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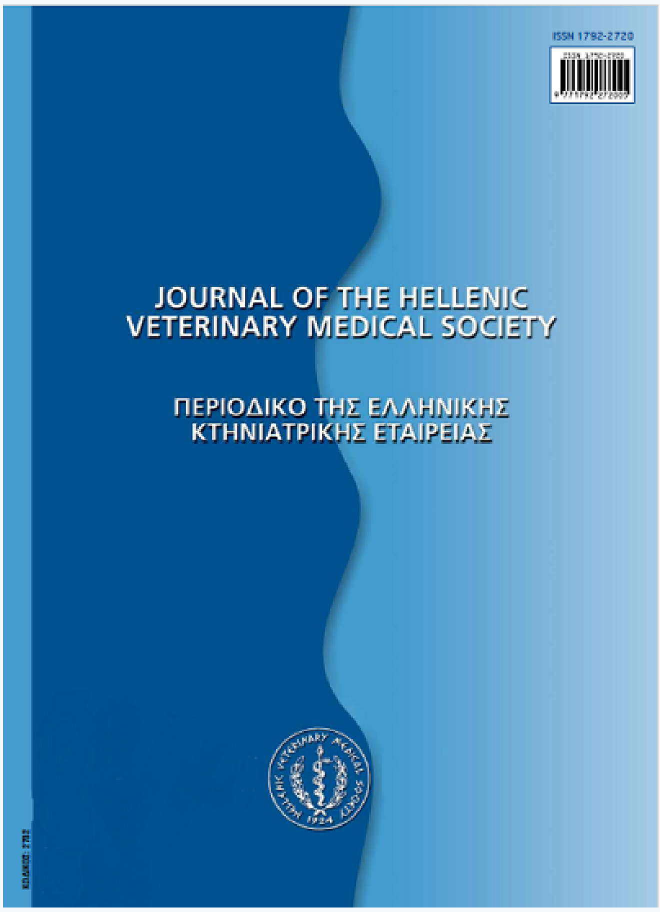

Η επίδραση των οργανικών οξέων στο οικοσύστημα του γαστρεντερικού σωλήνα και τις αποδόσεις των κρεοπαραγωγών ορνιθίων

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Review article
Ανασκόπηση

Effects of organic acids on the gut ecosystem and on the performance of broiler chickens

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ABSTRACT. Organic acids are studied as candidate alternatives to antibiotic growth promoters. Their action is related to the pH reduction of the intestinal digesta, affecting the gut ecosystem in numerous ways. Intestinal microbiota can be altered as a result of the remarkable antibacterial activity of organic acids and the growth enhancement of non-pathogenic beneficial micro-organisms, due to exclusive competition. Antibacterial activity has been widely reported for many poultry pathogens, such as *Salmonella* spp., *Escherichia coli*, *Clostridium perfringens*, *Campylobacter* spp., both *in vitro* and *in vivo*. However, it seems to depend on many factors concerning the weak acid used and the gut ecosystem. Apart from the microbiota, diet supplementation of organic acids has trophic effects on the intestinal mucosa, modifying the morphologic characteristics of intestinal villi and crypts and maintaining epithelial integrity. Furthermore, as found recently, organic acids have anti-inflammatory and immunostimulating properties. Diet acidification increases gastric proteolysis and the utilization of proteins and amino acids, affects pancreatic secretions and mineral absorption. There are also reports for an effect on appetite and palatability of the feed. All these properties attributed to organic acids have either a direct or indirect effect on the performance and health, even though the results presented for poultry lack consistency. Nonetheless, the benefits of organic acids can have practical application in the control of clinical and subclinical conditions, but more research is needed to study these perspectives.

