

# Organic acids in animal nutrition



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## Preamble

FEFANA is the European Association of Specialty Feed Ingredients and their Mixtures. With over 100 member companies from 28 European countries, it represents business operators active throughout the feed chain, such as the specialty feed ingredient producers, pre-mixtures manufacturers, users, importers and distributors.

Established in 1963, FEFANA has loyally represented and served the interests of its industry ever since, and it is recognised as a representative partner to national and international authorities and fellow organizations in the feed and food chain.

Specialty Feed Ingredients and their Mixtures are essential constituents of animal feed, balancing the nutritional quality of the feed and hence contributing to animal health and welfare. Quality and safety being two main cornerstones, we also focus on innovation and sustainability, which we believe to be present and future key features of our business.

With a unique framework, the association is able to draw on the exceptional knowledge of our membership, bringing together expertise and science. This is why we are able to provide you with such a valid publication.

Organic acids have been used in animal nutrition for several decades and are a key part of ensuring feed preservation and safety. As an industry, major advances have been made in developing novel products and solutions to control moulds, fungi and bacteria in order to improve livestock production in a challenging global environment.

This booklet is the work of the FEFANA Working Group Organic Acids and gives an overview of the state-of-the-art knowledge on the use and application of these products in animal nutrition.

FEFANA hopes you find the information herein useful and practical.

**Didier Jans**  
FEFANA Secretary General



## Introduction

Welcome to the FEFANA booklet on organic acids - a true team effort on the part of the FEFANA Working Group Organic acids. The aim of this working group is to promote, defend and represent the interests of the industry involved in the manufacture and marketing of organic acids, their salts and blends of these.

The booklet is meant for a broad public, including nutritionists, farmers, policy makers, NGO's, and researchers worldwide and intends to provide information on all relevant aspects of organic acids used in animal feed.

Organic acids and their salts can be envisaged as a multi-faceted diamond, representing many different functions in the feed and for the animal.

The booklet will highlight these functions as they have been proven by science, following a thorough introduction to the current organic acid market, the different types of manufacturing processes in place, and the EU regulatory framework.

The booklet will then describe potential synergies with other feed ingredients, the sustainability aspects of organic acids and finally, it will provide information on the safe handling and transport of organic acids.

The Working Group Organic Acids wish you a pleasant read and remain open to any suggestions or questions you may have.

**Hans van Dam**

Chairman Working Group Organic Acids

## 1. OVERVIEW OF THE EU ORGANIC ACID 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. Natural by-products of microbial fermentation, as well as occurring naturally in plants, organic acids have been used for thousands of years as food preservatives. This is why the industry has found them a natural choice and the general public perceives them as acceptable to optimise animal production [1]. They make a fundamental contribution to feed hygiene, as they suppress the growth of mould and bacterial pathogens, thus allowing better 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. This holds true for markets worldwide. The use of acidifiers has set standards in Europe and is expected to do so elsewhere, becoming a truly global opportunity, for pigs, poultry and aquaculture, for grain preservation and in addressing food and feed hygiene issues.

Organic acids are increasingly gaining worldwide acceptance in the animal nutrition industry. They have been used for more than 50 years to reduce bacterial growth and mould in feedstuffs and thus preserve hygienic quality. In feed legislation they are mainly registered as preservatives. However, due to their antimicrobial activity, organic acids and their salts not only help to preserve feed and silages, they are also effective in reducing bacterial content 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].

Health and performance-promoting effects have been demonstrated for a number of organic acids. Besides improving hygiene, the corresponding reduction in pathogen intake, stabilises the gut microflora eubiosis, while effects on feed digestion and absorption have been demonstrated in a number of studies. In animal husbandry, reduced feed conversion rates, improved daily gain and a reduced incidence of diarrhoea all contribute to enhanced economic return, through lower feed costs and reduced time to market weight [3].

As a group of chemicals, the organic acids are considered to be any organic carboxylic acid, including fatty acids and amino acids, with the general structure R-COOH. Common organic acids and their chemical structures and properties are shown in Table 1.

**Table 1**

Physico-chemical properties of some organic acids (after [4])<sup>1</sup>

Acid	Molecular formula	MM (g/mol)	Density (g/ml)	Phys. form	pKa	Solubility in water
Formic	HCOOH	46.03	1.22	liquid	3.75	∞
Acetic	CH <sub>3</sub> COOH	60.05	1.049	liquid	4.76	∞
Propionic	CH <sub>3</sub> CH <sub>2</sub> COOH	74.08	0.993	liquid	4.88	∞
Butyric	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> COOH	88.12	0.958	liquid	4.82	∞
Lactic	CH <sub>3</sub> CH(OH)COOH	90.08	1.206	liquid	3.83	v
Sorbic	CH <sub>3</sub> CH:CHCH:CHCOOH	112.14	1.204	liquid	4.76	s
Fumaric	COOHCH:CHCOOH	116.07	1.635	liquid	3.02	s
					4.38	
Malic	COOHCH <sub>2</sub> CH(OH)COOH	134.09		liquid	3.4	∞
					5.1	
Tartaric	COOHCH(OH)CH(OH)COOH	150.09	1.76	liquid	2.93	v
					4.23	
Citric	COOHCH <sub>2</sub> C(OH)(COOH)CH <sub>2</sub> COOH	192.14	1.665	solid	3.13	v
					4.76	
					6.4	

<sup>1</sup> 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, potassium diformate and ammonium formate) are widely used.

Their different strengths (in terms of their minimal inhibitory concentrations) and properties mean that it is very usual to find liquid blends of different acids.

Furthermore, for customer convenience, organic acids are available on the market in a variety of forms, mainly:

**Salts** - usually solids (except for example ammonium propionate and ammonium formate which are liquid), in order to minimise or improve some functional properties in the feed mill, such as corrosion, volatility and odour;

**Adsorbates** - liquid acids or mixtures of acids adsorbed onto a solid, inert substrate, usually silica or vermiculite.

Organic acids are applied directly into feedstuffs and compound feed: liquid acids and blends are sprayed onto the feed whereas solid acids and acid salts are added directly or via special premixtures. Organic acids can also be applied to silages and included in drinking water.

In 2010, a market report from *Global Industry Analysts* [5] indicated that the European market for feed acidifiers is the fastest growing feed additives market in the world, predicting growth of 6.6% between 2008 and 2012 and passing the €375 million mark in 2012. More recently, the same source reported that the global market for feed acidifiers is projected to reach €1.0 billion by 2015, driven by increasing demand in developing economies, stable demand for meat and meat products from developed economies and, of course, a swelling world population.

Per volume, the most commonly used organic acids in feed are propionic, fumaric, formic and lactic acid, with an overall Compound Annual Growth Rate (CAGR) of 4.5% (Table 2).

**Table 2**

Current world-wide market value for selected organic acids in animal nutrition

Organic acids and their salts	Current market value ( M€ )	EU % of world-wide demand
<b>TOTAL market</b>	<b>1.04</b>	<b>41%</b>
Formic	210	67%
Fumaric	240	33%
Lactic	170	36%
Propionic	330	30%
Other acids*	100	42%

\*Includes benzoic, butyric, sorbic, malic and citric acids; and their salts.

Within this picture, Europe represents the biggest market share, given its state of the art and cutting edge development in animal health and feed conversion rates. Table 3 and Figure 1 present the global market perspective of organic acids in € (Table 3 and Figure 1).

