed additive in the EU.

4.9 Use in poultry nutrition

In a meta-analysis with 51,960 broilers from 121 articles, it was found that organic acids supplemented in the feed improved feed conversion ratio (FCR) both under healthy and challenged conditions. However, the effect under challenged conditions was lower than for antibiotics. Average daily gain was improved by the use of blends of organic acids but not by single acids [75].

Under challenges conditions, such as *Eimeria* challenge with oral gavage of 10 x dose of *Eimeria* vaccine, combined with different types of diets (rye 10%, canola meal 7.5% and poultry meal 3%, and their combination), fat protected benzoic acid improved growth performance of broilers and the efficacy was greater when diet complexity was increased by combining viscous grain and low digestible protein ingredients [76].

Abd El-Ghany gives a comprehensive overview of applications of organic acids in poultry feed [77]. He lists a series of effects that organic acids and its derivatives can have in the area of growth performance and carcass traits, the utilisation of nutrients like minerals, the immune system and microbial control.

Haq. et al. summarized the effect of various organic acids on gut functioning, including pH value reduction and microbial modulation. Acids like fumaric acid and sorbic acid may alter the ratio lactobacilli – coliform bacteria in the ileum up to the caecum. Dry matter and protein digestibility may be improved by various acids; the optimum inclusion rate depends on the acid involved, with citric acid showing a positive effect up to 4% inclusion rate, while formic acid does not show positive effects beyond 1% inclusion rate [78].

Oguwuegbu et al. shows that dietary supplementation of benzoic acid at 5.0 g/kg optimally enhances broiler growth performance, FCR and carcass characteristics, but higher levels may induce metabolic stress.

Benzoic acid significantly improves gut morphology, epithelia thickness, leading to better nutrient absorption and gut integrity. The inclusion of benzoic acid at 7.5 g/kg promotes the growth of *Lactobacillus* and *Bifidobacteria* while reducing *Salmonella* and *E. coli*, supporting a healthier gut microbiota [79].

4.10 Use in ruminant nutrition

More recent research indicates positive effects of certain organic acids and derivatives on ruminant performance and rumen function. Short chain fatty acids (also called volatile fatty acids) acetic acid, propionic acid and butyric acids are already produced in large quantities by the rumen biota [80] and are not typically supplemented in the ruminant feed for zootechnical purposes.

Other organic acids and salts, however, have gained some traction by positive effects found in scientific studies on the functioning of the rumen.

In a meta-analysis by Malik et al. [81] fumaric acid, an intermediate in rumen metabolism, was found to positively affect propionic acid production and had a reducing effect on methane production, especially in small ruminants. In a meta-analysis by Rocha et al. [82] it was shown that malic acid/malate inclusion in the diet improved ruminal pH stability, supporting fibre digestion. Besides, it increased beneficial fermentation products, especially propionate, and improved blood glucose, while reducing signs of fat mobilisation.

4.11 Use in aquaculture nutrition

In intensive aquaculture production, losses due to bacterial diseases are a major concern to producers. Feeding medicated feeds is common practice to treat bacterial infections, so the prophylactic use of antibiotics as growth promoters in aquaculture production has also been standard practice, although to a lesser extent than in pig and poultry production. The use of antibiotic growth promoters is accused of being a major contributing factor in the emergence of antibiotic-resistant bacteria, through contamination of food products and the environment. Therefore, the practice of using prophylactic antibiotics to control intestinal infections in animal production that has been banned in EU countries is increasingly under public scrutiny and criticism in most other countries. Consequently, a wide variety of products ranging from plant extracts, prebiotics, probiotics and organic acids or their salts have been evaluated as alternatives to antibiotics, also in aquaculture. One of the earliest studies on the application of organic acids in aquaculture appeared in 1981. Rungruangsak and Utne [83] tested formic acid preserved fish silage in diets for rainbow trout, though with limited success. Since then, many studies have reported that organic acids can improve growth, feed utilisation, mineral availability and disease resistance in aquatic species.

This chapter gives a brief highlight of the application of organic acids in aquaculture, covering the most economically relevant fish groups and shrimp as covered in previous reviews [84, 85] The use of acidifiers has since reached almost all commercially relevant species of temperate and tropical fish and shrimp [86, 87]. Ng and Koh summarized their review: 'Due to the sheer number of aquatic animal species being farmed and under various culture conditions, the path to establishing dietary organic acids as part of a standard disease management programme in any aquaculture farm is anticipated to be a long one but crucial'. Many researchers are currently investigating the use of organic acids and their salts to improve performance and survival rates in fish and shrimp in aquaculture. For potassium diformate alone, the list of species in which acidifiers have been researched is extensive and includes grass carp, tilapia, salmon, trout, sea bass, pangasius, catfish (*Clarias*), gourami and pompano, as well as shrimp [88]. Other studies have also tested different organic acids and their salts in more species, including common carp [89], gilthead sea bream [90], European- [91] and Asian sea bass [92] as well as the giant freshwater prawn [93], among others.

Salmonids were among the first species in which organic acids showed promise in practice. In rainbow trout, adding formic acid or citric acid to fishmeal-based diets significantly improved mineral digestibility—especially phosphorus, magnesium and calcium—while blends of formic and sorbic acids enhanced weight gain and feed efficiency. Similar effects were seen in Arctic charr, where sodium lactate and sodium acetate improved growth and nutrient utilisation. Potassium diformate tended to enhance growth in Atlantic salmon [84]. Beyond performance, coated butyrate salts have been shown to improve gut structure and microbiota composition in trout, although growth effects varied with diet composition [94].

In another trial, sodium diformate (around 1.06%) enhanced the digestibility of protein, starch, and amino acids. Pellet quality also improved, although results depended on extrusion temperature [95].

In tilapia, studies have shown that specific acids such as citric, propionic and lactic acid can stimulate feeding activity at low concentrations, while blended acid–salt products can modestly improve growth performance. Although the growth effects are sometimes limited, organic acid blends consistently reduce harmful gut bacteria such as *Aeromonas hydrophila* and can improve survival rates during disease challenges, including *Streptococcus agalactia*e and *Vibrio anguillarum*. Dietary formate—whether alone or within blends—has been repeatedly reported to improve feed conversion, protein efficiency and resilience to bacterial infections [84].

A recent study [90] evaluated the inclusion of a mixture of formic acid and sodium formate at 0.3% in practical diets for juvenile gilthead sea bream (*Sparus aurata*) formulated with low fishmeal and high plant protein content. The supplementation resulted in improvements in intestinal morphology, including elongated mucosal folds and reduced submucosal thickness. Goblet cell size was diminished, and feed utilisation showed an upward trend of approximately 8%. Furthermore, survival rates following a Vibrio anguillarum challenge were significantly enhanced.

In another study, adding 0.5% monoglycerides of short- and medium-chain fatty acids blend (SCFA/MCFA) to the diet improved economic feed conversion (eFCR) and reshaped the gut microbiota. Beneficial bacteria like *Lactobacillus* and *Leuconostocaceae* increased, while harmful *Gammaproteobacteria* (including *Vibrio* and *Photobacterium*) declined. These shifts were confirmed through weighted UniFrac analysis [96]. A recent study [97] highlights a comparable effect on the modulation of microbiota composition in Black Sea Bream (*Acanthopagrus schlegelii*) using similar SCFA/MCFA blends.

Shrimp being fed a monoglyceride blend rich in monobutyrin (0.75–1.0%) over 60 days led to better final biomass, weight gain, and feed conversion. Immune responses also improved, with higher phenoloxidase and phagocytic activity. Survival after *Vibrio harveyi* challenge was significantly higher, and no adverse effects were seen in *Hepatopancreas* histology. Responses plateaued or declined at 1.5% [98].

Organic acid blends (1–2%) also showed promising effect by enhancing growth, phosphorus utilisation, hepatopancreatic health, and resistance to *V. harveyi* under challenge conditions [99].

Supplementing diets with tartaric acid (5–7.5 g/kg) improved growth, feed efficiency, lactic acid bacteria count, digestive enzymes, antioxidant levels, immune markers and survival after *V. parahaemolyticus* challenge [100].

In recent years, a growing number of comprehensive reviews have been published, evaluating organic acids and their derivatives as beneficial feed additives, and reporting positive effects on both the health and performance of various aquatic species. However, the magnitude and nature of these effects are highly dependent on several factors, including fish species, the specific type or blend of organic acids used, the basal diet composition, and the experimental conditions of each trial. Given the promising outcomes observed, it is expected that future research will further explore these compounds, aiming to unravel their mechanisms of action and refine recommendations for their application. This will be particularly important for developing targeted strategies, including the use of organic acids in combination with other feed ingredients, to maximise their benefits in aquaculture.

5. Synergies of organic acids and other feed ingredients

Given that the mode of action of organic acids in monogastrics involves a reduction in pH value in the upper gastrointestinal tract and a direct antibacterial effect against mainly Gram-negative bacteria, it might be anticipated that feeding organic acids and their salts might also have beneficial effects in diets containing other types of additives that benefit from lower pH value. Research into additive or synergistic interactions between organic acids and dietary enzymes, probiotics, prebiotics, essential oils and plant extracts has revealed improved productivity parameters in some cases in pigs and poultry, though it seems that such synergistic effects may be dependent on a range of dietary and/or environmental factors. It is therefore important to investigate the potential for synergy or antagonistic effects between additives if we are to make the best use of additives in the nutrition of monogastric livestock.

5.1 Complementary modes of action reap complementary benefits

Organic acids and their salts have been shown to exert their effects on feed preservation and zootechnical performance in pigs and poultry through the following modes of action (see above chapters):

Reducing feed and gastric pH value when used in non-protected form (unfavourable growth conditions for Gram-negative bacterial pathogens);

Favourable growth conditions for beneficial microflora (*lactobacilli*, *bifidobacteria*);

Optimise the pH value for digestive enzymes (especially in digestion of proteins and minerals; stimulate intestinal mucosal cell development and pancreatic enzyme secretion);

Bactericidal/bacteriostatic effects on Gram-negative bacterial pathogens, e.g. *E. coli*, *Salmonella*, *Campylobacter*, by permeating the bacterial cell wall and affecting internal cellular metabolism.

Since a number of other feed additives that lay claim to improving the health and/or growth of livestock share some commonality in their modes of action, it has been of interest to researchers for a number of years to establish whether different classes of additives could be formulated together, with the result that the performance benefits could exceed that of the individual additive administered alone – synergy. Therefore, several studies have been done to test effect of organic acids and their salts.