Keywords: broiler chickens, gut ecosystem, organic acids, performance

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ΠΕΡΙΛΗΨΗ. Τα οργανικά οξέα αποτελούν σημαντικό πεδίο έρευνας ως διαιτητικά προσθετικά εναλλακτικά των αντιμικροβιακών αυξητικών παραγόντων. Ο μηχανισμός δράσης τους σχετίζεται με τη μείωση του pH του γαστρεντερικού σωλήνα, επηρεάζοντας ποικιλοτρόπως το οικοσύστημα του γαστρεντερικού σωλήνα. Διαθέτουν αξιοσημείωτες αντιβακτηριδιακές ιδιότητες που τροποποιούν την εντερική μικροχλωρίδα και προάγουν την ανάπτυξη των ωφέλιμων, μη παθογόνων μικροοργανισμών, λόγω ανταγωνιστικού αποκλεισμού. Η αντιβακτηριδιακή δράση των οξέων αυτών έχει μελετηθεί εκτενώς για αρκετά παθογόνα των πτηνών, όπως *Salmonella* spp., *Escherichia coli*, *Clostridium perfringens*, *Campylobacter* spp., τόσο *in vitro* όσο και *in vivo*. Ωστόσο, η παραπάνω δράση φαίνεται πως εξαρτάται από παράγοντες που σχετίζονται με το εκάστοτε χρησιμοποιούμενο οξύ και με το μικροπεριβάλλον του γαστρεντερικού σωλήνα. Εκτός από την επίδρασή τους στην εντερική μικροβιακή χλωρίδα, τα οργανικά οξέα αποτελούν πηγή ενέργειας για τα εντεροκύτταρα, επηρεάζουν τα μορφολογικά χαρακτηριστικά των λαχνών και των κρυπτών του επιθηλίου και συμβάλλουν στη διατήρηση της ακεραιότητας του εντερικού βλεννογόνου. Επιπλέον, πρόσφατα αποδόθηκαν στα οργανικά οξέα αντιφλεγμονώδεις και ανοσοδιεγερτικές ιδιότητες. Η προσθήκη των οργανικών οξέων στην τροφή ή το νερό αυξάνει τη γαστρική πρωτεόλυση και επομένως την αξιοποίηση των πρωτεϊνών και των αμινοξέων, επηρεάζει τις παγκρεατικές εκκρίσεις και την απορρόφηση των μακροστοιχείων και ιχνοστοιχείων. Υπάρχουν επίσης αναφορές περί επίδρασης στην όρεξη και τα οργανοληπτικά χαρακτηριστικά της τροφής. Το σύνολο των παραπάνω ιδιοτήτων έχει άμεσο ή έμμεσο αντίκτυπο στις αποδόσεις και την υγεία των πτηνών, αν και σε ορισμένες περιπτώσεις τα αποτελέσματα σχετικών ερευνών είναι αντικρουόμενα. Παρόλα αυτά, από πλευράς πρακτικής εφαρμογής, η πτηνοτροφία μπορεί να επωφεληθεί από τη χρήση οργανικών οξέων σε διάφορες κλινικές και υποκλινικές παθολογικές καταστάσεις, όμως απαιτείται περαιτέρω έρευνα για να εξετασθούν οι προοπτικές αυτές.

Λέξεις ευρετηρίασης: αποδόσεις, κρεοπαραγωγά ορνίθια, οικοσύστημα γαστρεντερικού σωλήνα, οργανικά οξέα.

INTRODUCTION

The removal of antibiotic growth promoters (AGPs) from poultry diets in the countries of the European Union in 2006 has led the researchers to reconsider the complexity of the gut ecosystem and the need to clarify the continuous interaction among the feed ingredients, the host and the intestinal microbiota, as well as to find alternatives to AGPs (Chowdhury et al., 2009; Houshmand et al., 2011). Among the candidate alternatives widely studied are the organic acids. As a group these compounds include the saturated straight-chained monocarboxylic acids and their respective derivatives (unsaturated, hydroxylic, phenolic and multicarboxylic versions) and are often generically referred to as fatty acids, volatile fatty acids, weak or carboxylic acids (Cherrington et al., 1991).

The use of organic acids as feed additives has a long history in the food preservation process, preventing food deterioration and extending the shelf life of perishable ingredients (Theron and Lues, 2011). In animal feed industry, they were originally added to serve as antifungals, whereas in poultry, they have also been examined for antibacterial activity against *Salmonella* spp. contaminated feed

(Dixon and Hamilton, 1981; Thompson and Hinton, 1997). The dietary acidification was found to resemble the effect of AGPs in the gastrointestinal tract of farm animals (Senkoylu et al., 2007), so, many studies, especially on swine, have focused on examining the effect and mode of action of organic acids added in the feed. In poultry production, organic acids have not gained as much attention as in swine production, because there is lack of consistency in the results and great variability in the performance (Dibner and Buttin, 2002). However, organic acids have made great contribution to the profitability in poultry production affecting the intestinal microbiota, the mucosa and immune system of the host, the protein digestibility, pancreatic secretion, mineral utilization and as a result, the performance(Adil et al., 2010).