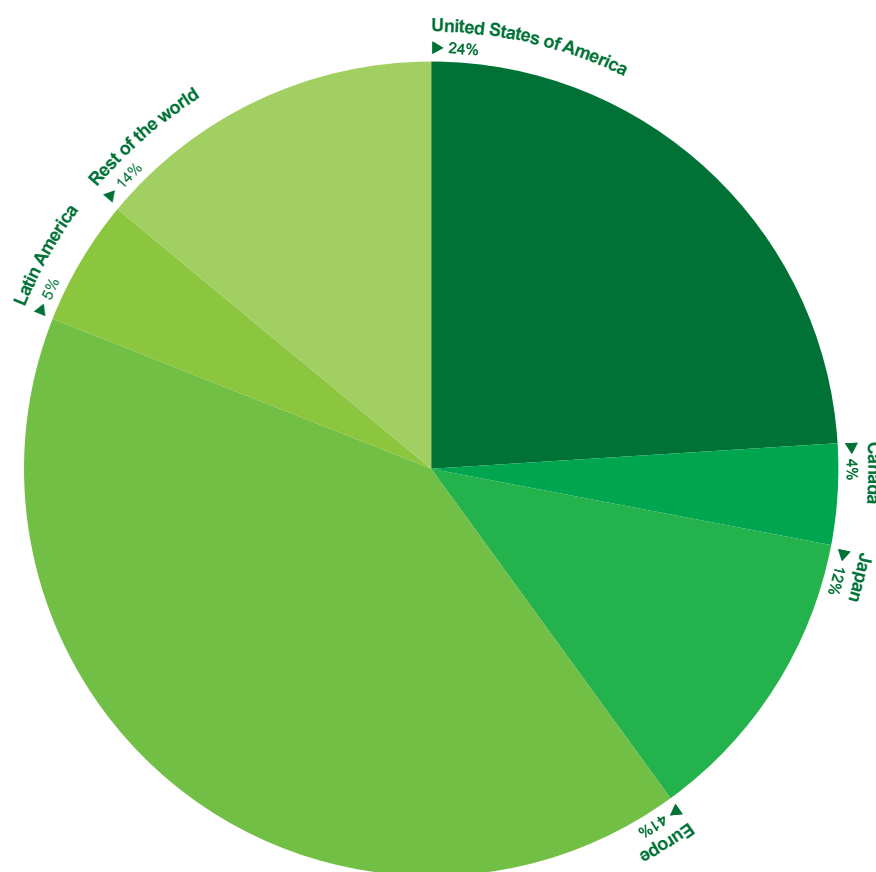
**Table 3**

Actual and projected market values of organic acids in animal nutrition by region (actual and projected values in mio €\*)

Region/country	2007	2013	2015	CAGR %
US	181	231	252	4.2
Japan	91	113	122	3.8
Europe	283	387	431	5.4
Latin America	42	53	58	4.0
Rest of the world	113	139	150	3.6
<b>Total</b>	<b>710</b>	<b>923</b>	<b>1013</b>	<b>4.5</b>

\* €-values derived from amounts in USD, with a mean exchange rate of 1.33\$/€ calculated over 2013. Source: [5]





**Figure 1** Regional importance of organic acids in animal nutrition (2013)

Our understanding of the modes of action of organic acids in the gastrointestinal tract has grown significantly in recent decades. The demonstrated modes of action of organic acids are listed in Table 4.

**Table 4**

Modes of action of organic acids and their salts (after [6])

Area targeted		Mechanism
Feed		Decrease in pH
		Antimicrobial effect (bacteria, yeast, fungi)
GIT <sup>1</sup>	Proton	Reduced buffering capacity of feed
		Decreases pH in the stomach
	Anion	Increases efficiency of pepsin (pH optima of 2.5 and 3.5)
		Antimicrobial effect
Intermediary metabolism		Antimicrobial effect
		Complexing agent (Ca <sup>2+</sup> , Mg <sup>2+</sup> , Fe <sup>2+</sup> etc.)
		Energy source

<sup>1</sup>GIT : Gastrointestinal tract.

The inclusion of organic acids and their salts 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.

More recently, the use of acids has been adopted by the poultry and aquaculture industries. The most recent developments also include combinations of organic acids with other synergistic agents such as essential oils and enzymes.

## 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 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.

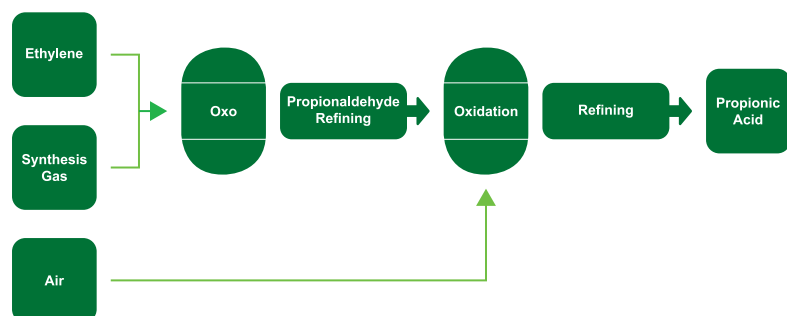
### 2.1 Production of organic acids by chemical synthesis

The chemical synthesis of organic acids is a long established process performed in bulk for several industrial applications, including the use as feed and silage additives.

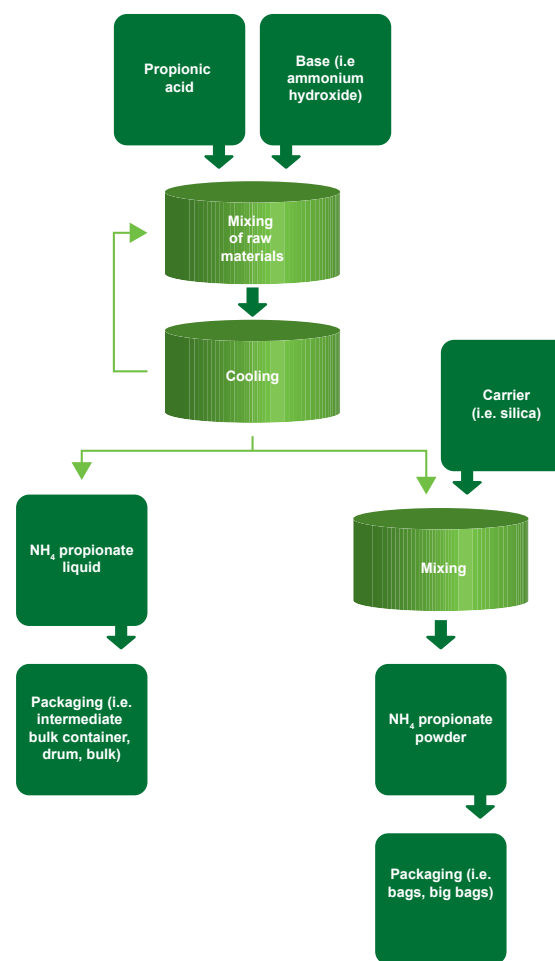
The production process of organic acids includes a reaction of the raw materials followed, if necessary, by separation of the acid from other fractions and a final distillation, to yield the acid at the required concentration. Examples of organic acids produced by chemical synthesis are formic acid, acetic acid, propionic acid, sorbic acid and fumaric acid.

