VETERINÁRIA NOTÍCIAS

REVIEW

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SHORT CHAIN ORGANIC ACIDS IN POULTRY: LITERATURE REVIEW

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Abstract

The importance of Brazil for the International Trade of poultry products induces legislators and interested companies to pay special attention to complying with international standard quality requirements. One of these requirements is the suppression of the indiscriminate use of antibiotics in poultry, as in the strategy of their use as a growth promoter. To meet this demand, the study of short-chain organic acids becomes interesting and their review essential. As good examples for the issue described, short chain organic acids such as citric acid, fumaric acid, sorbic acid and malic acid are extremely important due to their antimicrobial qualities, accessibility, and international acceptance. In addition to the individual effect, the blend of acids, that is, the association of acids for the role of growth promoter or performance enhancer, combines acids that act synergistically, enhancing their qualities. This study aims to address the physicochemical characteristics, effects,

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and qualities of short-chain organic acids, as well as their associated effects, encouraging further work that fills the scientific gaps in empirical knowledge about these additives.

KEYWORDS: fumaric acid, citric acid, malic acid

Resumo

O Brasil ocupa posição de destaque no cenário mundial de produção de proteína animal e devido a sua representatividade para o comércio internacional de produtos avícolas é imprescindível que o setor esteja empenhado em cumprir exigências de qualidade de padrão internacional. Uma dessas exigências é a supressão do uso indiscriminado de antibióticos na avicultura, como ocorre na estratégia de uso como promotor de crescimento. Para atender essa demanda o estudo dos ácidos orgânicos de cadeia curta se torna interessante e a revisão deles, essencial. Como bons exemplos para a questão descrita, os ácidos orgânicos de cadeia curta tal qual ácido cítrico, ácido fumárico, ácido sórbico e ácido málico são extremamente importantes devido às suas qualidades antimicrobianas, possibilidade de acesso e aceitação internacional. Além do efeito individual o blend de ácidos, ou seja, a associação de ácidos para a função de promotor de crescimento ou melhorador de desempenho combina ácidos que atuam sinergicamente potencializando suas qualidades. Este estudo objetiva abordar as características físico-químicas, efeitos e qualidades dos ácidos orgânicos de cadeia curta, bem como seu efeito associado, estimulando mais pesquisas sobre os reais efeitos desses compostos no organismo animal.

PALAVRAS-CHAVES: ácido fumárico, ácido cítrico, ácido málico

1. INTRODUCTION

Brazil is the largest exporter and one of the largest broiler producers in the world, in addition to being in the world ranking of consumer egg production (ABPA, 2021). As chicken is one of the most consumed proteins of animal origin in Brazil, it is important to maintain the quality of these products (ABPA, 2021). For distinct reasons, legislators in several countries have deliberately minimized and tried to control the use of antibiotics in poultry farming, as they represent a serious problem for the health of birds and, in particular, for the health of consumers. Therefore, the biggest challenge in poultry production is to explore the use of specific dietary supplements that allow the expression of their genetic potential for optimal performance.

The gastrointestinal tract of newborn chicks is immature and sterile. It starts to develop its function and its microflora when the bird starts to ingest food. At this time, the chick is very susceptible to pathogenic microorganisms. Under such circumstances, antimicrobial feed additives such as antibiotics are often used to suppress or eliminate harmful organisms in the intestine and to improve growth and feed efficiency. Organic acids, especially short-chain ones, reduce the pathogenic bacterial load in the digestive tract, improving animal performance, probably due to the direct effect of organic acids on the pH of the gastrointestinal tract, improving absorption by diffusion at the level of the intestinal epithelium (VINUS et al., 2017). There are effects of organic acids that are similar to (like) each other, such as the ability to acidify the food that generates several benefits, but some of these shared effects are more efficient and effective in some of them than in others.