These special properties of the organic acids as well as the practical perspectives of their use are the interesting aspects discussed in this review article.

ORGANIC ACIDS AND INTESTINAL MICROBIOTA

Intestinal microbiota producing organic acids

Bacterial genera, such as *Lactobacillus* spp., *Leuconostoc* spp., *Enterococcus* spp., *Pediococcus* spp., *Lactococcus* spp. produce lactic acid as the major metabolic end-product of carbohydrate fermentation and comprise the lactic-acid bacteria group. Strains of both *Lactobacillus* spp. and *Bifidobacterium* spp. are known as lactic-acid producing bacteria commonly used as probiotics. Lactic acid is a major component of bacterial fermentation and plays a key role in the metabolic pathway of bacteria (Floch, 2010). The main products are short-chain fatty acids (SCFAs). SCFAs, particularly propionate, acetate and butyrate, are produced in millimolar quantities in the gastrointestinal tract and characteristically occur in high concentrations in regions where strictly anaerobic microflora is predominant. Since only 10% of the chicken intestinal bacteria species have been characterized, the knowledge about the SCFAs producing microbiota is limited. However, the increasing interest in butyric-acid producing strains particularly has resulted in isolating a novel species from the chicken ceca, within a novel genus, for which the name *Butyricicoccus pullicaecorum* has been proposed (Eeckhaut et al., 2008).

Antibacterial activity of organic acids

Organic acids enter the gastrointestinal tract in their undissociated form. In this form they are lipid soluble and able to pass through the cell membrane of the bacterial cell. Once in the cytoplasm of the cell, the organic acids dissociate due to the alkaline environment and release protons (H+) that lower the pH of the cytoplasm. In an attempt to restore the balance, the bacterial cell increases the consumption of adenosine triphosphate (ATP), resulting in a great loss of energy (Paul et al., 2007). The anions released (RCOOH-) are responsible for less direct antibacterial activities such as damaging the cell membrane, causing leakage and interference in transport of nutrients and disrupting the synthesis of DNA and proteins (Alakomi et al., 2000; Davidson, 2001). However, the antibacterial result of adding an organic acid in the diet depends on many factors.

The pKa of the organic acid and the pH of the surrounding milieu

Organic acids are weak acids which mean that they can only be partly dissociated. In order to determine the pH value at which each organic acid is half dissociated, the term of pKa was introduced concerning every organic acid. pKa expresses the acidity of weak acids and along with pH, these values determine the amount of organic acid remaining in the undissociated form, capable of entering the bacterial cell. The antibacterial activity increases when pH reduces. Dibner and Buttin (2002) studied the antimicrobial activity of several organic acids at different pH values. At pH 7.3 little antimicrobial activity was observed whereas at pH 4 all acids had better activity against *Escherichia coli*.

The antimicrobial spectrum of each organic acid

Studies have shown that propionic acid has better antifungal properties than other acids, whereas lactic acid is more effective against bacteria. Though, formic acid has been reported to have a broader antibacterial spectrum (Partanen and Mroz, 1999; Haque et al., 2009). These differences are the reason why blends of organic acids are most commonly used in poultry feed. However, despite the fact that the organic acids spectrum has been widely studied for bacteria and some pathogenic fungi and yeast like *Aspergillus* spp. and *Candida albicans* respectively (Haque et al., 2009; Samanta et al., 2010), there is no available data for the effect of organic acids on poultry pathogenic protozoa like *Eimeria* spp., *Cryptosporidium* spp. and *Histomonas meleagridis*.

The bacterial mechanisms of resistance to organic acids

Russell (1992) claimed that some microorganisms are more resistant to organic acids because they are capable of allowing their internal pH to decline. Russell and Dien-Gonzalez (1998) attributed the resistance of Gram-positive bacteria to organic acids to higher intracellular potassium concentration that provides counteraction for the anions. Also, acid-tolerant bacteria, like *Lactobacillus* spp. and *Bifidobacterium* spp. seem to be growth promoted by short-chain fatty acids. That growth promoting effect was further confirmed using an organic acid blend of orthophosphoric, formic and propionic acid (Samanta et al., 2010). On the other hand, pathogenic Gram-negative bacteria, like *E.coli*, *Salmonella* spp. and *Campylobacter* spp. are acid-sensitive and therefore, much more affected by the weak acids. In spite of this fact, there is an emerging potential that acid-sensitive bacteria can adapt in an acidified environment, surviving the acid shock through the production of protective proteins (Foster, 2001).