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

#### Oxo Propionic Acid Process



**Figure 2** Production of propionic acid



**Figure 3**

Production of the ammonium salt of propionic acid

Neutralisation can be complete (e.g., calcium and sodium salts) but also partial, resulting in a buffered liquid (e.g. ammonium and sodium salts). An exception to this is the 50% buffered formate products, which are crystalline (e.g. potassium diformate).

## 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 yogurt. Examples of organic acids resulting from fermentation are lactic and citric acids.

The following materials are generally required for the production of organic acids by fermentation:

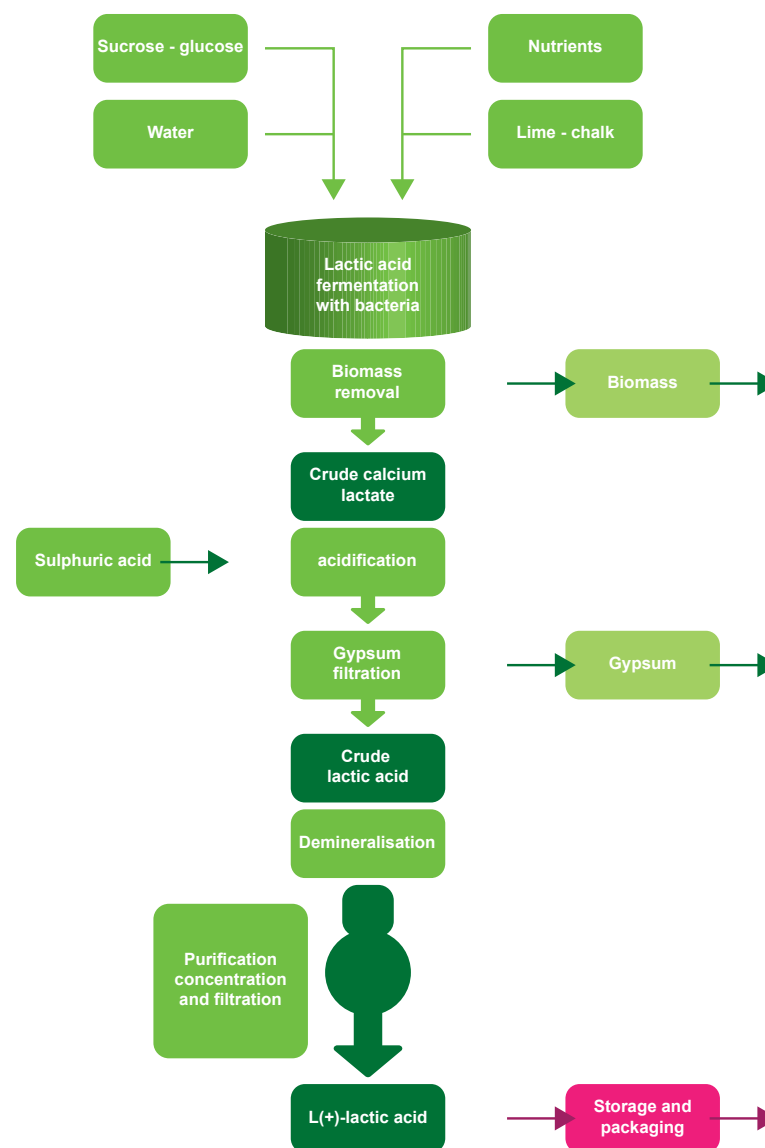
- Substrates/raw materials;
- Nutrients for the growth of bacteria;
- Neutralising agents;
- Micro-organisms.

### 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 4**  
Production of lactic acid

### Fermentation

An aqueous solution of sugar/carbohydrates is added together 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. In order to stabilise the pH, calcium hydroxide (lime) is added.

### Biomass removal

When fermentation is finished, biomass is removed from the broth by filtration (it is 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 added, producing gypsum (calcium sulphate) and lactic acid.

### Gypsum filtration

Gypsum is then separated from the broth by filtration (gypsum is used in the building industry among others).

### Demineralisation

At this stage, the lactic acid obtained 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 premixes can be packaged in pails, drums, Intermediate Bulk Containers (IBC) or bulk.

### 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 a number of reasons why protection of organic acids is of interest for the animal nutrition industry. The right delivery system for the right product is essential in adding value to the product, not only in terms of its benefits, but also for ease of use for the customer in the feed mill. In order 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, which lead to different manufacturing processes, but currently, many of these methods are closely guarded by the specific companies manufacturing these types of products.

## 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 often 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 take into account the inherent corrosiveness of the various organic acids and their salts.

## 3. ORGANIC ACIDS AND THEIR SALTS IN FEED LEGISLATION

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

- Promote safety of feed and food, of animal health and the environment;
- Promote consumers' rights 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

In order 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 animal, the consumer, the factory worker and the environment by the European Food Safety Authority (EFSA). The additive can only be authorised after a positive evaluation. 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 limit in feed, restrictions to the possible routes of application (feed, water), or specific provisions for more sensitive animal species.

Regarding those organic acid salts that are currently listed as a feed material, in general, we recommend that the conditions of use should be in line with those stated in the authorisations of the intact acid (or equivalent molar basis).

As will be further elaborated in Chapter 7, some organic acids are corrosive or irritant and are consequently classified under 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 also apply.

### 3.2 Information to consumers

The consumers' right to information on the feed products they acquire 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 ('labelling').

### 3.3 Efficacy of organic acids and salts

The proof of efficacy of organic acids and their salts is covered in the registration procedure set out within the Feed Additive Regulation and, where organic acid salts are used 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 the Additive Regulation). Organic acids are registered within a number of 'Functional Groups':

- **Preservatives**  
Protect feed against deterioration caused by micro-organisms or their metabolites;
- **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.

Registration within 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 marketed on the basis of this functionality. The **EU Register of Feed Additives**<sup>1</sup> provides an up-to-date overview of authorised feed additives in the various functional groups.

The so-called 'Zootechnical Additives' deserve special attention in this context. According to EU-legislation, 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, pro-

<sup>1</sup> [http://ec.europa.eu/food/food/animalnutrition/feedadditives/comm\\_register\\_feed\\_additives\\_1831-03.pdf](http://ec.europa.eu/food/food/animalnutrition/feedadditives/comm_register_feed_additives_1831-03.pdf)

tected citric and sorbic acids (including phytochemicals) and sodium benzoate. Most of these additives are registered for use in pigs, while only one preparation can be used in poultry. Furthermore, registration of all these compounds are holder specific and can therefore only be placed on the market by the producer/registration holder or by licensed, authorised companies.

As a general rule, the minimum amount of evidence that is needed for sufficient proof of efficacy is 3 trials 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. In contrast to the procedure for feed additives, the evidence for efficacy of organic acid salts classified as feed materials is assessed by the competent authorities of the EU Member States instead of by EFSA and does not need to be done prior to marketing. 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.

### 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 Additive Regulation (Reg. (EC) No. 1831/2003), whereas those of feed materials and compound feeds can be found in the Marketing of Feed Regulation (Reg. (EC) No 767/2009). The main difference between these two legal frameworks 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 salts used 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.