5.2 Combinations of organic acids

It is well known that although short chain organic acids are effective singly their effects on different microorganisms are not the same. The data on minimum inhibitory concentration (MIC) shown in Figure 9 gives example of single acid effect against different pathogenic bacteria.

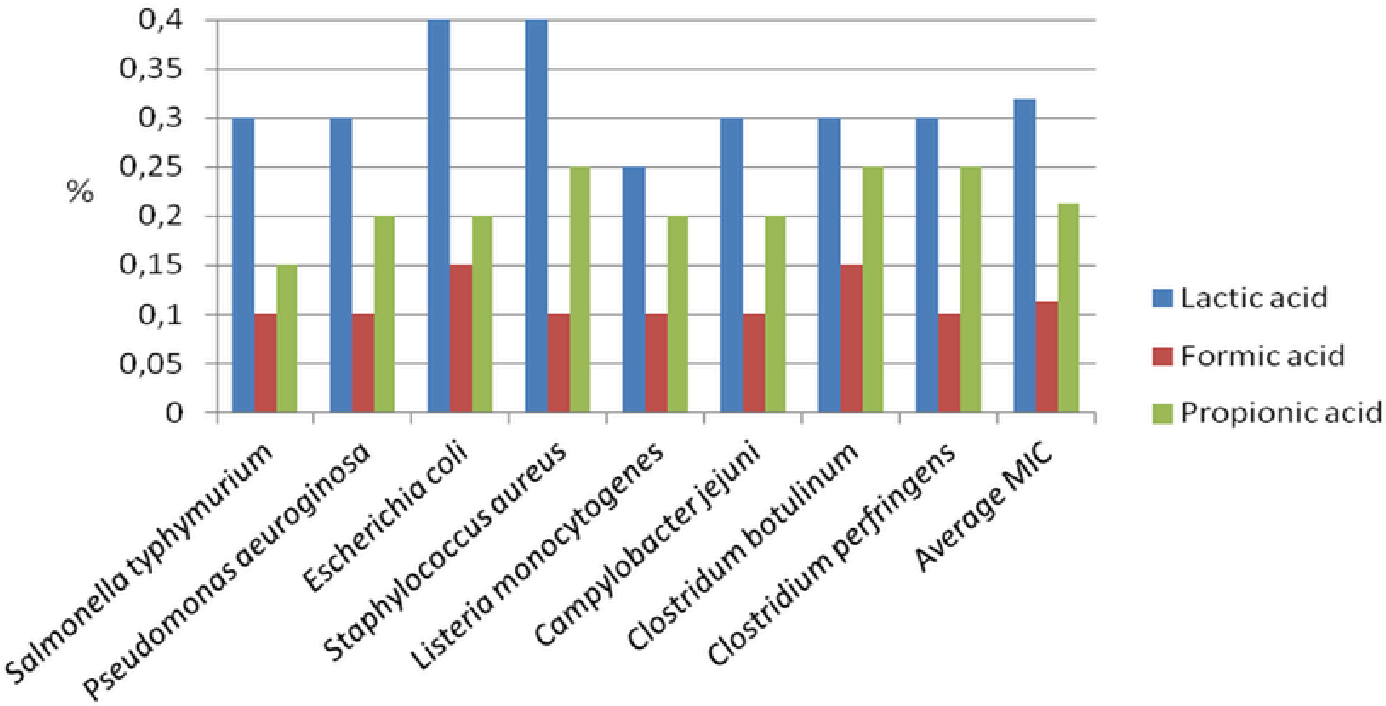


FIGURE 9. MINIMUM INHIBITORY CONCENTRATIONS (MIC) (IN %) OF SCFA (plot of [101])

The use of blends of acids is common to achieve a greater effect on a wider range of organisms. It is also known that there is a synergistic effect of combining acids. For example, lipophilic acids such as propionic acid may make the bacterial cell membrane more permeable to other acids such as formic acid. Much research has been done on *Salmonella* reduction using blends of organic acids. Hinton and Linton [102] reported significant results when a blend of formic and propionic acids was added to feed. Different formic and propionic acid blends added to feed were demonstrated to have differing effects on *Salmonella Pullorum* in poultry [103]. Koyuncu et al. [104] showed a reduction of *Salmonella* in contaminated feeds and feed ingredients to be different with single acid, blends of acids and blends of acids with acid salts - the blends being shown to be the most effective in complete feeds compared to the single acid.

Malicki et al. [105] studied the effect of a blend of formic and propionic acids on fishmeal contaminated with *E. coli*. The effect was dose dependent, but the authors concluded that the reduction in bacterial levels with the blend was greater than that reported for formic or propionic acid alone [106, 107]. The deduction can thus be made that formic and propionic acids act synergistically to reduce *E. coli* levels.

Chaveerach et al. [108] studied the effects of single and blends of different acids at various pH values on *Campylobacter* spp. Their results showed that blends of formic, acetic and propionic acids gave higher *Campylobacter* reduction rates than correspondingly higher levels of the single acids at the same pH value, clearly demonstrating a synergistic effect.

Further studies indicate synergistic effects of various organic acids, e.g. formic and propionic acids on broiler performance [109].

5.3 Organic acids in combination with other feed ingredients

5.3.1 Combination with phytase

In the late 1990's a considerable body of work was generated on the effects of exogenous phytase on the digestibility of phytate phosphorus from plant-based feed ingredients. The optimal pH of phytase from *Aspergillus niger* (the most common source of exogenous dietary phytase used in animal diets) ranges from 2.5 to 6.0, depending on the fermentation method used [110]. Phytases produced by solid-state fermentation tend to have lower pH value optima compared to those produced by submerged fermentation methods, but all work better in an acidic environment. Furthermore, a lower intestinal pH value contributes to increased solubility of phosphorus and phytate, thereby enhancing phosphorus absorption from plant-based sources. Additionally, organic acids can bind various cations along the digestive tract, acting as chelating agents, which can also lead to improved absorption of minerals [111]. Therefore, the combined use of dietary acids with various phytases would be expected to confer benefits to all monogastric species.

Chemical acidification of a phytase-supplemented swine diet using formic acid was shown to further enhance the release of plant-derived phytate-bound phosphorus, thereby increasing pre-ceacal phosphorus digestibility compared to the use of phytase without acidification [112]. Han et al. [113] found that using citric acids with microbial phytase in growing pigs had a 'collective' effect in replacing inorganic phosphorus supplementation, i.e. the animals were able to benefit more from the phosphorus released by digestion of phytase in plant feedstuffs (wheat middlings). Subsequent data from growing-finishing pigs [114] show that the apparent total tract digestibility of total phosphorus was increased by the addition of a combination of microbial phytase and lactic acid, to a greater extent than was calculated as the sum of the stimulatory effects of the single additions, thus clearly demonstrating synergy between the two additives.

Similarly, Callesen [115] reported synergistic effects on weight gain and feed conversion when potassium diformate and phytase were used in the same diet. Studies in early weaned piglets also showed an additional benefit of acetic acid combined with phytase in promoting improved mineral digestibility [116]. Dusel et al. also demonstrated that the combination of phytase and a mixture of formic acid and sodium formate in pig diets led to the greatest reduction in phosphorus excretion while simultaneously achieving the highest weight gains — both during the nursery and finishing phases [117].

Similar effects have been reported in poultry. A study from Sacakli et al. [118] demonstrated that it may be possible to reduce the use of supplemental inorganic phosphorus in the diet by adding phytase and/or organic acids. This is supported by Krieg et al., who describe factors influencing the degradation of phytate. The addition of formic acid increased the degradation of phytate (InsP6), thereby enhancing the availability of plant phosphorus for broiler chickens. The highest level of phytate degradation was achieved through the combination of phytase, calcium carbonate as calcium source, and formic acid [119].

Although there are significantly fewer studies on the use of phytase and its combination with organic acids in aquaculture compared to swine and poultry, a trend is emerging that suggests acidification may also be beneficial in aquaculture when phytase is added.

Similarly, dietary microbial phytase and citric acid synergistically enhanced nutrient digestibility and growth performance in Indian carp [120]. This also confirms a recent study by Malý et al. where phosphorus digestibility was increased through the combination of phytase and citric acid. This approach has the potential to positively influence the impacts of carp farming on the environment by reducing P excreted by fish. Additionally, the combination of these additives improved fish feed utilisation, increased specific growth rate, and improved breeding production parameters in aquaculture [121].

5.3.2 Combination with carbohydrases

Exogenous feed enzymes are widely utilised in commercial monogastric diets to enhance nutrient and energy digestibility by mitigating the effects of anti-nutritional factors and releasing additional nutrients. Non-starch polysaccharides (NSP) are such anti-nutritional ingredients components found in plant-based feedstuffs, for which monogastric animals lack endogenous enzymes, thus hindering efficient nutrient utilisation, including energy, protein or amino acids. Various NSPs are present in typical monogastric diets in differing concentrations and forms, both soluble and insoluble. For instance, wheat primarily contains soluble NSPs in the form of arabinoxylan, while barley and oats are rich in β-glucan. In copra meal, mannan is prevalent, which can also be found in protein sources like soybean meal [122]. This diversity underscores the rationale for combining exogenous NSP enzymes to effectively target these various substrates.

The nutritional effects of NSP on monogastric animals are varied and, in some instances, significant. It is widely acknowledged that the main disadvantages of NSP are related to the viscous nature of these polysaccharides, their physiological and morphological effects on the digestive tract, and interaction with the intestinal flora [122]. Although the mechanisms by which NSP enzymes operate differ from those of organic acids, their effects within the digestive tract of monogastric animals share some similarities, suggesting the potential for additive or even synergistic interactions when these additives are combined.

Moreover, the specific pH value optimum of both endogenous and exogenous enzymes plays a crucial role in determining their effectiveness. The combination of enzymes and organic acids may offer additional benefits in this regard.

In a study by Kim et al., the effects of NSP-cleaving enzymes (xylanase+ß-glucanase and mannanase) alone or in combination and in combination with a formic acid/sodium formate mixture in broilers were investigated. The results indicated that all feed additives either numerically or significantly improved final body weight at day 35 and increased apparent metabolisable energy (AME), with the most pronounced improvements observed when the additives were used in concert [123]. These findings suggest that an appropriate combination of carbohydrases and organic acids could exert a synergistic impact on growth and intestinal fermentative capacity, by effectively targeting NSP present in wheat-based diets.