After the ban on the use of antibiotics as growth promoters by the European Union (EU), Brazil as one of the largest chicken exporters in the world had to adapt to these measures, in this sense nutritionists and researchers tried other alternatives to improve the performance of poultry. One of these alternatives was the use of organic acids as food additives in poultry production (VINUS et al., 2017). Although in Brazil this ban is still partial (BRASIL, 2020), there is a tendency for this ban to become total, many companies have sought and made use of these additives (BEZERRA et al., 2017). Organic acids and their salts are considered safe and have been approved by most EU member states to be used as feed additives (VINUS et al., 2017; HABIBU et al., 2018). In this sense, this review aims to discuss the main organic acids used in poultry farming and demonstrate their benefits to poultry production.

2. CHARACTERIZATION AND PROPERTIES OF ORGANIC ACIDS

Organic acids are substances that contain one or more carboxyl in their molecular structure with a general structure R-COOH (MENTEN et al., 2014). Thus, short-chain fatty acids and some amino acids are considered organic acids, in addition to other substances that also fall under this classification (HAJATI, 2018). Short-chain organic acids are simple monocarboxylic acids, such as formic, acetic, propionic, and butyric acids or carboxylic acids with a hydroxyl group, such as lactic, malic, tartaric, and citric acids, or short-chain carboxylic acids containing double bonds such as acids fumaric and sorbic (MENTEN et al., 2014).

Not all of these acids have effects on the intestinal microflora. Organic acids with specific antimicrobial activity are short-chain acids (one to seven carbons) and are widely distributed in nature as normal constituents of plant or animal tissues (MENTEN et al., 2014). Short-chain organic acids such as citric, fumaric, sorbic, malic, formic, acetic, propionic, butyric, lactic, and tartaric acids are the most commonly used in poultry diets (KIM et al., 2015) in liquid or powder form, whose characterization is found in table 1. They are also formed by the microbial fermentation of carbohydrates, mainly in the large intestine. Sometimes they are found as their sodium, potassium, or calcium salts.

One of the important chemical properties of acids is the pKa calculated by -LogKa, with Ka being the acid dissociation constant. The pKa indicates whether a substance is easy to lose protons (DUTRA, 2021), which can be defined as the pH value of a solution, in which the concentration of the acidic species is equal to the basic one. So, the more acidic the substance (that is, the greater the Ka), the lower its pKa value (PREVIDELO et al., 2006) Most organic acids with antimicrobial activity have a pKa between 3 and 5 (MENTEN et al., 2014), that is, the pH at which 50% of the acid is in its dissociated form, which is its active form.

Table 1. Characterization of the most common short-chain organic acids in poultry feed.

Acid	Chemical name	Formula	pK_a
Citric	2-hydroxy-1,2,3-propanetricarboxylic acid	COOHCH2C(OH)(COOH)CH2COOH	3.13
Fumaric	2-butenedioic acid	COOHCH:CHCOOH	3.02
Sorbic	2,4-hexadienoic acid	CH3CH:CHCH:CHCOOH	4.76
Malic	Hydroxybutanedioic acid	COOHCH2CH(OH)COOH	3.40
Formic	Methanoic acid	HCOOH	3.75
Acetic	Ethanoic acid	CH3COOH	4.76
Propionic	Propanoic acid	CH3CH2COOH	4.88
Butyric	Butanoic acid	CH3CH2CH2COOH	4.82
Latic	3-hydroxypropanoic acid	CH3CH(OH)COOH	3.83
Tartaric	2,3-dihydroxybutanedioic acid	COOHCH(OH)CH(OH)COOH	2.93

Source: Adapted from Hajati, 2018.

Organic acids are defined as proton donors while bases are receptors. Weak acids and bases are those that do not remain ionized in an aqueous medium, they are common in biological systems and play an important role in the metabolism (MENTEN et al., 2014). The effect on gram-negative bacteria is greater if the organic acid is not dissociated. Due to this mode of action, it is necessary to associate organic acids with dissociation at different pH values, so that the antimicrobial action is prolonged over a wider pH range (LÜCKSTÄD et al., 2004). Furthermore, from a nutritional point of view, as the energy content of organic acids is fully available during metabolism, this can be taken into account when calculating the energy of the rations (Table 2).

Table 2. Energy content of short-chain organic acids.

Acido organic	EM (MJ/Kg)
Citric	10.29
Malic	9.79
Formic	11.34
Acetic	12.19
Propionic	17.78
Butyric	22.43
Latic	14.53

Source: Adapted from Hajati, 2018.