The relevant conditions of use (such as inclusion rate per target species and including 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 apply for those additives and premixtures that are classified as corrosive or irritant.

### 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 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, or the partial waiver on compositional information on the compound feed label. 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. As European manufacturers of organic acids and their salts, we continually strive to be such professional partners to our customers.

#### 4. ROLES OF ORGANIC ACIDS IN ANIMAL NUTRITION AND THEIR MODES OF ACTION

##### **Disclaimer**

*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 a given jurisdiction defines whether a certain application is approved for use in feed.*

Organic acids have been investigated in 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, a sign 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 encapsulation.

This has yielded a wealth of data on the functionality of organic acids in the feed and in the animal. It gives an insight in 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' 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 and the gastrointestinal tract. Improvements in feed hygiene and digestion consequently lead to improved zootechnical performance (4.2 to 4.6);

### Section B - Effect of organic acids on zootechnical performance

Extensive scientific research has led to a number of improvements in zootechnical performance (4.7 and 4.8).

#### 4.1 General modes of action of organic acids

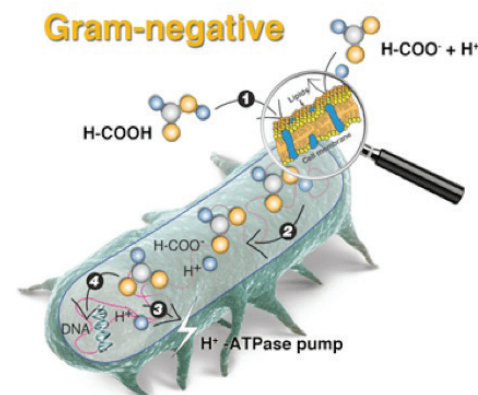
For many applications, efficiency of organic acids is determined by the same underlying modes of action. These are mainly based on the antimicrobial effects of organic acids on microorganisms and the beneficial effects that this triggers.

The mode of action of organic acids appears to be the same regardless 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 pKa value and the ambient pH level. 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. 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 in the GI tract, providing evidence for the mode of action described above. Consequently, organic acids may still display efficacy where strong, inorganic acids (that only reduce pH and cannot enter the cell) do not.

This process is depicted in Figure 5.



**Figure 5**

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 more simple 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 pass easily across. This explains why formic acid is more effective as an antibacterial but less so as a mould inhibitor.

As a consequence 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 and their salts against various microbial species is 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 drenched into 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.

## Section A

### Effects of organic acids in animal nutrition

#### 4.2 Preservation and mould inhibition

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 the spores which allow the mould to reproduce and also give the 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, mould growth will occur at higher moisture levels, with temperatures between 20 and 30 °C. However, some species have adapted to grow at lower or higher temperatures in order 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 in order 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: 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 any mould controlling agent already present to prevent mould growth should these optimal 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 5.

**Table 5**

Effect of mould contamination on nutrient content of grain (in [7])

Grain type	Moisture content (in %)	Mould contamination	Fat content (%)
Maize	13.0	No	3.8
	15.1	Yes	2.4
Sorghum	12.1	No	2.5
	15.0	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 in itself due to a much-reduced energy content of the grain (Table 6).

**Table 6**

Effect of maize quality on performance of young chicks [8]

Maize quality	Weight gain (g/3 weeks)	Feed Conversion Ratio	Metabolisable Energy (MJ/kg)
Good	738	1.80	11.5
Mouldy	612	2.15	10.7

Tables 5 and 6 clearly show the need for mould control in feeds and feed ingredients. The products typically added 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 in order 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 commonly used products for mould inhibition are acetic acid, propionic acid and its salts and sorbic acid.

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

Enterobacteria are also an issue which, although not visible, can affect the feed quality, potentially reducing feed intake and animal performance.

#### 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 of these products have a place, 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 individual acids. The use of a powder product in this situation would be virtually useless, due to difficulty in application and dispersion and slower fungicidal rates. However, a powder product is more suitable for addition into compound feed where it can be more easily mixed and dispersed.

Buffered products are often favoured over un-buffered acids as they are easier to handle, having a more neutral pH and reduced corrosiveness. Propionic acid is corrosive to both machinery and

operators and requires special and careful handling. Buffered acids also favour release of the acid over time, giving a longer period of protection which is useful for feeds and feed ingredients which are likely to be stored for a period of time.

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 contain them, however, and it is down to individual choice as to whether or not they are included on a continual basis as an insurance policy against the detrimental effects of moulds, bacteria and yeasts.

### 4.3 Reduction of pathogenic bacteria in feed

There is a growing interest in organic acids with respect to food safety, especially for reducing *Salmonella* shed and spread. Dietary acidification with organic acids has been shown to contribute to environmental hygiene, protecting feed raw materials and compound feed from zoonotic agents like *Salmonella*. Moreover, constant treatment with organic acids has a residual protective effect in feed, which helps to reduce recontamination and also to reduce the contamination of milling and feeding equipment. In poultry production, organic acids have mainly been used to sanitise the feed, considering problems with *Salmonella* infections [9, 10, 11, 12].

#### 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 sanitisation of the feed or water and also the possibility of modifying the gut microflora. Single acids and acid blends have been used in poultry diets to improve feed efficiency and also to reduce *Salmonella* contamination in feed and this approach has been extended to treatment of drinking water. Selection of a single acid or an acid blend, however, requires an understanding of the mode of action of the acids in order 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 treatments varies between different feed materials. The types of feed materials which might be contaminated are:

- Feed materials (raw material - single commodity)  
raw material treatment;
- Compound feedingstuffs (complementary feed and complete feed)  
mash feed treatment; pelleted feed treatment.

The efficacy of the acid treatments (formic acid and different blends of formic acid, propionic acid and sodium formate) varies significantly between different feed materials [13]. The strongest reduction was seen in pelleted and compound mash feed (2.5 log<sub>10</sub> reduction) followed by rapeseed meal (1 log<sub>10</sub> reduction) after five days of exposure. However, in soybean meal, the acid effects were limited (less than 0.5 log<sub>10</sub> 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 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 in *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<sub>10</sub> 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* sp. 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 min, while the death of 90% of the microor-

ganisms was achieved after the 3 hour incubation with 0.5-0.7 mol/l of the acid. In another experiment, propionate 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 the 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 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 of dairy farmers to optimise crop utilisation and dairy cow feeding programs [23].

The principle of silage production dates back 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 with 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 im-

proved 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 to live either with or without oxygen. The most dominant group of facultative aerobic bacteria is the Enterobacteriaceae, which are capable of fermenting sugars and converting 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 and the drop in pH levels in the silage favour the growth of lactobacilli, strictly anaerobic bacteria.