5.3.3 Combination with plant extracts

Plant extracts have comparable modes of action to antimicrobials, working against both Gram-negative and Gram-positive bacteria. They are also reported to have stimulatory effects on the intestinal mucosa and on secretion of digestive enzymes.

The industry has already long adopted the combined use of organic acids and plant extracts, as the list of submitted and registered feed additives in the EU Register of Feed Additives shows. Several such additives which are registered as zootechnical additives, combine, for instance, benzoic acid and essential oils for poultry or protected microbeads containing citric acid, sorbic acid, thymol and vanillin, as previously discussed. Thus, their efficacy has been clearly demonstrated to EFSA with scientifically significant studies.

Langhout [124], for example, suggested that a combination of organic acids and plant extracts would be beneficial in poultry feeding, as the organic acids appear to be particularly active in the feed, crop and gizzard, whereas essential oils appear to work more in the later segments of the intestinal tract. A combination of both products might therefore result in a stronger product and improve the digestive process throughout the gastrointestinal tract. These concepts have been proven in several studies with pigs and poultry [125, 126, 127].

Other plant extracts such as saponins, which can act as surfactants, may also affect the bacterial cell membrane, potentially enhancing the efficacy of organic acids. The combination of plant extracts with organic acids is now becoming popular, with many products available on the market. Zheng et al., [128] concluded from their research that combination formulae of thymol and lactic acid, thymol and acetic acid, carvacrol and thymol and carvacrol and eugenol showed good synergistic effects, based on the Fractional Inhibitory Concentration (FIC) index against spoilage bacteria isolated from fresh produce. Zhou et al., [129] studied thymol and carvacrol, the key active ingredients of oregano oil, combined with acetic acid against *Salmonella Typhimurium*. In the presence of acetic acid, the antibacterial activity from the combinations of thymol (100 mg/l) plus acetic acid (0.10%) and of carvacrol (100 µl/l) plus acetic acid (0.10%) achieved those reached with 400 mg/liter thymol and 400 µl/l carvacrol alone. This demonstrates a powerful synergistic antimicrobial effect.

5.3.4. Combination with Butyrate and Medium Chain Fatty Acids (MCFA)

Organic acids have been used regularly in combination with Butyrate and with Medium Chain Fatty Acids (MCFA). Butyrate has a straight chain with four carbon atoms, and for the purpose of this booklet is considered with the fatty acids, not the organic acids. MCFA also contain straight chain organic/fatty acids with a chain length of 6 carbon atoms or more and are obtained from hydrolisation of coconut or palm kernel fat.

Blends of short chain fatty acids (e.g. formic acid, propionic acid) (SCFA) and medium chain fatty acids (e.g. caprylic acid, lauric acid) (MCFA) are more effective than organic acids alone. MCFA have higher pK_a values than SCFA in general and a higher molecular weight. MCFA are larger molecules compared to SCFA, which results in slower breakdown and uptake of the molecules. Therefore, these molecules will reach the lower part of the gastrointestinal tract and can exert an antimicrobial effect, while SCFA are no longer present at the beginning of the small intestine. In addition, the longer MCFA (C10-C12-C14) are known to affect the peptidoglycan layer of Gram-positive bacteria (e.g. *Clostridium, Enterococcus caecorum, Streptococcus suis*), making it permeable to organic acid molecules, enabling them to enter and disrupt internal cell metabolism. Therefore, with the inclusion of MCFA in the diet, a broader antimicrobial spectrum is achieved. This shows that the combination of SCFA and MCFA results in a synergetic effect, reducing both Gram-negative and Gram-positive bacterial counts. Further studies have shown that a combination of SCFA and MCFA in the diet results in improved animal performance in piglets [130] and broilers [131]. Dijksterhuis et al. found synergistic antifungal effects of a combination of ammonium propionate and medium-chain fatty acids [132].

On butyrate, scientific literature indicates that butyric acid is mainly used by enterocytes, the first layer of cells of the wall of the intestinal tract [133]. Especially colonocytes (gut wall cells in the large intestine) have a preference for butyrate as substrate. The substrates are fully absorbed and oxidised in by the gut wall cells so that no butyrate is present in the peripheral blood [134]. The same authors indicate that in the digestive tract, butyrate can act directly (upper gastrointestinal tract or hindgut) or indirectly (small intestine) on tissue development and repair. This was also found by Kotunia et al. who observed some trophic effects of butyrate on the intestinal epithelium, like increase in the villi length and crypt depth [135].

Also, other functions of butyrate in the gastrointestinal tract have been observed [134, 136] that all support the functioning of the gut, like improving gut development, reduction of inflammation, and modulation of gut microbiota. Ninwinska et al. reviewed the effects on calves and found that butyrate stimulates rumen development [137].

Bedford and Gong summarised research on Sodium butyrate. They found that levels around 1 mg/kg Sodium butyrate in the feed ration seem to have positive effects in many monogastric species like piglets, poultry and calves fed milk replacer [136]. We conclude that this may reflect the requirement by enterocytes. At this inclusion rate, there are often slight positive effects on performance, which can be explained from its supply of nutrition of the gut and of other effects on gut functioning.

Boas et al. found that the functionalities of organic acids and of butyrate were additive in the nutrition of piglets, leading to a superior numerical growth improvement of the combination of the two [138].

Zentek et al. used fumaric and lactic acids combined with caprylic and capric acids in weaning piglets and found benefits to the gastrointestinal microbial ecology as well as bacterial metabolites in the digestive tract [139]. Dahmer et al. studied the impact of an agglomerate of sodium diformate and glycerol monolaurate on performance of weanling pigs in comparison to administration of zinc oxide, finding increased average daily gain immediately post-weaning, with no difference between the treatments [140].

6. Sustainability aspects of organic acids

Sustainable development has captured the attention of society, regulatory authorities and industry alike and is now seen as a necessity, rather than a luxury. To bring these various aspects into a logical framework, we first need to describe what systems are in place to assess 'sustainability'.

Many criteria can be considered to play a role in sustainability, from relatively narrow, specifically defined criteria like carbon, ammonia or methane emissions, to much broader concepts such as Life Cycle Assessments (LCA). There tends to be a great deal of confusion as to what sustainability really means and therefore many institutions and NGOs are working to develop valid definitions and systems for assessment. Also, for organic acids and their derivatives, it makes a difference up to which point the LCA is determined. The footprint of the product up to its delivery to the feed compounder or the farmer (cradle to gate) would usually add to the total footprint of the final product like milk or meat. However, when the beneficial effects of organic acids and their derivatives are included in its assessment (which then influences the cradle to farm gate – being the animal produced), the partial LCA could then result in a lower or even negative footprint, depending on the size of the productivity enhancing effect in the animal.
As a harmonising principle, we consider 3 pillars of sustainable development in which other, narrower, criteria can find a niche, according to the approach followed by the United Nations [141]. These three pillars are as follows (with examples in parentheses where organic acids can play a role):

Social aspects (reduction of zoonoses; preventing the development of antibiotic resistance by reducing farm antibiotic use; increasing the nutritional value of low-quality ingredients thus enabling allocation of higher quality ingredients to human food; improving animal welfare);

Environmental aspects (contributing to lower mineral addition to feed thereby reducing eutrophication in the environment; reducing ammonia emissions);

Economic aspects (increased efficiency of animal production; increasing nutritional value of low-quality ingredients thereby increasing flexibility of feed formulation; reduction in feed material and compound feed losses caused by microbial spoilage).

6.1 Assessment of sustainability

We describe below the benefits of applying organic acids in the feed to food chain. This information may serve as inputs for sustainability models.

Currently, organic acids can be categorised in 2 groups, the "free organic acids" (FOA) and "protected organic acids" (POA). Overall, FOA are generally known as 'acidifiers', which aid in preserving the microbial quality of water for drinking or feed and, depending on the type of acid, to a certain extent support the upper intestinal gut health of livestock animals, as they are quickly released into the digestive tract after intake. On the other hand, because of the special formulation technology often used, including POA in the feed contributes to an improvement in animal performance, often with other additional benefits, such as animal welfare, through supporting the gut flora of livestock animals until the end of their intestinal tract.

6.2 Economic and ecological benefits of organic acid use in feed

A third of all food produced is wasted, making the food chain the third biggest carbon emitter, according to the United Nations. On a yearly basis, 1.3 billion tonnes of food, worth an estimated €550 billion is wasted, along with all the energy, water and chemicals used to produce it [142]. The Food and Agriculture Organisation of the United Nations (FAO) further identify food waste and loss as targets to assist economies everywhere in the transition to a green economy.

In India, for example, some 21 million tonnes of wheat annually perishes due to inadequate storage and distribution, equivalent to the entire production of Australia [143]. In Pakistan, losses amount to about 16% of production or 3.2 million tonnes annually, where inadequate storage infrastructure leads to widespread rodent infestation problems. However, per capita, much more food is wasted in industrialised nations. In Europe, an estimated 95-150 kg per year is wasted per capita.

6.3 Promoting sustainability in the food chain

With such a high percentage of wasted food products, engineers need to act now to promote sustainable ways to reduce waste from the farm to the supermarket and the consumer [143]. Agricultural practices, including the use of additives and various nutritional solutions in feed, can help to lower this wastage in several ways and thus have an impact on developing sustainable production. Free organic acids can be used in the feed chain for preservation purposes, to maintain high standards of feed quality and reduce losses due to fungal or bacterial contamination. Protected organic acids can be applied to enhance growth and support the efficiency of feed conversion, allowing a more sustainable feed consumption and food production.

6.4 Grain and feed preservation

Losses in feed due to mould and bacterial spoilage appear as mentioned above, not only in sub-optimal hygienic conditions, but also under the heavily regulated hygienic conditions prevalent in Europe. With spiralling oil prices and the concomitantly environmentally unfriendly and increasingly unsustainable solution to preservation of grain by drying, high moisture grain preservation with organic acids, such as propionic and formic acids and their salts, or sodium benzoate, may contribute towards an environmentally friendly, but still secure storage of the yearly grain harvest. An eco-efficiency analysis performed by one of the world's largest organic acid producers has indeed shown that feed grain preservation using propionic acid has economic and ecological benefits over other methods, including drying and airtight preservation in silos. Most organic acids currently in use have the legal status of 'preservative' in the EU register of feed additives, reflecting their high acceptance in this regard.

6.5 Feed efficiency

Between 50 and 80% of the total cost of poultry and pig production in Europe relate to feed. It therefore makes sense to improve the efficiency of turning feed into growth. In Germany, the largest economy in the EU, more than 70 kg of pork and poultry are consumed per capita per year, showing the scale of the need to improve feed efficiency, cost of production and environmental impact.