Organic acids have the potential to reduce stomach pH and can improve the digestibility of nutrients such as: proteins, calcium, phosphorus, magnesium, and zinc (LANGHOUT, 2000) and increase the activity of enzymes such as phytase which, associated with citric acid, can improve weight gain and aid bone mineralization in phosphorus-restricted chickens (VIEIRA et al., 2017). Protein digestibility can be improved in the presence of organic acids due to their acidifying effect on the diet, reducing stomach pH, thus increasing the action of pepsin on peptide digestion, reducing the rate of stomach emptying, increasing the digestion of peptides. In this sense, by improving the digestibility of phytic phosphorus (LANGHOUT, 2000) and proteins (VIEIRA et al., 2017), organic acids can reduce the excretion of phosphorus and nitrogen, mitigating the potential pollutant of excreta from birds. Furthermore, due to the ability of organic and inorganic acids to lower the pH of the diet, they can improve the conservation of ingredients and feed, reduce the proliferation of pathogens (MENTEN et al., 2014)

The potent antimicrobial action occurs through the dissociation of the acid within the bacterial cytoplasm, non-dissociated, lipophilic organic acids present in the intestinal lumen travel across the bacterial cell membrane by diffusion (DINABANDHU et al., 2009). Malic acid and acetic acid are low molecular weight hydrophilic organic acids and therefore can easily travel across the membrane and subsequently show considerable antimicrobial activity (ESWARANANDAM et al., 2004). Its antimicrobial activity is mainly attributed to its ability to lower the pH of the bacterial cytoplasm (MANI-LÓPEZ et al., 2012), which generally occurs more intensively in acidic foods. Under acidic conditions, the protonated form of organic acid is present in larger amounts. This protonated form can penetrate the bacterial cell membrane and subsequently dissociate into its more alkaline interior and reduce the pH, due to cationic release (H+) (VAN IMMERSEEL et al., 2006). Excess cations (H+) are expelled from the cell cytoplasm, leaving dissociated anions capable of interrupting enzymatic reactions and nutrient transport systems for the bacteria (DINABANDHU et al., 2009). This leads to substantial damage to cell cytoplasm and inhibition of the body's metabolic machinery without apparent damage to the cytoplasmic membrane (VAN IMMERSEEL et al., 2006). In addition to depressing the internal pH of the bacterial cell cytoplasm, it is postulated that undissociated organic acid interferes with substrate transport by affecting membrane permeability, chelating metal ions necessary for microbial growth, interrupting the flow of protons, and thus negatively affecting the energy production by the microorganism (ESWARANANDAM et al., 2004).

In poultry farming, organic acids have variable results, which according to Viola et al. (2008) is due to their physicochemical characteristics, mode of action, environmental condition, dose used, buffering capacity of the ingredients used in the rations and the responses evaluated. Associations between organic acids of different pKa ensure microbial action to a greater extent in the intestinal tract, as the pH changes throughout the gastrointestinal tract (3.5 to 4.0 in the crop and proventriculus, 6.4 in the duodenum, 6.6 in the jejunum and 7.2 in the ileum).

In addition to being used in the diet, organic acids are widely used in food preservation and can be applied in the meat industry. As an example of peracetic and lactic acids with an effect on the development of Salmonella spp. during the slaughter of broiler chickens. The latter showed an effect applied to chiller water artificially contaminated with Salmonella spp.. Although not allowed by Brazilian law, the use of lactic acid in chicken slaughterhouses is already a practice adopted in the United States of America (ZABOT et al., 2018).

3. CITRIC ACID

It is a low-cost, but efficient and effective organic acid, accepted in the European Union and in Brazil, with no restrictions on its use in food nationwide. (BRASIL, 2013). It acts directly in the feed, preventing bacterial deterioration and pathogenic bacteria in the gastrointestinal tract as it creates an acidic environment with a pH between 3.5 and 4.0 favoring the development of lactobacilli (CHOWDHURY et al., 2009). Consequently, it increases protein and fiber digestibility, improving weight gain, feed conversion efficiency and mineral adsorption. Reduces need for available phosphorus by making it more accessible (SHARIFUZZAMAN et al., 2020). This is due to the chemical reaction dissolution of phosphorus sources through citric acid, which is one of the best among organic and inorganic compounds for this function, both because of its ease of availability and its mineral stability. (SCHÜTZE et al., 2020).