### Phase 3 - Lactic acid fermentation phase

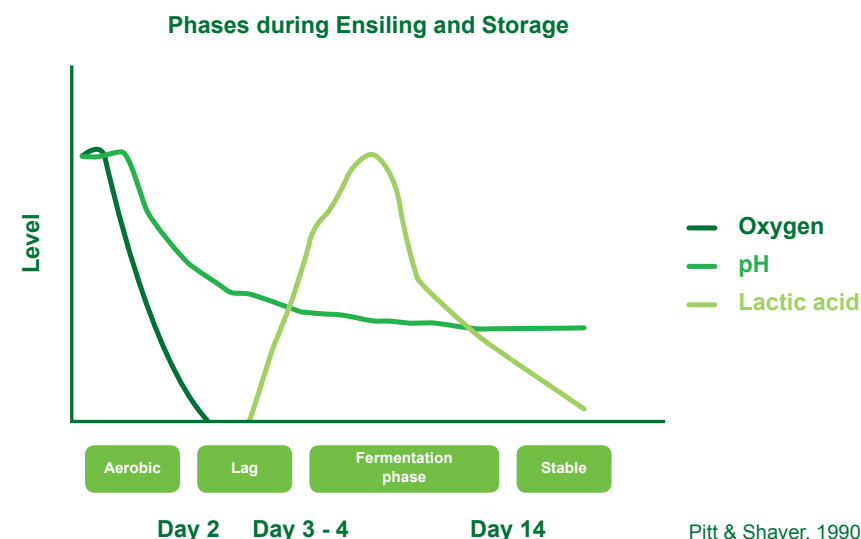
In the third phase, lactic acid fermentation takes the place of Enterobacteria fermentation. The most dominant species are now the Lactobacilli, of which some are homo-fermentative and others are hetero-fermentative. From the perspective of silage preservation, the first category is preferred as they 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 [27]. Wilting grass prior to ensiling seems to increase the number of lactic acid producing bacteria [32].

### Phase 4 - Stable phase

After approximately 2 weeks' time the pH of the silage has dropped to 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 si-

lages 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 6.



**Figure 6**

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 malfermentation 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 suboptimal 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 in order to maintain nutritional quality of the silage. This is usually caused by elevated microbial levels in the silage, e.g. of yeasts and moulds.

The amount of effluent coming from silage is another quality criteria; effluent involves 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 increase the utilisation of crude protein and energy from forages, mainly through changes in the solubilisation of protein and the fermentation of soluble sugars into volatile fatty acids (VFA's). In practice, proteolysis can only be restricted by a very rapid decrease in silage pH [36] ] or by applying heat during wilting [37, 38]. 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 [39, 37]. Preserving silage crude protein and readily available carbohydrates may stimulate microbial protein synthesis and, as a result, microbial fermentation in the rumen [40]. 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 in inhibiting proteolysis than natural fermentation as the pH of the silage is rapidly reduced [41]. Several trials 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 a total inhibition of plant protease activity and a significant inhibition 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 the silage and reduces spoilage of ammonia-nitrogen to the environment [42]. 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 [43]. In most experiments there was no significant difference between buffered and un-buffered organic acids in silage preservation [41]. 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 corn in silage clamps or egg bags [44]. 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 [45]. 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 used also in crimping technology to rapidly lower the pH of crimped grain to 4. Propionic acid can also be used as this is considered as the best preservative against mould growth [46, 47].

## 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 amount of harmful microorganisms in both the feed and gastrointestinal tract of animals.

### 4.5.1 What is an acidity regulator?

The ability of organic acids to reduce activity of harmful bacteria is among others related to ambient pH value and the pKa of the respective acid [48]. The pH measures the acidity of a solution ( $\text{pH} = -\log [\text{H}^+]$ ), which means that the higher the hydrogen ion ( $\text{H}^+$ ) concentration, the lower the pH value and therefore the more acidic the environment. The pKa-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 pH. If the ambient pH is higher than the pKa-value, more acid molecules will be dissociated, which results in quicker pH reduction. Therefore, acids with a low pKa-value will be more effective in reducing pH.

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 pH even at low doses, both in the feed and in the stomach. Acids with more than one proton can also be very efficient acidifiers, depending on the pKa-value of the second (and if present, third) proton.

### 4.5.2 Effect of acidification in the feed and gastrointestinal tract

Dietary organic acids act as antimicrobials in the 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 is outside this range, growth is disturbed. Therefore, organic acids can be applied to reduce pH in feed. In addition, organic acids are able to 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 count 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 monogastrics is low (2.5 - 4.0), as a result of hydrochloric acid (HCl) present in the stomach. The low pH inhibits the growth of harmful microorganisms and therefore reduces the number of microbes that can proceed to colonise the intestines. Gastric pH is higher in newly weaned piglets compared to older pigs. Therefore, these animals have a higher need for support, in decreasing stomach pH by including organic acids in the die [48]. Because of the low pH in the stomach, organic acids with a low pKa, such as formic, fumaric or lactic acids, are effective in reducing the pH in this environment. Furthermore, at the low pH of the stomach, 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. Therefore, acidification of the diet may also promote protein digestion, especially in young animals [51]. The level of pancreatic juice secreted by the pancreas is based on the pH value of the stomach content. When stomach content pH is lowered by the addition of organic acids in the diet, more pancreatic juice is produced. Since pancreatic juice contains digestive enzymes, breakdown of carbohydrates, proteins and fats will be further improved.

There is no evidence to date for organic acids reducing intestinal pH. However, many studies have shown positive effects of 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 pKa, 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 been 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 as a result 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 in-

creased availability of chloride for pepsinogen activation, improving protein digestion. Inorganic acids are sometimes added to organic acid blends at low doses, to make use of their strong acidifying ability.

## 4.6 Applications in drinking water

Water is the most crucial nutrient for livestock. Animals normally ingest at least twice as much water as feed. At higher environmental temperatures, water intake may be further increased. The high performance animals in modern animal production are increasingly less tolerant of stressors such as poor water quality. The application of organic acids to drinking water 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 controls undesirable microorganisms by reducing the pH, as well as through direct activity on the microorganism. The pH is reduced by the acid releasing protons (H<sup>+</sup>) 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 will be decreased rapidly, because of the quick release of protons. Buffered acids have a weaker effect on pH reduction. Buffered acids are acids mixed with a conjugate base. The conjugate base in this mixture neutralises released protons, therefore the pH reduction will be less compared with single acids. Internal data have shown that buffering acids leads to a higher final pH of a solution than when unbuffered acids are used. This is of importance in the acidification of drinking water, because when the pH 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 water pH. Therefore, using a synergistic blend of free and buffered acids is the most favourable strategy for product efficacy.

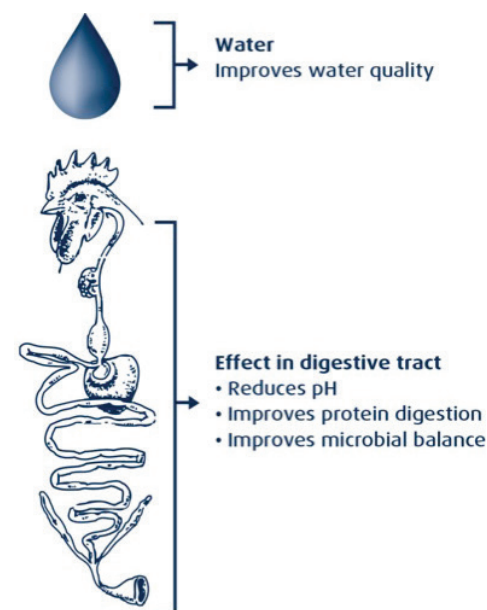
Acetic, lactic, propionic and formic acids are those mainly used as additives in drinking water [53]. Açıkgöz et al. [54] found that water pH was reduced from 7.4 to 4.5 when formic acid was added. Field results showed that a commercial product based on propionic acid eliminated Salmonella from the drinking water, while over 80% of samples in the control group were positive [53]. Another study showed that a combination of formic and propionic acids increased weight gain and improved feed conversion ratio in broilers [55]. A combination of organic acids with different pKa values is usually more effective in inhibiting microbial growth in water. Formic acid, for example, has a very low pKa (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 pKa. More molecules of the acids with a higher pKa 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 surface of the inside of 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 get blocked, restricting water intake by the animals and therefore negatively affecting growth and production performance and animal welfare. Products based on organic acids products can be applied through drinking water to reduce biofilm formation and therefore to reduce microbial uptake by the animal through this route.