Controlling the balance of the intestinal microbiota is the foundation of a profitable modern livestock operation. The maintenance of a stable microflora within the digestive tract is a key aspect in achieving optimal feed efficiency; it also impacts food safety and animal welfare.

A holo-analysis of organic acids' use in pig nutrition [72] has revealed that feed efficiency (FCR) can be improved by an average of 3.7%. Assuming an overall FCR of 2.2 from the piglet to the fattener and a slaughter weight of 100 kg, this average improvement of feed efficiency of 3.7% would result in a saving of 8 kg feed per pig. Comparable calculations could be applied to poultry production, which is expected to become the largest source of meat worldwide in the next few years. For example, when broilers would have lifetime improvement of 8 points FCR (e.g. from 1.89 to 1.81) this would result in a feed saving of 180 g/bird.

The use of organic acids for sanitising feed or water can reduce mortality on the farm and thereby indirectly improve the overall efficiency of feed use and cost of production.

6.6 Efficient use of scarce resources

6.6.1 Phosphorus

The phosphorus situation has many similarities with that of oil. Unlike oil, however, there are no substitutes for phosphorus, since it is vital as a fertiliser in intensive agriculture. Studies suggest that high-grade phosphorus reserves will be depleted within 50-100 years [144]. More than 90% of the world's phosphorus supply is controlled by only 5 countries, none of which are in Europe.

Improving phosphorus digestibility can reduce phosphorus excretion into the environment and reduce the requirement for inorganic phosphorus in the diet. Since phosphorus is one of the most expensive ingredients in the diet, reducing its use can also improve the diet's overall cost-effectiveness. Dietary acidifiers are known to improve phosphorus digestibility, by creating more acidic pH in the proximal digestive tracts of pigs and poultry. One such study [145] shows a 4% increase in P digestibility in weaner pigs, from the use of dietary potassium diformate, reducing output in faeces and urine by 8%. In aquaculture, inclusion of acidifiers in the diet, e.g. citric acid, also improves P digestibility.

It is highly likely that the combined use of an acidifier and phytase will have a further impact in all monogastric livestock in this respect, as outlined in the chapter on synergy.

6.6.2 Nitrogen

A further threat to the environment is the eutrophication of water bodies. As water is an increasingly scarce resource, any impact on the reduction of its use or contamination will contribute to improved sustainability in our food production as we race to feed an increasing, and increasingly urban, population. Eutrophication is mainly caused by phosphorus and nitrogen, which leaches into ground water, rivers and lakes. Nitrogen is crucial to protein synthesis. Therefore, efficient use of this element is crucial to optimise feed efficiency and reduce environmental impact. Reducing the pH in the proximal digestive tract through the addition of organic acids to the diet improves the activation of pepsinogen to pepsin, improving protein digestibility and nitrogen retention. Roth et al. [146] reported a 2% increase in nitrogen digestibility resulting from the addition of an organic acid salt in diets for weaned piglets, with an associated reduction in faecal and urinary nitrogen output of more than 10%. Similarly, significant improvements in N-retention have also been reported in aquaculture and poultry.

6.7 Perspective of organic acids in sustainability

Continued profitability of the modern industrialised food chain requires minimal tolerance to wasted resources, with maximal potential to re-use non-product outputs elsewhere in the chain and minimising the excretion of valuable resources into the environment. In this sense, the use of feed additives such as organic acids, which preserve feedstuffs or improve the conversion of feed ingredients into animal food products (meat, eggs, milk, or fish), should be considered an integral part of any strategy to improve sustainability in the livestock production chain.

7. Safety aspects of organic acids and their salts

An organic acid is an organic compound with acidic properties, and they have a corrosive or irritant nature. The most common organic acids are the carboxylic acids, whose acidity is associated with their carboxyl group –COOH. Alcohols, with –OH, can act as acids but they are usually very weak. The relative stability of the conjugate base of the acid determines its acidity. In general, organic acids are weak acids and do not dissociate completely in water, whereas the strong mineral acids do. Lower molecular mass organic acids such as formic and lactic acids are miscible in water, but higher molecular mass organic acids, such as benzoic acid, are insoluble in molecular (neutral) form. Exceptions to these solubility characteristics exist in the presence of other substituents that affect the polarity of the compound.

Organic acids and their derivatives are widely used substances but may represent certain hazards for handling (user safety) or the environment. FEFANA members active in organic acids and derivatives employ the highest safety standards and have committed to classifying safety hazards of these products and mixtures according to internationally recognized procedures and communicating hazards in the chain.

For the EU, toxicological and eco-toxicological hazards of organic acids and their derivatives according to REACH, Reg. (EC) No. 1907/2006 are therefore taken as reference, as well as the classification and labelling rules according to the Regulation on classification, labelling and packaging of substances and mixtures, Reg. (EC) No. 1272/2008, also known as CLP. The latter legal EU act aligns with the Globally Harmonised System of Classification and Labelling of Chemicals (GHS). The GHS is an internationally agreed-upon system, created by the United Nations. It is designed to replace the various classification and labelling standards used in different countries, by using consistent criteria for classification and labelling on a global level and to inform users about these hazards through standard symbols and phrases on the packaging labels and through safety data sheets (SDS). For mixtures of organic acids, well defined calculation criteria are applied to arrive at the proper classification of the mixture.

7.1 Classification of most commonly used organic acids and salts

Within the EU, organic acids and their salts are classified according to the CLP of the purest forms or highest concentrations of substance.

The acidic nature of the organic acids and their salts is very well represented by their corrosive and irritant classification. In general, lower concentrations will reduce the corrosiveness of the product. The amounts present in feed are sufficiently low not to bear any health hazard to animals. Handling and storage of higher concentration acids (and some salts) however requires appropriate equipment, such as stainless-steel storage tanks and approved personal protective gear.

TABLE 6. EXAMPLES OF CLASSIFICATION OF ORGANIC ACIDS AND SALTS FOR THEIR PUREST FORMS UNDER EU LEGISLATION[1]

Chemical substance (minimum purity)	**Classification under GHS / CLP Reg. (EC) No. 1272/2008**
Citric acid (99%)	Signal word: Warning GHS07: Exclamation mark H319: Causes serious eye irritation H335: May cause respiratory irritation
Formic acid (99%)	Signal word: Danger GHS02: Flame GHS05: Corrosion GHS06: Skull and crossbones H226: Flammable liquid and vapour H290: May be corrosive to metals H302: Harmful if swallowed H314: Causes severe skin burns and eye damage H331: Toxic if inhaled
Calcium diformate (98%)	Signal word: Danger GHS05: Corrosion H318: Causes serious eye damage
Sodium formate	Not classified as hazardous
Lactic acid (80%)	Signal word: Danger GHS05: Corrosion H315: Causes skin irritation H318: Causes serious eye damage
Propionic acid (99%)	Signal word: Danger GHS05: Corrosion H314: Causes severe skin burns and eye damage

[1] See ECHA Homepage - ECHA Information from registration dossiers or, if not available, from Classification and Labelling Inventory (most notified classification); February 2026.

As can be seen from the examples in Table 6, the salt of an organic acid is usually less corrosive in nature than its corresponding acid or not classified as hazardous (e.g. sodium formate). Moreover, mono-, di-, and triglycerides of fatty acids ate generally not classified as hazardous under the CLP Regulation. In many cases, the efficacy of an organic acid can be maintained even when part of the acid is in the form of its salt or ester. In this way, a reduction in corrosiveness can be obtained that positively affects CLP and ADR (road transport) classification. Besides this, the degree of vaporisation may also be reduced, rendering higher recovery of the acid in the feed.

7.2 Transport of organic acids and salts

Carrying goods involves the risk of traffic accidents. If the goods are dangerous, there is also the risk of incidents, such as spillage, leading to hazards such as fire, explosion, chemical burn or environmental damage. Transport of chemicals, whether by road, rail, air or water, is regulated on various levels (country, region, environment, workers). To ensure consistency between all these regulatory systems, the United Nations developed mechanisms for transport conditions for all modes for transport (United Nations Recommendations on the Transport of Dangerous Goods, TDG).

The respective regulations are known by the following abbreviations:

Transport by road: ADR - Agreement concerning the international carriage of Dangerous goods by Road

Transport by rail: RID - Regulations concerning the International Carriage of Dangerous Goods by Rail

Transport by sea: IMDG - International Maritime Dangerous Goods code

Transport by air: IATA - International Air Transport Association

Transport by inland waterway: ADN– Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways

All dangerous goods need to be classified so that all organisations in the supply chain, including the emergency authorities, know and understand exactly the hazard. Dangerous goods are assigned to different classes depending on their predominant hazard. The main identification of the goods is by the UN number, further categorisation is on the packaging. UN numbers are four-digit numbers. Some hazardous substances have their own UN numbers while sometimes groups of chemicals or products with similar properties receive a common UN number (e.g. corrosive liquid, acidic, organic, not otherwise specified, have UN3265). A chemical in its solid state may receive a different UN number from the liquid phase if their hazardous properties differ significantly; substances with different levels of purity (or concentration in solution) may also receive different UN numbers. Non-hazardous substances do not have a UN number.

Acronyms / Glossary

- ABC: Acid binding capacity
- ADG: Average daily gain
- AND: Transport by inland waterway
- ADR: Transport by road
- BC: Buffering capacity
- CLP: Classification, Labelling and Packaging
- DSD: Dangerous Substance Directive
- EC: European Commission
- ECHA: European Chemical Agency
- EFSA: European Food Safety Authority
- EU: European Union
- FA: Formic acid
- FAMI-QS: Quality and Safety System for Specialty Feed Ingredients and their Mixtures
- FCR: Feed conversion ratio
- GHS: Globally Harmonised System of Classification and Labelling of Chemicals
- GIT: Gastrointestinal tract
- HACCP: Hazard Analysis and Critical Control Points
- IATA: Transport by air
- IBC: Intermediate Bulk Containers
- IMDG: Transport by sea
- LCA: Life Cycle Analysis
- MCFA: Medium Chain Fatty Acids
- MIC: Minimum Inhibitory Concentration
- PA: Propionic Acid
- pH: Acidity level
- pK_a: Acid dissociation constant
- REACh: Registration, Evaluation, Authorisation and Restriction of Chemicals
- RID: Transport by rail
- SCFA: Short Chain Fatty Acids
- SF: Sodium Formate
- TDG: Transport of Dangerous Goods

List of Tables and Figures

References

References in Chapter 1

1. Lückstädt, C. (2011). Principles for the use of organic acids in animal nutrition – Preface. In: Standards for acidifiers (Ed. C. Lückstädt). Nottingham University Press, UK, p. ix.
2. Feedinfo News Service (2011). Use of 'reliable' organic acids tipped to grow worldwide. Accessed 14.02.2011 at feedinfo.com.
3. Freitag, M. (2007). Organic acids and salts promote performance and health. In: Acidifiers in Animal Nutrition: A guide for feed preservation and acidification to promote animal performance (Ed. C. Lückstädt). Nottingham University Press, UK, pp. 1-11.
4. Foegeding, P.M. and Busta, F.F. (1991). Chemical food preservatives. In: Disinfection, Sterilization and Preservation. Pp. 802-832. S.S. Block (Ed.). Lea and Febiger, Philadelphia, USA.
5. Kirchgessner, M. and Roth, F.X. (1988) Ergotrope Effekte durch organische Säuren in der Ferkelaufzucht und Schweinemast. [Energy value of organic acids in the rearing of piglets and the fattening of pigs] Übersichten zur Tierernährung 16: 93-108.