By reducing the intestinal, cecal and gizzard pH in broiler chicks, citric acid reduces the pathogenic microbial load inducing a better immune response and can compensate the performance in broiler chickens by mitigating about 10% of the energy and protein level in the diet (SHARIFUZZAMAN et al., 2020). This is because citric acid is the final common oxidative pathway for carbohydrates, fats, and amino acids, therefore the most important metabolic pathway in providing energy to the body (AKRAM, 2014).

Regarding the use of citric acid (2%) in the feeding of quails challenged with Salmonella enteritidis, Colvara (2018) did not observe effects of supplementation on performance and obtained unsatisfactory results in relation to the peel, with lower thickness and percentage of peel, although the acid has been efficient in maintaining the intestinal morphology of birds. On the other hand, Vieira (2018), using meta-analytical techniques, showed that supplementation together with phytase and citric acid promoted greater weight gain and better bone mineralization in broilers than supplementation with phytase alone. Thus, in combination with phytase, it allows for a reduction in (to reduce) the inclusion of inorganic phosphorus with possible economic benefit.

4. FUMARIC ACID

Fumaric acid or dicarboxylic acid is present in all organisms and the exogenous is rapidly metabolized, not accumulating in animal tissues and products, however, as it participates in the tricarboxylic acid cycle in a dominant way, its addition to the diet can change the apparent metabolizable energy for broiler chickens (DUREK et al., 2014). For this production, food supplementation with fumaric acid increases weight gain and improves feed conversion of birds raised at neutral temperature (DING et al., 2020).

Supplementation with fumaric acid decreases the effects of poor performance of broilers at high temperatures, but this is not related to increased feed intake, but rather to a better feed conversion, resulting in greater daily weight gain. The improvement in weight gain is probably due to the bactericidal effect of this acid and the better energy metabolism (DING et al., 2020). In addition, there are more factors that can contribute to the results of organic acid application, such as the ingredients, their chemical properties, and the microbial load in the environment (KIM et al., 2015).

Fumaric acid supplementation (2%) in diets of quail (Coturnix coturnix japonica) challenged with Salmonella enteritidis, resulted in a reduction in bacterial excretion, improved egg quality, egg conversion and production, without affecting hen performance (COLVARA, 2018).

Pirgozliev et al. (2008) investigated the effect of fumaric acid on broilers fed a wheat-based diet. They observed that broilers fed the diet containing 1% fumaric acid had lower weight gain and feed efficiency ($P < 0.001$), while those fed with 1.5% fumaric acid had higher food efficiency ($P < 0.05$) compared to broilers that ate the control diet. Furthermore, there was a decrease ($P < 0.05$) in the number of microbial colonies when dietary organic acids were added to the feed. Broilers fed diets supplemented with organic acid had fewer coliform colonies than broilers fed the control diet ($P < 0.05$).

Fumaric acid improved the performance of broiler chickens under heat stress through hematological and biochemical regulation (DING et al., 2020). These authors observed an increase in final weight, average daily gain and production efficiency factor in birds fed a diet containing fumaric acid (5, 10 and 15 g/kg) exposed to heat stress when compared to the group fed a diet without the additive. The mortality rate of broilers submitted to high temperatures was reduced in the groups with 1.0% and 1.5% of fumaric acid supplementation when compared to the group without supplementation. In addition, the birds supplemented with fumaric acid had significantly higher erythrocyte counts, hemoglobin concentration and serum total protein, albumin, globulin, total cholesterol, and high-density lipoprotein cholesterol compared to the group subjected to heat stress. These results demonstrate that the improved growth performance may be related to better hematological parameters and serum biochemical states in broilers under heat stress through acid consumption.