#### 4.6.3 Effect in the digestive tract

The addition of organic acids to drinking water may aid in an antimicrobial strategy and support digestion in the digestive tract of the animal (Figure 7). Lower pH in the stomach, as a result 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 Salmonella 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 7**

Addition of organic acids to the water may aid in antimicrobial action and support of digestion in the digestive tract of the animal

#### 4.6.4 Supply of organic acids via water for drinking

In piglets, the stresses associated with weaning are known to disturb the balance of intestinal microflora and adversely affect gastrointestinal functions. 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 mature pigs feed intake will drop during a period of disease. They will, however, maintain water intake. Therefore, supplementing organic acids through water is a very useful strategy to control microbial uptake and to support gut health during periods of lower feed intake. The optimal pH value of drinking water to aim for is 3.8 - 4.2, since from within this range, the growth of Enterobacteria will be reduced.

In poultry, feed is usually withdrawn for several hours before slaughter in order 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 the drinking water 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 drinking water 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 drinking 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. Feed chain hygiene products based on organic acids are referred to by FEFANA as: "substances which favourably affect the hygiene of the feed chain by acting on the feed or water and/or in the animals by protecting against particularly harmful micro-organisms and/or their metabolites."

##### 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 a number of pathogens can be spread by feed, in particular *Salmonella* spp. and *Listeria monocytogenes*. Evidence for transmission of other pathogens via feed is scarce [59]. Although not all *Salmonella* serotypes isolated from feed are found to cause clinical disorders in animals, they may all be pathogenic to humans [59]. 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, in order 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

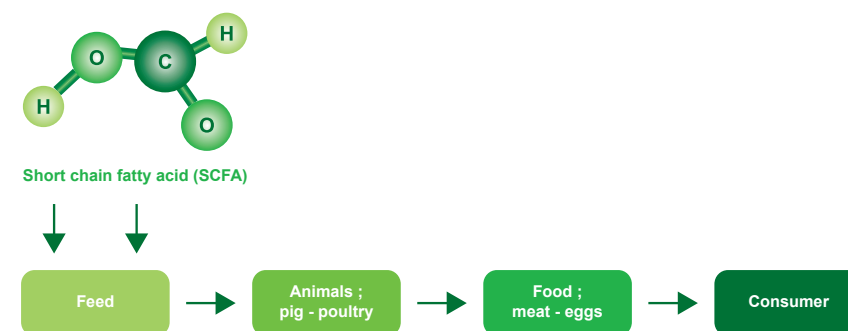
where they dissociate and disturb metabolism. Proliferation of pathogens is reduced, resulting in lower counts in the intestinal tract.

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 [60]. 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 [61]. It was found that lactic acid delayed the multiplication of an enterotoxigenic *E. coli* and reduced the mortality rate of animals [62]. Studies have also shown that various organic acids can reduce colonisation by *Salmonella* 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 [60]. Different studies have shown that caecal colonisation of *Salmonella* is reduced when butyric acid is added in the feed [63, 64]. Preventing initial *Salmonella* 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 [60].

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 8).



**Figure 8**

Organic acids support the control of bacteria in the total feed to food chain

## Section B

### Effect of organic acids on zootechnical performance

#### 4.8 Potential use in pig nutrition

In animal nutrition, acidifiers and their salts exert their performance-promoting effects via three different mechanisms: through the feed, in the gastro-intestinal tract (GIT) and on metabolism [65]. 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 [66] 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* [67], which may also have an impact on animal performance.

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

Partanen [68] evaluated the effects of dietary organic acids on performance (average daily gain, feed intake and feed:gain ratio) in weaned piglets and fattening pigs, through a meta-analysis of data collected from the literature. The findings are summarised in Table 7.

**Table 7**

Multifactorial analysis of the effect of organic acids and salts in piglets (adapted from [68])

	Formic acid	Fumaric acid	Citric acid	Potassium diformate
<b>Experiments/ Observations</b>	6 / 10	18 / 27	9 / 19	3 / 13
<b>Acid levels, g/kg feed</b>	3 - 18	5 - 25	5 - 25	4 - 24
<b>Feed intake, g/d</b>				
Control	667 ± 87	613 ± 148	534 ± 276	764 ± 9
Experimental	719 ± 75	614 ± 152	528 ± 302	823 ± 38
<b>Weight gain, g/d</b>				
Control	387 ± 65	358 ± 99	382 ± 121	479 ± 4
Experimental	428 ± 62	374 ± 101	396 ± 127	536 ± 26
<b>Feed to gain, kg/kg</b>				
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
<b>Unbiased effect size, d</b>				
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	0.46a ± 0.16	-0.08b ± 0.10	-0.20b ± 0.13	0.59a ± 0.14
P ≤*	0.01	0.42	0.14	0.001

\*Probability values for a comparison with the control diet.

<sup>a,b</sup> 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. [69]. In 2008, it was applied to a dataset collected from all available published material on the use of acidifiers in pigs [70], using nearly 500 studies that had been published on the use of 158 acids and their salts. The dataset used for the 'Acipig' model covers the results of all the published, negatively controlled trials 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 negatively controlled trials 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 acids in pig diets improves the productivity parameters of greatest importance to economic success. The magnitudes of the improvements were: 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 [71], 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, indi-

cates beneficial physiological effects within the pig that improve feed component digestibility and availability.

#### 4.9 Potential use in aquaculture

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. As far as we know, only a few studies have been produced on the use of organic acids in aquafeeds. One of the earliest studies on the application of organic acids in aquaculture appeared in 1981. Rungruangsak and Utne [72] tested formic acid preserved fish silage in diets for rainbow trout, though with limited success. Since then, several studies have reported that some organic acids, particularly citric and formic acids and their salts can improve growth, feed utilisation, mineral availability and disease resistance in aquatic species, including such economically important fish as salmonids, tilapia, catfish and shrimp.

This chapter gives a brief highlight of the application of organic acids in aquaculture, covering the economically most relevant fish groups, salmonids and tilapia, as covered in previous, extensive reviews [73, 74].