References in Chapter 4.2

6. Bartov, I., Paster N. and Lister, N., (1982). The nutritional value of moldy grains for broiler chicks. Poultry Science 61: 2247 – 2254.
7. Bartov, I. (1983). Effects of propionic acid and of copper sulphate on the nutritional value of diets containing moldy corn for broiler chicks. Poultry Science 62:2195-2200.

Further Reading

Kashket, E.R. (1985). The proton motive force in bacteria: an assessment of methods. Ann. Rev. Microbiol. 39: 219-242.

Kashket, E.R. (1987). Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance. Microbiology Reviews 46:233-244.

Russell, J.B. (1992). Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling. Journal of Applied Bacteriology 73: 363 – 370.

References in Chapter 4.3

8. France, 2007. Arrêté ministriel du 23 avril 2007, annexe IV modifié par l'Arrêté du 10 mars 2010 - art. 3 & 4).
9. Hinton, M. and Linton, A.H. (1988). Control of Salmonella infections in broiler chickens by the acid treatment of their feed. Veterinary Record 123: 416-421.
10. Iba, A.M. and Berchieri, A. (1995) Studies on the use of a formic acid-propionic acid mixture to control experimental Salmonella infection in broiler chickens. Avian Pathology 24:303–311.

11. Berchieri, A. and Barrow, P.A. (1996) Reduction in incidence of experimental fowl typhoid by incorporation of a commercial formic acid preparation into poultry feed. Poultry Science 75:339–341.
12. Thompson, J.L. and Hinton, M. (1997) Antibacterial activity of formic and propionic acids in the diet of hens on *Salmonellas* in the crop. British Poultry Science 38:59–65.
13. Koyuncu S., M.G. Andersson, C. Löfström, P.N. Skandamis, A. Gounadaki, J. Zentek and Häggblom, P. (2013). Organic acids for control of *Salmonella* in different feed materials. BMC Veterinary Research 9:81
14. EFSA (2008). Microbiological risk assessment in feedingstuffs for food-producing animals. Scientific Opinion of the Panel on Biological Hazards (Question No EFSA-Q-2007-045). Adopted on 5 June 2008. Parma. (The EFSA Journal, 720).
15. Davies, R.H. and Hinton, M.H. (2000): *Salmonella* in Domestic Animals. In: Salmonella in Domestic Animals, pp. 285–300. C. Wrey and A. Wrey (editors) CABI Publishing, New York, USA.
16. Berge, A.C. and Wierup, M. (2012). Nutritional strategies to combat *Salmonella* in mono-gastric food animal production. Animal: An International Journal of Animal Bioscience, 6(4):557–564.
17. Van Immerseel, F., Cauwerts, K., Devriese, L.A., Haesebrouck, F. and Ducatelle, R. (2002): Feed additives to control *Salmonella* in poultry. World's Poultry Science Journal 58(4):501-513.
18. Humphrey, T.J. and Lanning, D.G. (1988). The vertical transmission of Salmonella and formic acid treatment of chicken feed. Epidemiology and Infection 100: 43-49.
19. Cherrington, C.A., Hinton M. and Chopra, I. (1990). Effect of short-chain organic acids on macromolecular synthesis in *Escherichia coli*. Journal of Applied Bacteriology 68:69-74.
20. Cherrington C.A., Hinton M., Mead G.C. and Chopra I. (1991). Organic acids: chemistry, antibacterial activity and practical applications. Advances in Microbial Physiology 32:87-108.
21. Shin, R., Suzuki M. and Morishita, Y. (2002). Influence of intestinal anaerobes and organic acids on the growth of enterohaemorrhagic *Escherichia coli* O157:H7. J. Med. Microbiol., 51: 201-206.
22. Malicki, A., Zawadzki, W., Bruzewicz, S. and Czerski, A. (2004). Effect of formic and propionic acid mixture on *Escherichia Coli* in fish meal stored at 12 C°. Pakistan Journal of Nutrition 3(6): 353-356.

Further Reading

Russell, J. B. and Diez-Gonzalez, F. (1998). The effects of fermentation acids on bacterial growth. Advances in Microbial Physiology 39: 205-234.

References to Chapter 4.4

23. Harrisson, J.H., Blauwekiel, R. and Stokes, M.R. (1994). Fermentation and utilisation of grass silage. Journal of Dairy Science 77:3209-3235.

References in Chapter 4.4

24. Wattiaux, M. (2002). Introduction to Silage Making. In: Dairy Updates. Feeding No 502. The Babcock Institute. University of Wisconsin.
25. Holmes, B.J. and Muck, M.E. (2000). Preventing silage storage losses. University of Wisconsin, Madison. Proceedings of the School of Veterinary Medicine.
26. Oude-Elferink, S.J.W.H., Driehuis, F. and Gottschalk, J.E. (2001). Silage fermentation processes and their manipulation. FAO Plant Production and Harvest Technology.
27. Muck, R.E. (1988). Factors influencing silage quality and their implications for management. Journal of Dairy Science, 71:2992-3002.
28. Ruppel, K. (1997). Economies of silage management practices: What can I do to improve the bottom line of my ensiling business? In: Proceedings in Silage: Field to Feedbunk. Heskey, Pennsylvania: p. 125-136.
29. Lindgren, S., Petterson, K., Kaspersson A., Jonsson A. and Lingvall, P. (1985). Microbial dynamics during aerobic deterioration of silages. Journal of the Science of Food & Agriculture 36:765-774.
30. Fenton, J. (1987). An investigation into the sources of lactic acid bacteria in grass silage. Journal of Applied Bacteriology 62: 181-188.
31. Langston, C.W., Wiseman, H.G., Gordon, C.H., Jacobsen, W.C., Melin, C.G. and Moore, L.A. (1962a). Chemical and bacteriological changes in grass silage during the early stages of fermentation I. Chemical changes. Journal of Dairy Science, 45: 396-402.
32. Langston, C.W., Wiseman, H.G., Gordon, C.H., Jacobsen, W.C., Melin, C.G. and Moore, L.A. (1962b). Chemical and bacteriological changes in grass during the early stages of fermentation II. Biological changes. Journal of Dairy Science 45: 618-624.
33. Pitt, R.E. and Shaver, R.D. (1990). Processes in the preservation of hay and silage. In: Proceedings of the Dairy Feeding Systems Symposium (NRAES-38), Harrisburg, Pennsylvania.
34. McDonald, P., Henderson, A. R. and Heron, S.J.E. (1991). The Biochemistry of Silage. Second Edition. Lincoln, UK. Chalcombe Publications. 340p.
35. Jones D.I.H. and Jones, R. (1995). The Effect of Crop Characteristics and Ensiling Methodology on Grass Silage Effluent Production. Journal of Agricultural Engineering Research 60: 73-81.
36. Alves, S.P., Cabrita, A.R.J., Jerónimo, E., Bess, R.J.B. and Fonseca, A.J.M. (2011). Effect of ensiling and silage additives on fatty acid composition or ryegrass and corn experimental silages. Journal of Animal Science 89: 2537-2545.
37. Charmley, E. and Veira, D.M. (1990). Inhibition of proteolysis in alfa-alfa silage using heat at harvest- Effects on digestion in the rumen, voluntary intake and animal performance. Journal of Animal Science. 68: P 2042-2051.
38. Wilson, R.J. and Wilkins R.F. (1973). Formic acid as a silage additive. The Journal of Agricultural Science 81(1): 117 – 124.
39. Thompson D.J., Beever, D.E., Lonsdale, C.R., Haines, M.J., Cammell, S.B and Austin, A.R. (1981). The digestion by cattle of grass silage made with formic acid and formic acid-formaldehyde. British Journal of Nutrition 46(1): 193-207.

40. Fernandez-Lorenzo, B. and O'Kiely, P. (2008). Alternatives to formic acid as a grass silage additive under two contrasting ensilability conditions. Irish Journal of Agricultural and Food Research 47: 135-149.
41. Tamminga, S., Ketelaar, R. and van Vuuren, A.M. (1991). Degradation of nitrogenous compounds in conserved forages in the rumen of dairy cows. Grass Forage Science 46: 427-435.
42. Jaakkola, S., Kaunisto, V. and Huhtanen, P. (2006a). Volatile fatty acid proportions and microbial protein synthesis in the rumen of cattle receiving grass silage ensiled with different rates of formic acid. Grass and Forage Science 61: 282-292.
43. Wilkerson, V.A., Glenn, B.I. and McLeod, K.R. (1997). Energy and nitrogen balance in lactating cows fed diets containing dry or high moisture corn in either rolled or ground form. Journal of Dairy Science 80: 3463-3468.
44. Jaakkola, S., Saarisalo, E. and Kangasniemi, R. (2005). Ensiled high moisture barley or dry barley in the grass silage-based diet of dairy cows. In: Park, R.S. & Stronge, M.D. (eds). Proceedings of theXIVth International Silage Conference, Belfast, Northern Ireland. Wageninger Academic Publishers. p. 184.
45. Sebastian, S., Philip, L.E., Fellner, V. and Idziak, E.S. (1996). Comperative assessment of bacterial inoculant propionic acid treatment of aerobic stability and microbial populations of ensiled high moisture ear corn. Journal of Animal Science 74: 447-456.
46. Higgins, C., and Brinkhaus, F. (1999). Efficacy of several organic acids against mold. The Journal of Applied Poultry Research. 8: 480-487.