The form of the organic acids

When ingested, organic acids disappear in the gastrointestinal tract, being unable to reach parts of the intestine where pathogens inhabit. Hume et al. (1993) demonstrated that most of the propionic acid originating from the treated feed is metabolized and absorbed in the foregut of the chicken (crop, gizzard and proventriculus) and does not reach the small intestine or the cecum in sufficient quantities to be effective. Organic acids have a strong antibacterial effect against *Salmonella* spp. and *E.coli* in the crop which is a major colonization site, but it is desirable to reach further down the intestinal tract in a sufficient concentration. van Immerseel et al. (2004) tried microencapsulation and coating of propionic, formic, acetic and butyric acid in micropearls to allow the slower and selective release of the acids in the intestine of young chickens. The same authors compared the efficacy of uncoated and coated butyric acid in controlling *Salmonella* colonization early after oral inoculation of SPF layer chickens with *Salmonella enteritidis*. Coated butyric acid significantly decreased caecal colonization 3 days after the oral challenge, while the powder form of butyric acid had no effect (van Immerseel et al., 2005). These results are in agreement with those of Fernandez-Rubio et al. (2009), who compared unprotected sodium butyrate and partially protected sodium butyrate for their efficacy against *S. enteritidis*. The partially protected form had a great effect even at the late phase of infection, remaining active all along the gastrointestinal tract. Thormar et al. (2006) reported greater bacterial inhibition when monoglycerides of fatty acids were used, because they are released only under the action of lipase in the small intestine.

Organic acids against important poultry pathogens

Many researchers have studied the effect of organic acids against *Salmonella spp*. in poultry. Formic acid alone or in combination with propionic acid at concentrations of 0.6 % managed to prevent *Salmonella gallinarum* infection (Berchieri and Barrow, 1996). The same combination had a bactericidal effect for *Salmonella enteritidis* when tested *in vitro* with hen's crop contents (Thompson and Hinton, 1997). In an experiment with broiler chickens, Izat et al. (1990) found reduced number of *Salmonella* spp. in caecal contents following addition of either 0.36% calcium formate or 0.5% formic acid. Waldroup et al. (1995), in contrast, found that formic and propionic acid blend, citric, lactic, fumaric acid in concentrations up to 2% offered no protection for *Salmonella tymphimurium* caecal colonisation. In the last decade, butyric acid was intensively studied for its role in *Salmonella* infections in poultry. van Immerseel et al. (2004) reported the decrease of *S. enteritidis* invasion in caecal epithelial cells in vitro after pretreating the cells with butyric acid. On the contrary, pretreatment with acetic acid resulted in increase of invasion. Invasion of intestinal epithelial cells is an important step in the pathogenesis of *Salmonella*-mediated enteritis and requires a set of genes encoded on the *Salmonella* pathogenicity island1 (SPI1). Gantois et al. (2006) managed to show that butyrate down-regulates SPI1 gene expression, enlightening one of the mechanisms causing reduced invasion. Fernandez-Rubio et al. (2009) studied the protective effect of sodium butyrate against *S. enteritidis* at gastrointestinal and systemic levels and found significantly reduced levels of colonization in the crop, the ceca and the liver.

E. coli was decreased with the inclusion of propionic acid in broilers feed (Izat et al., 1990). Samanta et al. (2010) reported a slight reduction of *E.coli* in broilers fed a blend of orthophosphoric, formic, propionic acid and calcium propionate in powder form for 35 days.

In an attempt to control Poult Enteritis and Mortality Syndrome of turkeys, where *E. coli* seems to play a key role, Roy et al. (2002) tried propionic acid as feed additive and observed the sporadic growth of *E. coli* type 1 and 0114 colonies, with the addition of 2.5% propionic acid.