### 4.9.1 Salmonids

A study with rainbow trout showed that the apparent digestibility of phosphorus could be significantly increased in fish that were fed a fishmeal-based diet supplemented with 10 ml/kg formic acid. Magnesium and calcium digestibility also increased with the addition of formic acid (4 or 10 ml/kg). Another trial comparing the growth of trout fed diets supplemented with 0, 0.5, 1.0 and 1.5% of an organic acid blend (sorbic acid and formic acid and its salt) or 40 mg/kg of flavomycin indicated that weight gain significantly increased in fish fed diets with the 1.0 or 1.5% acid blend. The growth of fish fed the antibiotic diet was similar to that of fish fed the 1.5% acid blend diet, but the latter tended to have better feed conversion ratio. In Arctic charr, supplementation of commercial diets with 1% Na-lactate or Na-acetate significantly improved weight gain and feed conversion ratio. Feed intake was not affected by inclusion of these compounds, but the addition of 1% sodium acetate improved the digestibility of protein and lipid. A more recent study with Atlantic salmon showed that inclusion of fish meal enriched with 0.8 or 1.4% potassium diformate (KDF) tended to improved growth. Other authors, investigating the effects of dietary acidification on the availability of minerals in fishmeal in rainbow trout, found that 50 g/kg citric acid significantly increased the apparent digestibility of Ca, P, Mg, Fe, Mn and Sr in those diets. Further reports particularly emphasised the significantly increased P retention, which indicates improved mineral retention in fish fed acidified diets.

### 4.9.2 Tilapia

Various concentrations of organic acids (citric, propionic, acetic and lactic acids) have been evaluated for their effects on the feeding behaviour of Nile tilapia. The results indicated that citric acid at concentrations of  $10^{-2}$  -  $10^{-6}$  M, propionic acid at  $10^{-4}$  -  $10^{-6}$  M and lactic acid at  $10^{-2}$  -  $10^{-5}$  M stimulated feeding activity. Comparing performance, diets supplemented with an organic acid/salt blend

(calcium formate, propionate, lactate and phosphate and citric acid) at 0, 0.5, 1.0 and 1.5% or 0.5% oxytetracycline showed no significant differences in weight gain or feed conversion among treatments, although the group fed the 1.5% acid/salt blend diet gained 11% more than the negative control. A feeding study to compare the effects of various dietary levels (0, 0.1, 0.2 or 0.3%) of a commercial organic acid blend and 0.2% formate in red hybrid tilapia showed that total faecal and adherent gut bacterial count, particularly *Aeromonas hydrophila*, significantly decreased in fish fed the organic acid blend or formate-containing diets and that the 0.3% organic acid blend was as effective as 0.2% formate. Cumulative mortality 15 days after a challenge with *Streptococcus agalactiae* was significantly reduced in fish fed diets supplemented with the organic acid blend or formate. Another study reported that formate at dietary levels of 0.2, 0.3 or 0.5% significantly improved growth and feed conversion in Nile tilapia. Mortality at 15 days post challenge with *Vibrio anguillarum* (challenge after 10 days of feeding) was lower in the group fed the formate-containing diets. Most recently, a study using 0, 0.3 and 0.5% sodium formate and formic acid (SFF) yielded a non-significant growth improvement in tilapia fed diets supplemented with 0.3 or 0.5% SFF. A similar trend was observed for feed conversion, where the value for the diet containing 0.3% SFF was significantly better than that of the control. Protein efficiency ratio and protein retention efficiency were also significantly improved with this dietary treatment.

### 4.9.3 From research to practice

Available information on the beneficial effects of dietary inclusion of organic acids, their salts or their combination on fish performance is still relatively scarce and appears to vary depending on many factors such as:

- Fish species, fish size or age;
- Type and levels of organic acids, salts or their combination;
- Composition and nutrient content of experimental diets;

- 
- Buffering capacity of dietary ingredients;
  - Culture and feeding management;
  - Water quality.

Despite the discrepancy among data of published studies, it appears that organic acids and/or their salts have potential as dietary supplements to improve growth performance, feed utilisation efficiency and nutrient digestibility of aquaculture species. However, as their use is much more recent than it is in poultry and especially in pig production, more research is needed to better understand the mechanisms underlying the potential beneficial effects of these compounds or their mixtures.





## 5. SYNERGY BETWEEN ORGANIC ACIDS AND OTHER FEED INGREDIENTS

Given that the mode of action of organic acids in monogastrics involves a reduction in pH 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. 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:

#### Reducing feed and gastric pH

(unfavourable growth conditions for Gram negative bacterial pathogens);

#### Favourable growth conditions for beneficial microflora

(lactobacilli, bifidobacteria);

#### Optimise pH for digestive enzymes

(especially in digestion of proteins and minerals; stimulate intestinal mucosal cell development and pancreatic enzyme secretion);

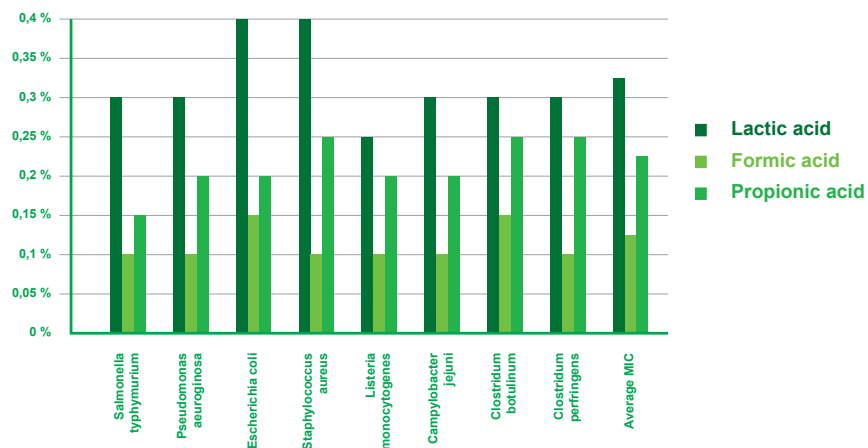
## Bactericidal/bacteriostatic effects on Gram negative bacterial pathogens

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 communality 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 Organic acid in combination with other organic acids

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



**Figure 9**

Minimum inhibitory concentrations (mic) (in %) of scfa (plot of [75]).

The use of blends of acids is common in order 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 [76], 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 [77]. Koyuncu et al. [78] 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. [79] 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 [80, 81]. The deduction can thus be made that formic and propionic acids act synergistically to reduce *E. coli* levels.

Chaveerach et al. [82] studied the effects of single and blends of different acids at various pH levels 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, clearly demonstrating a synergistic effect.

Further studies indicate synergistic effects of various organic acids, e.g. formic and propionic acids on broiler performance [83].

### 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 [84]. Phytases produced by solid-state fermentation tend to have lower pH optima compared to those produced by submerged fermentation methods, but all work better in an acidic environment. Therefore, the combined use of dietary acids with various phytases would be expected to confer benefits to all monogastric species.

Han et al. [85] 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 [86] 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 [87] 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 acids (acetic acid) combined with phytase in promoting improved mineral digestibility [88].

Similar effects have been reported in poultry. A study from Sacakli et al. [89] demonstrated that it may be possible to reduce the use of supplemental inorganic phosphorus in the diet by adding phytase and/or organic acids. Similarly, dietary microbial phytase and citric acid synergistically enhanced nutrient digestibility and growth performance in Indian carp [90]. A recent trout study with sodium diformate and phytase indicates similar results.

### 5.3.2 Combination with non-starch polysaccharidases and xylanase

Recent industry information from one of the leading organic acid producers as well as an enzyme supplier confirm that the com-

bination of non-starch polysaccharide degrading enzymes and acids may have at least an additive effect, since most of these enzymes prefer slightly acidic conditions in the gastrointestinal tract, which can be stabilised with a properly dosed organic acid.