References in Chapter 4.5

47. Canibe, N., Engberg, R.M. and Jensen, B.B. (2001). An overview of the effect of organic acids on gut flora and gut health. In: Proceedings of the workshop: alternatives to feed antibiotics and coccidiostats in pigs and poultry (AFAC). Oslo, Norway, 13-16 October 2001.
48. Lawlor, P.G., Lynch, P.B., Caffrey, P.J., O'Reilly, J.J., and O'Connell, M.K. (2005). Measurements of the acid-binding capacity of ingredients used in pig diets. Irish Veterinary Journal 58(8): 447-452.
49. Partanen, K., Niemi, J. and Karhula, T. (2010). Organic acids in pig diets. In: Recent Advances in Animal Nutrition, pp. 257-285. Eds.: Garnsworthy, P.C. and Wiseman, J.
50. Kim, Y.Y., Kil, D.Y., Oh, H.K. and Han, In.K. (2004). Acidifier as an alternative material to antibiotics in animal feed. International Symposium on Recent Advances in Animal Nutrition during the 11th Animal Science Congress, Asian-Australasian Association of Animal Production Societies. Kuala Lumpur, Malaysia. September 5-9.
51. Tung C.M. and Pettigrew J.E. (2006). Critical review of acidifiers. National Pork Board. Available at: http://www.pork.org/Documents/PorkScience/ReviewOfAcidifiers.pdf. Accessed 10 April 2009.
52. Dersjant-Li, Y. (2010). Impact of dietary cation anion difference in fish and pigs: a comparative study. Thesis Wageningen University. Ponsen & Looijen, Wageningen. ISBN: 90-5808-297-0.

References in Chapter 4.6

53. Van Immerseel, F., De Zutter, L., Houf, K., Pasmans, F., Haesebrouck, F. and Ducatelle, R. (2009). Strategies to control salmonella in the broiler production chain. World's Poultry Science Journal 65(3): 367-391
54. Açikgöz, Z., Bayraktar, H. and Altan, Ö. (2011). Effects of formic acid administration in the drinking water on performance, intestinal microflora and carcass contamination in male broilers under high ambient temperature. Asian-Australian Journal of Animal Science 24(1): 96–102.
55. Qing-Yang, Ji., Wenqiong Wang, Haodong Yan, Hengxian Qu , Yang Liu, Yi Qian and Ruixia Gu (2023). The Effect of Different Organic Acids and Their Combination on the Cell Barrier and Biofilm of Escherichia coli. Foods 12: 3011.
56. Kim, Y.Y., Kil, D.Y., Oh, H.K. and Han, In. K. (2004) Acidifier as an alternative material to antibiotics in animal feed. International Symposium on Recent Advances in Animal Nutrition during the 11th Animal Science Congress, Asian-Australasian Association of Animal Production Societies. Kuala Lumpur, Malaysia. September 5-9.
57. Corrier, D.E., Byrd, J.A., Hargis, B.M., Hume, M.E., Bailey, R.H. and Stanker, L.H. (1999). Presence of Salmonella in the crop and ceca of broiler chickens before and after preslaughter feed withdrawal. Poultry Science 78: 45-49.

References in Chapter 4.7

58. EFSA (2008). Microbiological risk assessment in feedingstuffs for food-producing animals, scientific opinion of the Panel on Biological Hazards. The EFSA Journal 720: 1-84.
59. Van Immerseel, F., Russell, J.B., Flythe, M.D., Gantois, I., Timbermont, L., Pasmans, F., Haesebrouck and Ducatelle, R. (2006). The use of organic acids to combat Salmonella in poultry: a mechanistic explanation of the efficacy. Avian Pathology 35(3): 182-188.
60. Canibe, N., Engberg, R.M. and Jensen, B.B. (2001). An overview of the effect of organic acids on gut flora and gut health. Proceedings of the Workshop: Alternatives to Feed Antibiotics and Coccidiostats in Pigs and Poultry (AFAC). 13-16 October, Oslo, Norway.
61. Thompson, J.L. and Lawrence, T.L.J. (1981). Dietary manipulation of gastric pH in the profilaxis of enteric disease in weaned pigs. Some field observations. Veterinary Record 109: 120-122.
62. Van Immerseel, F., De Zutter, L., Houf, K., Pasmans, F., Haesebrouck, F. and Ducatelle, R. (2009). Strategies to control Salmonella in the broiler production chain. World's Poultry Science Journal 65(3): 367-391.
63. Partanen, K., Niemi, J., Karhula, T. (2010). Organic acids in pig diets. In: Recent Advances in Animal Nutrition. Garnsworthy P.C. and Wiseman, J. Nottingham University Press, Nottingham, UK.
64. Yousaf, M.S., Goodarzi Boroojeni, F., Vahjen, W., Männer, K., Hafeez, A., Ur-Rehman, H., Keller, S., Peris, S., and Zentek, J. (2017). Encapsulated benzoic acid supplementation in broiler diets influences gut bacterial composition and activity. British Poultry Science 58(2): 122-131.

65. Junpeng Jia, Ruiling Liu, Rongfeng Tang, Jian Lin, Qian Yang. (2024). Benzoic Acid potentiates intestinal IgA response in broiler chickens against *Salmonella enterica Serovar Typhimurium* infection. Poultry Science 103(12): 104505.

Further Reading

Koyuncu, S., Andersson, M.G., Löfström, C., Skandamis, P.N., Gounadaki, A., Zentek, J. and Häggblom, P. (2013). Organic acids for control of *Salmonella* in different feed materials. BMC Veterinary Research 9: 81.

References in Chapter 4.8

66. Freitag, M. (2007) Organic acids and salts promote performance and health in animal husbandry. In: Acidifiers in Animal Nutrition. C. Lückstädt (Ed.). Nottingham University Press, UK. 1-11.
67. Kirchgessner, M. and Roth, F.X. (1988) Nutritive effects of organic acids in piglet rearing and pig fattening. Übersichten zur Tierernährung 16: 93-108.
68. Øverland, M., Granli, T., Kjos, N.P., Fjetland, O., Steien, S.H. and Stokstad, M. (2000). Effect of dietary formates on growth performance, carcass traits, sensory quality, intestinal microflora, and stomach alterations in growing-finishing pigs. Journal of Animal Science 78:1875-1884.
69. Partanen, K.H. (2001). Organic acids -their efficacy and modes of action in pigs. In: Gut Environment of Pigs. A. Piva, K.E. Bach Knudsen, and Lindberg, J.E. (Eds.), pp. 201-218, University Press, Notingham, UK.
70. Mellor, S. (2008). Holoanalysis – information in action for organic acids. Feed Compounder August, 21-23.
71. Rosen, G.D. (2008). Abstracts of the British Society of Animal Science, Poster presentation 085.
72. Cole D.J.A., Beal, R., M. and Luscombe, J.R. (1968). The effect on performance and bacterial flora of lactic acid, propionic acid, calcium propionate and calcium acrylate in the drinking water of weaned pig. Veterinary Record 83: 459-464.
73. Wang, H., Long, W., Chadwick, D., Zhang, X., Zhang, S., Piao, X., Hou, Y. (2022). Dietary acidifiers as an alternative to antibiotics for promoting pig growth performance: A systematic review and meta-analysis. Animal Feed Science and Technology, 289: 115320.
74. Xu, B., Fu, J., Zhu, L., Li, Z., Jin, M., and Wang, Y. (2021). Overall assessment of antibiotic substitutes for pigs: a set of meta-analyses. Journal of Animal Science and Biotechnology, 12:3. 15 pp.

References in Chapter 4.9

75. Polycarpo, G.V., Andretta, I., Kipper, M., Cruz-Polycarpo, V.C., Dadalt, J.C., Rodrigues, P.H.M., and Albuquerque, R. (2017). Meta-analytic study of organic acids as an alternative performance-enhancing feed additive to antibiotics for broiler chickens. Poultry Science, 96: 3645–3653.

76. Yan, F., Chen, J., Kuttappan, V., and Vazquez-Anon, M. (2018). Efficacy of protected benzoic acid in broilers subject to Eimeria challenge as affected by diet type. PSA Annual meeting July 23-26, 2018. San Antonio (TX), United States.
77. Abd El-Ghany, W.A. (2024). Applications of Organic Acids in Poultry Production: An Updated and Comprehensive Review. Agriculture,14: 1756.
78. Haq, Z., Rastogi, A., Sharma, R.K., and Khan, N. (2017). Advances in role of organic acids in poultry nutrition: A review. Journal of Applied and Natural Science 9(4): 2152-2157.
79. Ogwuegbu, M.C., Chukwudi, P., Okafor-Paul, C., Edeh, H.O., Ani, A.O., Okpanachi, U., Mthiyane, M.N. (2025). Effect of varying levels of benzoic acid on growth performance, haemato-biochemical indices, carcass traits, gut morphology and intestinal microbiota of broiler chickens. Journal of Applied Poultry Research 34: 100556.

References in Chapter 4.10

80. Nozière, P., Glasser, F., Sauvant, D. (2011). In vivo production and molar percentages of volatile fatty acids in the rumen: a quantitative review by an empirical approach. Animal, 5(3): 403–414.
81. Malik, M.I., Capucchio, M.T., Tunkala, B.Z., Muneeb, M., Ncho, C.M., Hao, L., Cheng, L. (2025). The effect of fumaric acid on ruminant enteric methane emission and ruminal volatile fatty acids concentration: a meta-analysis. Journal of Animal Science, 103, skaf362.
82. Rocha, L.T. da, Del Valle, T.A., Skonieski, F.R.; Pereira, S.N., Oliveira, P.S.d. Facco, F.B. Viégas, J. (2025). Exploratory Meta-Analysis of the Effect of Malic Acid or Malate Addition on Ruminal Parameters, Nutrient Digestibility, and Blood Characteristics of Cattle. Animals, 15: 2177.

References in Chapter 4.11

83. Rungruangsak, K. and Utne, F. (1981). Effect of different acidified wet feeds on protease activities in the digestive tract and on growth rate of rainbow trout. Aquaculture22: 67-79.
84. Lückstädt, C. (2008). The use of acidifiers in fish nutrition. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 3(44): 1-8.
85. Ng, W.K. and Koh, C.B. (2011). Application of organic acids in aquafeeds: impacts on fish growth, nutrient utilisation and disease resistance. In: Standards for Acidifiers C. Lückstädt (Ed.). Nottingham University Press, Nottingham, UK, 49-58.
86. Ng, W.K. and Koh, C.B. (2017). The utilization and mode of action of organic acids in the feed of cultured aquatic animals. Reviews in Aquaculture 9: 342-368.
87. Hoseinifar, S.H., Sun, Y.Z. and Caipang, C.M. (2017). Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view. Aquaculture Research 48: 1380-1391.
88. Chen, J., He, S., Zhang, Z., Li, J., Zhang, X., Li, J, Xu, J., Zheng, P., Xian, J. and Lu, Y. (2024) Application of organic acids salts as feed additives in some aquatic organisms: Potassium diformate. Fishes 9(85): 1-11.