5. MALIC ACID

Malic acid is an organic dicarboxylic acid with antimicrobial activity (OLAIMAT et al., 2018; REN et al., 2020). It can be produced industrially and has the advantage of not having a pollutant or residual effect and being little toxic (CHI et al., 2016). Malic acid is commonly recognized as a mild-tasting acid and has wide application in the food industry (CHI et al., 2016), which allows its application in animal feed to improve the performance of animals and the quality of their products (REN et al., 2020), without prejudice to feed palatability.

The antimicrobial effect of malic acid on Salmonella in chicken breast at 4°C was evidenced by Olaimat et al. (2018) resulting in complete elimination of the bacteria after 21 days of storage. However, the combination of malic acid and acetic acid was more effective in improving the safety of chicken meat. Related results were obtained by Fernández et al. (2020), who reported an effective reduction in Salmonella counts in chicken breast after immersion in a 3% malic acid solution for 15 seconds with minimal changes in chicken meat quality and sensory characteristics compared to control samples. Even malic acid (5 and 10%) proved to be efficient in preventing biofilm by *Salmonella enteritidis* at 12°C (MENEZES; MORAES, 2017).

Ren et al. (2020) observed that drinking water supplemented with malic acid for five days for slaughter-age birds reduced Campylobacter excretion by 1.05-1.55 log, suggesting that there is an effective reducing effect on the excretion of microorganism. Malic acid had no adverse effects on broiler performance, including body weight, intestinal indices, and intestinal microflora. However, it was found that the quality of the chicken meat was improved, with a significant increase in moisture content by 5.54% and a reduction in fat by 1.60%.

Supplementation with malic acid via drinking water for broiler chickens suffering from respiratory diseases showed a surprising result, improving survival rates by 20% in the treated group compared to the control group (REN et al., 2020).

6. ORGANIC ACID ASSOCIATIONS (blend)

The association between organic acids has also been shown to be beneficial in industrial poultry, reducing Salmonella excretion regardless of the administration route. Pickler et al. (2012) observed a reduction in the presence of Salmonella in the crop and cecum of infected broilers supplemented with two commercial mixtures of organic acids (lactic, fumaric, citric and formic) in the feed and fumaric and citric acids in drinking water in salmonella-infected birds.

On the other hand, Calaça et al., (2019) showed that the association between acetic acid (5%), formic acid (12%) and propionic acid (4%), replacing anticoccidials and antimicrobials, was beneficial in maintaining health intestinal tract of broiler chickens and in the control of *Salmonella enteritidis* e *Eimeria tenella*. There was a reduction in the frequency of bacterial isolation in lymphoid organs and in the number of oocysts excreted in feces and in the intestinal lesion scores caused by *E. tenella*.

Colvara (2018), using a blend of organic acids (propionic, lactic, and formic acids) in the diet of Japanese quails (*Coturnix coturnix japonica*) challenged with *Salmonella enteritidis*, observed less bacterial excretion and better egg quality without affecting bird performances, in which quails produced eggs with higher weight, higher percentage of albumen and better Haugh unit. Fouladi et al. (2018) showed that diets containing lactic acid alone or in combination with other acids reduced the population of Salmonella sp. in the gastrointestinal tract of Japanese quail with positive effects on bird performance and egg characteristics.

Literature has proven that organic acids are effective in controlling Salmonella (PICKLER et al., 2012; COLVARA, 2018; CALAÇA et al., 2019). This suggests that organic acids, by competitive exclusion by inducing the growth of beneficial bacteria, can reduce coliform counts and increase lactic acid bacteria in the intestinal microbiota (PIRGOZLIEV et al., 2008).

In addition to the antibacterial effect, formulations containing citric, malic, and phosphoric acids showed effective viricidal action, being able to inactivate the avian influenza virus in experimental dilutions of 200 and 50 times under conditions of hard water and organic matter, respectively. This demonstrates its use as a disinfectant to prevent the spread of viral diseases in animals (CHAE et al., 2018).

7. FINAL CONSIDERATIONS

Several studies have revealed the effects of short-chain organic acids on birds in their isolated form or in combination with other acids. The association of organic acids seems to be the most promising way to obtain effective results, especially as to the control of enterobacteria such as salmonella and replacement of antimicrobials.

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