### 5.3.3 Combination with plant extracts/essential oils

Essential oils 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 essential oils/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 zootechnicals, combine, for instance, benzoic acid and essential oils for poultry or protected citric and sorbic acids and phytochemicals, as previously discussed. Thus, their efficacy has been clearly demonstrated to EFSA with scientifically significant trials.

Langhout [91], for example, suggested that a combination of organic acids and essential oils 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 [92, 93, 94].

Other plant extracts such as saponins which act can as surfactants, may also affect the bacterial cell membrane, potentially enhancing the efficacy of organic acids.

The combination of essential oils with organic acids is now becoming popular with many products available on the market. Zheng et al., [95] 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., [96] 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 Medium Chain Fatty Acids (MCFA)

For a number of years, organic acids have been used regularly in combination with Medium Chain Fatty Acids (MCFA). MCFA have larger molecules compared to organic acids, which results in slower breakdown and uptake of the molecules. Therefore, these molecules will reach the lower part of the intestinal tract and can exert an antimicrobial effect, while organic acids are no longer present at the beginning of the small intestine. In addition, MCFA are known to affect the peptidoglycan layer of Gram positive bacteria, 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 organic acids and MCFA results in a synergetic effect, reducing both Gram negative and Gram positive bacterial counts. Further studies have shown that a combination of organic acids and MCFA in the diet results in improved weight gain and feed conversion ratio.

## 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 Analyses (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.

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 [97]. 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

To date, no single internationally recognised calculation model is available to assess the sustainability of organic acids in the feed to food chain. Various attempts have been made to develop an overall model that brings together all the relevant aspects of sustainability, but thus far there is no agreement among scientists about the correct presumptions or exact calculation rules. Therefore, we describe below the benefits of applying organic acids in the feed to food chain. This information may serve as inputs for future sustainability models or existing partitioned models that only partly relate to sustainability. 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 drinking water 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 [98]. 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 [99]. 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 [99]. 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 of feed and grain 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 drying, wet grain preservation with organic acids, such as propionic 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 acids 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 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 fatter 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, in a trial in broilers with a granulated premixture of protected nature identical flavouring compounds with benzoic acid, an improvement in FCR from 1.89 to 1.81 resulted in a feed saving of 180 g/bird. Similar results with protected acids are expected in aquaculture, which is currently the fastest growing meat production sector worldwide.

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 [100]. 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 [101] 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. [102] 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 feed-stuffs 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  $\text{-COOH}$ . Alcohols, with  $\text{-OH}$ , 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 salts are widely used substances and most of them have been assessed regarding their toxicological and eco-toxicological hazards according to REACH, Reg. (EC) No. 1907/2006. In connection with REACH, the Regulation on classification, labelling and packaging of substances and mixtures, Reg. (EC) No. 1272/2008, also known as CLP, aligns previous EU legislation on classification, labelling and packaging of chemicals to the GHS (Globally Harmonised System of Classification and Labelling of Chemicals). 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.

Since 2010, all substances marketed as such have to be classified and labelled according to GHS. Mixtures are granted a longer transition period and will have to be classified and labelled according to GHS from 1st June 2015.





## 7.1 Classification of most commonly used organic acids and salts

Within the EU, organic acids and their salts are classified according to the Dangerous Substances Directive 67/548/EEC (DSD) and according to the CLP of the purest forms or highest concentrations of substance. Some examples are shown of the classification under either legislation are given in Table 8; the information on all organic acids can be obtained from the FEFANA website<sup>2</sup>, which provides up-to-date data supported by the organic acid industry and is applied consistently. The information is taken from the European Chemicals Agency (ECHA) and is extracted from REACH registration dossiers. Where an organic acid is not yet registered according to REACH, the information is taken from the Classification and Labelling (C&L) Inventory of ECHA; this inventory is a compilation of all existing classifications of a substance. Where information from this C&L inventory is shown, the classification most often mentioned was taken.

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.

<sup>2</sup> [www.fefana.org/clp-ghs/](http://www.fefana.org/clp-ghs/)

**Table 8**

Examples of classification of organic acids and salts for their purest forms under eu legislation<sup>3</sup>

Chemical substance (minimum purity)	Classification under DSD 67/548/EEC	Classification under GHS/CLP Reg. (EC) No. 1272/2008
Citric acid (99%)	Indication of danger: Irritant, Xi R36 - Irritating to eyes	Signal word: Warning GHS07: Exclamation mark H319: Causes serious eye irritation.
Formic acid (99%)	Indication of danger: Corrosive R10 – Flammable R35 - Causes severe burns	Signal word: Danger GHS02: flame GHS05: corrosion H226: Flammable liquid and vapour. H314: Causes severe skin burns and eye damage.
Calcium diformate (98%)	Indication of danger: Irritant, Xi R41 - Risk of serious damage to eyes	Signal word: Danger GHS05: corrosion H318: Causes serious eye damage.
Lactic acid (80%)	Indication of danger: Irritant, Xi R38: Irritating to skin R41 - Risk of serious damage to eyes	Signal word: Danger GHS05: corrosion H315: Causes skin irritation H318: Causes serious eye damage.
Propionic acid (99%)	Indication of danger: Corrosive R34 - Causes burns	Signal word: Danger GHS02: flame GHS05: corrosion H226: Flammable liquid and vapour. H314: Causes severe skin burns and eye damage.

<sup>3</sup> See ECHA (<http://echa.europa.eu>); Information from registration dossiers or, if not available, from Classification and Labelling Inventory (most notified classification); September 2013.

As can be seen from the examples in Table 8, the salt of an organic acid is usually less corrosive in nature than its corresponding acid. 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. 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). In order to ensure consistency between all these regulatory systems, the United Nations developed mechanisms for transport conditions for all modes for transport (TDG).

The respective regulations are known by the following abbreviations:

**Transport by road**, ADR;

**Transport by rail**, RIS;

**Transport by sea**, IMDG;

**Transport by air**, IATA;

**Transport by inland waterway**, ADN.

The respective regulations are known by the following abbreviations:

All dangerous goods need to be classified so that all organisations in the supply chain, including the emergency authorities, know and understand exactly what the hazard is. 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 simply do not have a UN number.

Since most organic acids are corrosive or irritant in nature it is worthwhile to review the transport classification with regard to corrosiveness. CLP distinguishes between skin and eye corrosion and the transport regulations also include metal corrosion in the criteria. Table 9 shows the main rules for assigning a 'corrosive' classification to goods for transport.

**Table 9**

Main criteria for assigning adr classification for organic acids

Skin	Eye	Metal	ADR
Corrosive	Corrosive	Corrosive or not corrosive	Yes
Irritant	Corrosive	Corrosive	Yes
Irritant	Corrosive	Not corrosive	No
Irritant	Irritant	Corrosive	Yes
Irritant	Irritant	Not corrosive	No

Here, a clear relationship between moisture content and mould contamin

## Acronyms / Glossary

- AND: transport by inland waterway
- ADR: transport by road
- 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 Concentration Rate
- GHS: Globally Harmonised System of Classification and Labelling of Chemicals
- HACCP: Hazard Analysis and Critical Control Points
- IATA: transport by air/BC: Intermediate Bulk Containers
- IMDG: transport by sea
- LCA: Life Cycle Analysis
- MCFA: Medium Chain Fatty Acids
- MIC: Minimum Inhibitory Concentration
- PA: Propionic Acid
- REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals
- RIS: transport by rail
- SF: Sodium Formate

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