89. Krome, C., Schuele, F., Jauncey K. and Focken, U. (2018) Influence of a sodium formate/formic acid mixture on growth of juvenile common carp (*Cyprinus carpio*) fed different fishmeal replacement levels of detoxified Jatropha curcas kernel meal in practical, mixed diets. Journal of Applied Aquaculture 30: 137-156.

90. Torrecillas, S., Aboelsaadat, E., Carvalho, M., Acosta, F., Monzón-Atienza, L., Gordillo, Á., & Montero, D. (2024). Dietary supplementation of a combination of formic acid and sodium formate in practical diets promotes gilthead sea bream (*Sparus aurata*) gut morphology and disease resistance. Aquaculture Reports 35: 101951.

91. Wassef, E.A., Abdel-Momen, S.A.G., Saleh, N.E.S., Al-Zayat, A.M. and Ashry, A.M. (2017) is sodium diformate a beneficial feed supplement for European seabass (*Dicentrarchus labrax*)? Effect on growth performance and health status. Egyptian Journal of Aquatic Research 43: 229-234.

92. Reyshari, A., Mohammadiazarm, H., Mohammadian, T. and Mozanzadeh, M.T. (2019) Effects of sodium diformate on growth performance, gut microflora, digestive enzymes and innate immunological parameters of Asian sea bass (*Lates calcarifer*) juveniles. Aquaculture Nutrition 25: 1135-1144.

93. Lückstädt, C. (2021) Dietary sodium diformate improves growth performance of the giant freshwater prawn under controlled conditions in the Philippines. Aquafeed: Advances in Processing & Formulation 13(1): 41-42.

94. Lin, X., Zhang, C., Cao, K., Li, Z., Zhao, Z., Li, X., & Leng, X. (2023). Dietary sodium butyrate changed intestinal histology and microbiota of rainbow trout (*Oncorhynchus mykiss*), but did not promote growth and nutrient utilization. Aquaculture Nutrition, Article ID 3706109.

95. Morken, T., Kraugerud, O. F., Barrows, F. T., Sørensen, M., Storebakken, T., & Øverland, M. (2011). Sodium diformate and extrusion temperature affect nutrient digestibility and physical quality of diets with fish meal and barley protein concentrate for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 317(1–4): 138–145.

96. Rimoldi, S., Gliozheni, E., Ascione, C., Gini, E., & Terova, G. (2018). Effect of a specific composition of short- and medium-chain fatty acid 1-monoglycerides on growth performances and gut microbiota of gilthead sea bream (*Sparus aurata*). PeerJ 6: e5355.

97. Ullah, S., Feng, F., Zhao, M., Zhang, J., & Shao, Q. (2025). Comparative effects of dietary supplementations with microencapsulated sodium butyrate, glycerol monolaurate and tributyrin on growth, immunity, and gut health in black sea bream. Animals 15(6): 810.

98. Novriadi, R., Khotimah, N., Kamarudin, A. S., Herawati, V. E., Prayitno, S. B., Windarto, S., Man, C., Barri, A., & Staehler, P. (2024). Dietary use of monoglycerides blend in plant-based diets influences growth performance, hepatopancreatic histology, immunity and disease resistance of Pacific white shrimp (*Penaeus vannamei*) against *Vibrio harveyi*. Aquaculture Studies 24(6): AQUAST1964.

99. Romano, N., Koh, C.-B., & Ng, W.-K. (2015). Dietary microencapsulated organic acids blend enhances growth, phosphorus utilization, immune response, hepatopancreatic integrity and resistance against *Vibrio harveyi* in white shrimp, *Litopenaeus vannamei*. Aquaculture435: 228–236.

100. Yousefi, M., Naderi Farsani, M., Afzali-Kordmahalleh, A., & Ahani, S. (2024). Dietary tartaric acid improves growth performance, gut microbiota, digestive enzyme activities, hemolymph immunity, antioxidant markers, and disease resistance against *Vibrio parahaemolyticus* in Pacific white shrimp. Journal of Marine Science and Engineering 12(1): 83.

References in Chapter 5

101. Strauss, G. and Hayler, R. (2001). Effects of organic acids on microorganisms. Kraftfutter 4: 1-4.

102. Hinton, M. and Linton, A.H. (1988). Control of Salmonella infections in broiler chickens by the acid treatment of their feed. Veterinary Record 123: 416-421.

103. Al-Tarazi, Y.H. and K. Alshawabkeh, (2003). Effect of Dietary Formic and Propionic Acids on *Salmonella Pullorum* Shedding and Mortality in Layer Chicks after Experimental Infection. Journal of Veterinary Medicine B 50: 112–117

104. Koyuncu, S., Andersson, M.G., Löfström, C., Skandamis, P.N., Gounadaki, A., Zentek, J. and Häggblom, P. (2013). Organic acids for control of *Salmonella* in different feed materials. BMC Veterinary Research 9: 81.

105. Malicki, A., Zawadzki, W., Bruzewicz, S., Graczyk, S. and Czerski, A. (2004). Effect of formic and propionic acid mixture on *Escherichia Coli* in fish meal stored at 12ºC. Pakistan Journal of Nutrition 3(6): 353-356.

106. Cherrington, C.A., Hinton, M. and Chopra, I. (1990). Effect of short-chain organic acids on macromolecular synthesis in *Escherichia coli*. Journal of Applied Bacteriology 68: 69-74.

107. Cherrington, C.A., Hinton, M., Pearson, G.R. and Chopra, I. (1991). Short-chain organic acids at ph 5.0 kill *Escherichia coli* and *Salmonella* spp. without causing membrane perturbation. Journal of Applied Bacteriology 70: 161-165.

108. Chaveerach, P., Keuzenkamp, D.A., Urlings, H.A.P., Lipman, L.J.A. and van Knapen, F. (2002). In Vitro study on the effect of organic acids on *Campylobacter jejuni/coli* populations in mixtures of water and feed. Poultry Science 81: 621–628

109. 1Al-Khassi, A.G. and Mohssen, M.A. (2009). Comparative study between single organic acid effect and synergistic organic acid effect on broiler performance. Pakistan Journal of Nutrition 8(6): 896-899.

110. Bhavsar, K., Buddhiwant, P., Soni, S.K., Depan, D., Sarkar, S. and Khire, J.M. (2013). Phytase isozymes from Aspergillus niger NCIM 563 under solid state fermentation: Biochemical characterisation and their correlation with submerged phytases. Process Biochemistry. 48 (11): 1618-1625

111. Jongbloed, A. W., et al. (2000). The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. Livestock Production Science 67(1): 113-122.

112. Klein, N., et al. (2023). Effects of dietary calcium source, acidification, and phytase on precaecal phytate degradation and phosphorus digestibility in pigs. Proceedings Society Nutritional Physiology 32: 90.

113. Han Y.M., Roneker, K.R., Pond, W.G. and Lei, X.G. (1998). Adding wheat middlings, microbial phytase, and citric acid to corn-soybean meal diets for growing pigs may replace inorganic phosphorus supplementation. Journal of Animal Science 76: 2649-2656.
114. Kemme, P.A., Jongbloed, A.W., Mroz, Z., Kogut, J. and Beynen, A.C. (1999.) Digestibility of nutrients in growing-finishing pigs is affected by Aspergillus niger phytase, phytate and lactic acid levels: 2. Apparent total tract digestibility of phosphorus, calcium and magnesium and ileal degradation of phytic acid. Livestock Production Science58(2): 119-127.
115. Callesen, J. (1999). Commercial feed products and feed mixes for piglets. Report 409 Lansuvalget for Svin, Denmark.
116. Valencia, Z. and Chavez, E.R. (2002). Phytase and acetic acid supplementation in the diet of early weaned piglets: effect on performance and apparent nutrient digestibility. Nutrition Research 22(5): 623-632.
117. Dusel, G., et al. (2022). Reduzierung der N/P-Ausscheidung durch den Einsatz von Enzymen. Forum angewandte Forschung in der Rinder- und Schweinefütterung. Soest, Verband der Landwirtschaftskammern: 204-208.
118. Sacakli, P., Sehu, A., Ergün, A., Genc, B. and Selcuk, Z. (2006). The effect of phytase and organic acid on growth performance, carcass yield and tibia ash in quails fed diets with low levely of non-phytate phosphorus. Asian-Australian Journal of Animal Science 19(2): 198-202.
119. Krieg, J., et al. (2021) Effects of calcium level and source, formic acid, and phytase on phytate degradation and the microbiota in the digestive tract of broiler chickens. Animal microbiome3: 23.
120. Baruah, K., Sahu, N.P., Pal, A.K., Jane, K.K,, Debnath, D. and Mukherjee S.C. (2007). Dietary microbial phytase and citric acid synergistically enhances nutrient digestibility and growth performance of *Labeo rohita* (Hamilton) juveniles at sub-optimal protein level. Aquaculture Research 38: 109-120.
121. Malý, O., Zugrková, I., Radojičić, M., Pošva, E., & Mareš, J. (2023). Increasing phosphorus digestibility in common carp (*Cyprinus carpio L.*) farming using phytase and citric acid. Aquaculture Research: 4781600.
122. Choct, M. (1997). "Feed Non-Starch Polysaccharides: Chemical Structures and Nutritional Significance." Feed Milling International June: 13-26.
123. Kim, E., et al. (2023). Apparent metabolizable energy and soluble fibre utilisation in broiler chickens fed wheat diets with supplemental carbohydrases and organic acid. Proceedings European Symposium on Poultry Nutrition. WSPA. Rimini, Italy. Page 294 (poster).
124. Langhout, P. (2000). New additives for broiler chickens. World Poultry 16: 22-27.
125. Zhang, K.Y., Yan, F., Keen, C.A. and Waldroup, P.W. (2005). Evaluation of micro-encapsulated essential oils and organic acids in diets for broiler chickens. International Journal of Poultry Science 4(9): 612-619.
126. Abd El-Hakim A.S., Cherian, G. and Ali, M.N. (2009). Use of organic acid, herbs and their combination to improve the utilisation of commercial low protein broiler diets. International Journal of Poultry Science 8(1): 14-20.

127. Rounsavall, J.W., Newman, C.A., Neher, F. and Laurenz, J.C. (2007). Improving the Performance of Weaned Pigs with Natural Products. In: Acidifiers in Animal Nutrition (Lückstädt Ed.). Nottingham University Press, Nottingham, UK pp. 55-62.

128. Zheng, L., Bae, Y.M., Jung, K.S., Heu, S. and Lee, S.Y. (2013). Antimicrobial activity of natural antimicrobial substances against spoilage bacteria isolated from fresh produce. Food Control 32: 665 – 672.

129. Zhou, F., Ji, B., Zhang, H., Jiang, H., Yang, Z., Li, J., Li, J., Ren, Y. and Yan, W. (2007). Synergistic effect of thymol and carvacrol combined with chelators and organic acids against Salmonella typhimurium. Journal of Food Protection, 70(7): 1704–1709.

130. Long, S.F., Xu, Y.T., Pan, L., Wang, Q.Q., Wang, C.L., Wu, J.Y., Wu, Y.Y., Han, Y.M., Yun, C.H., Piao, X.S. (2018). Mixed organic acids as antibiotic substitutes improve performance, serum immunity, intestinal morphology and microbiota for weaned piglets. Animal Feed Science and Technology, 235: 23-32.

131. Nguyen, D.H., Lee, K.Y., Mohammadigheisar, M., and Kim, I.H. (2018). Evaluation of the blend of organic acids and medium-chain fatty acids in matrix coating as antibiotic growth promoter alternative on growth performance, nutrient digestibility, blood profiles, excreta microflora, and carcass quality in broilers. Poultry Science, 97: 4351-4358.

132. Dijksterhuis, Jan, Kleinhoven, Pim, Van Kuijk, Sandra, Wolters, Anouk H.G., and Bruinenberg, Paul G. (2024). Synergistic antifungal effects of the preservative ammonium propionate and medium chain fatty acids against dormant and germinating conidia, germ tubes and hyphae of *Aspergillus chevalieri*, a feed spoilage fungus. International Journal of Food Microbiology, 422: 110802.

133. Hamer, H.M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F.J., and Brummer, R.-J. (2008). Review article: the role of butyrate on colonic function. Alimentary Pharmacology & Therapeutics, 27: 104-119.

134. Guilloteau, P., Martin, L., Eeckhaut, V., Ducatelle, R., Zabielski, R., and Van Immerseel, F. (2010). From the gut to the peripheral tissues: the multiple effects of butyrate. Nutrition Research Reviews, 23: 366–384.

135. Kotunia, A., Wolinski, J., Laubitz, D., Jurkowska, M., Romé, V., Guilloteau, P., Zabielski, R. (2004). Effect of sodium butyrate on the small intestine development in neonatal piglets feed by artificial sow. Journal of Physiology and Pharmacology, 55 (suppl): 59-68.

136. Bedford, A., and Gong, J. (2018). Implications of butyrate and its derivatives for gut health and animal production. Animal Nutrition 4: 151-159.

137. Ninwinska, B., Hanczakowska, E., Arciszewski, M.B., and Klebaniuk, R. (2017). Review: Exogenous butyrate: implications for the functional development of ruminal epithelium and calf performance. Animal,11(9): 1522-1530.

138. Boas, A.D.C.V., Budiño, F.E.L., Neto, M.A.T., Schmidt, A., Dadalt, J.C., Monferdini, R.P., Sitanaka, N.Y., Moraes, J.E., and Pizzolante, C.C. (2016). Organic acids in diets of weaned piglets: performance, digestibility and economical viability. Arq. Bras. Med. Vet. Zootec., 68(4): 1015-1022.

139. Zentek, J., Ferrara, F., Pieper, R., Tedin, L., Meyer, W. and Vahjen, W. (2013). Effects of dietary combinations of organic acids and medium chain fatty acids on the gastrointestinal microbial ecology and bacterial metabolites in the digestive trat of weaning piglets. Journal of Animal Science 91(7): 3200-3210.
140. Dahmer, P.L., Harrison, O.L. and Jones, C.K. (2022). Effects of formic acid and glycerol monolaurate on weanling pig growth performance, fecal consistency, fecal microbiota and serum immunity. Translational Animal Science 6: 1-11.

References in Chapter 6

141. United Nations Sustainable Development Platform (2013). United Nations.
142. Food and Agricultural Organisation of the United Nations (2011). Global Food Losses and Food Waste. FAO, Rome, 38 pp.
143. Institution of Mechanical Engineers (2013). Global Food Waste Not Want Not. IMECHE: 35 pp.
144. GPRI (Global Phosphorus Research Initiative) (2011). Sustainable Phosphorus Futures. http://phosphorusfutures.net/. Accessed 16th May 2014.
145. Roth, F.X., Windisch, W. and Kirchgessner, M. (1998a). Mineral metabolism (P, K, Mg, Zn, Mn Cu) and piglets supplied with potassium diformate. Agribiological Research 51(2): 1-8.
146. Roth, F.X., Windisch, W. and Kirchgessner, M. (1998b). Effect of potassium diformate on nitrogen metabolism and nutrient digestibility in piglets at graded dietary lysine levels. Agribiological Research 51: 167-175.

Acknowledgements

The first edition of this booklet was the result of the efforts and commitment of the members of the FEFANA Working Group Organic Acids.

The second, revised and extended edition of this booklet was facilitated by the help of the following experts: Christian Lückstädt, ADDCON; Sébastien Oguey, ADM; Harri Otto Miettinen, BASF; Elke von Hemendahl, BASF; Julia Trautwein, BASF; Liza van den Eede, EASTMAN; Tania Soenens, EASTMAN; Maja Sakkers, KEMIN; Sabina Díaz Martinez, NOVUS; Hans van Dam, TROUW NUTRITION (NUTRECO) (editor).

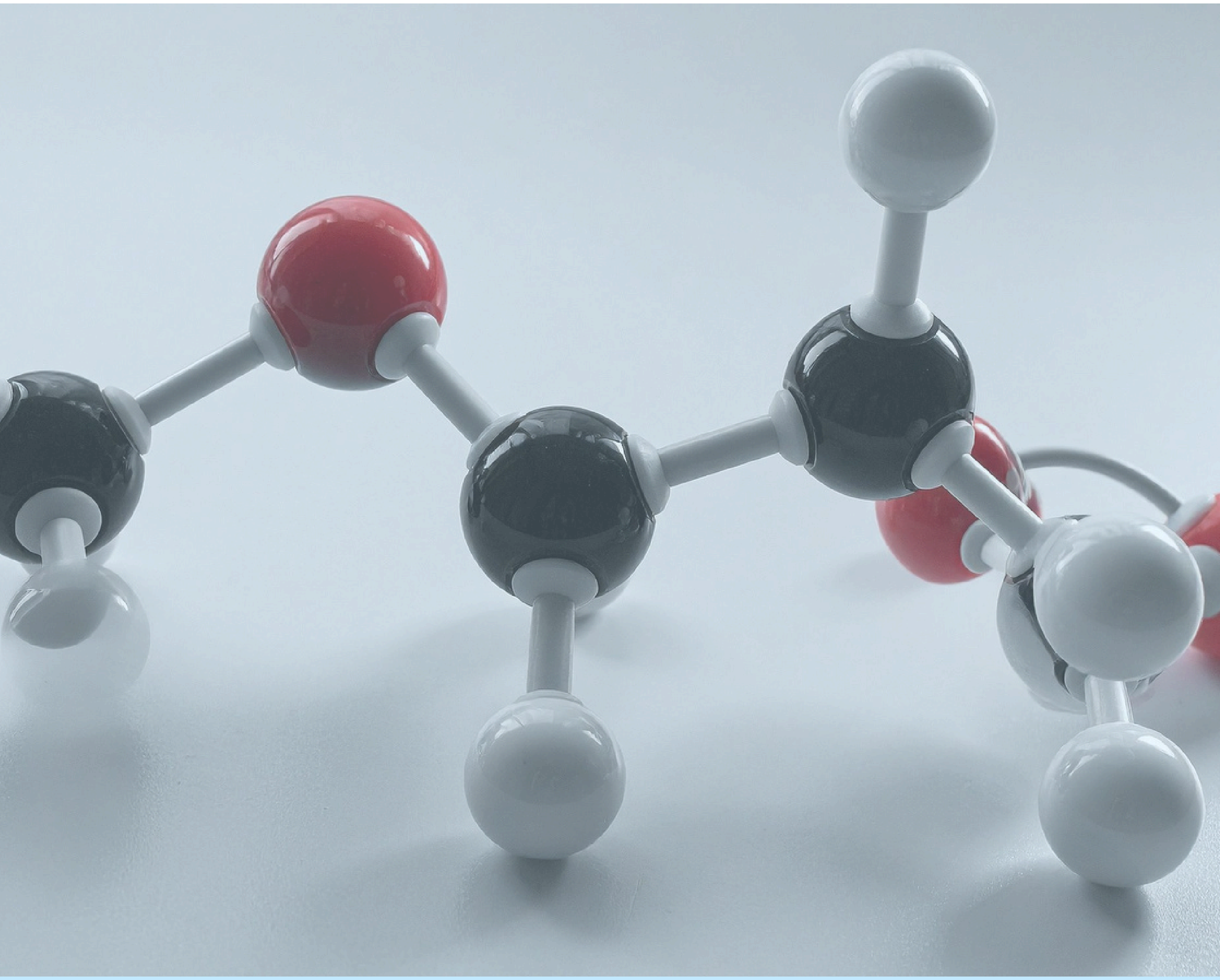
For the pictures courtesy of:
- ADDCON (figure 4);
- SELKO (figure 6).

Disclaimer

This booklet is intended to provide, to the best of our knowledge, basic information for anyone interested in gaining a better understanding of organic acids in animal nutrition. However, FEFANA accepts no responsibility for any use made of the information provided herein, whether by the general public or by any operator in the food and feed chain.
For more detailed information on specific uses, please refer to relevant technical documentation and safety data sheets provided by suppliers.

FEFANA

Specialty Feed Ingredients Industry



FEFANA asbl, Avenue des Arts 56, 1000 Brussels, Belgium

Website: fefana.org
Email: info@fefana.org
Tel: +32 (0)2 639 66 60

EU Transparency Register ID: 20132976103-18