Organic acids have also been tried to control *Campylobacter* spp. colonies. Chaveerach et al. (2002; 2004) reported that SCFAs as well as a commercial organic acid product were able to keep water free from *Campylobacter* spp. and decrease their number in the caecal content. Emulsions of 1-monoglyceride of capric acid in *Campylobacter*-spiked chicken feed reduced significantly the count of viable bacteria (Thormar et al., 2006). Neal-McKinney et al. (2012) studied the mechanism of lactobacilli inhibition of *Campylobacter jejuni in vitro* and assumed that growth inhibition *in vitro* was due to the effect of lactic acid. Then, on broiler chickens *in vivo*, the most important finding of this study was that *Lactobacillus* can dominate the metabolic activity of *Campylobacter jejuni* through the production of inhibitory organic acids.

The most challenging pathological condition, however, seems to be necrotic enteritis, since the ban of AGPs has resulted in outbreaks of the disease and even worse, in lack of ways to control the subclinical cases. Gauthier et al. (2007) evaluated the effect of two microencapsulated blends of organic acids and natural identical flavours in controlling necrotic enteritis in broilers. The first microencapsulated blend consisted of fumaric, malic, citric and sorbic acid and managed to lower the mortality rate of the infected chickens significantly. The second blend consisted of fumaric acid, calcium formate and calcium propionate and failed to reduce mortality of chickens. The authors assumed that the lower mortality rate in the first group was due to the lower *C. perfringens* numbers in the small intestine and ceca of the broilers. Kocher and Choct (2008) used two mixes of acetic, lactic, fumaric and benzoic acid to test whether the proliferation of *C. perfringens* could be controlled, but the results were not that encouraging, especially when compared to those of antibiotics. The antimicrobial activity of n-butyric acid and its derivatives against *C. perfringens* was studied *in vitro* and measured at two bacterium concentrations and two inoculations involving ambient aerobic and anaerobic conditions. The growth inhibition of *C. perfringens* caused by butyric acid was greater when a moderate initial inoculation concentration (10^5 cfu mL^{-1} of this bacterium) was used instead of a higher initial concentration (10^7 cfu mL^{-1}). Under both aerobic and anaerobic conditions, 50% monobutyrin maintained inhibition rate greater than 90%, suggesting that this monoglyceride could be used to control *C. perfringens* (Namkung et al., 2011). Sodium butyrate was also studied alone or in combination with essential oils to control necrotic enteritis. When given alone, sodium butyrate had no positive effect either on performance or on gross pathological and histopathological lesions (Jerzsele et al., 2012). These findings are contrasting those of Timbermont (2010) who observed beneficial effects of sodium butyrate in the control of necrotic enteritis. Taking into account the complexicity of the disease, the variance in the results can be justified. In order to demonstrate the effects of organic acids on necrotic enteritis more *in vitro* and *in vivo* studies are needed. Since necrotic enteritis is interdependent with *Eimeria* spp., it would be very useful to know any possible effect of organic acids on coccidia. There have been attempts to study the anticoccidial effect of organic acids, based on performance, mortality rates, lesion scoring and oocyst shedding (Leeson et al., 2005; Taherpour et al, 2012). The results indicate a complex potential role of organic acids hence, more data both *in vitro* and *in vivo* are necessary to reach to conclusions.

ORGANIC ACIDS AND INTESTINAL MUCOSA

SCFAs have a proven trophical effect on intestinal mucosa, first described by Frankel et al. (1994). Tappenden et al. (1994) managed to show that systematic SCFAs can rapidly upregulate the expression of proglycagon and early response genes (c-myc, c-jun and c-foc). Proglycagon-derived peptides are strongly correlated with cellular proliferation in the intestine, while early response genes control cell division, growth, differentiation and apoptosis. Among the three major SCFAs, butyrate seems to have the most stimulating effect on enterocytes proliferation, followed by propionic acid (Scheppach et al., 1995). Apart from that, butyric acid is the most preferred source of energy for colonocytes and has been shown to decrease intestinal epithelial permeability by increasing the expression of tight junction proteins (Van Immerseel et al., 2010). That was also reported by Van Deun et al. (2011), who studied the effect of butyrate on Caco-2 cells under a *Campylobacter jejuni* invasion pressure. Butyrate protected the undifferentiated cells better that the differentiated, but pretreatment of differentiated Caco-2 cells with butyrate for 48 hours also inhibited the invasion. The mass paracellular translocation was also prevented indicating that the tight junctions displayed sufficient integrity. Leeson et al. (2005) compared the effect of 0.2 % butyric acid and bacitracin on crypt depth, finding a significant decrease in duodenal crypt depth of bacitracin treated birds, but no significant difference between the butyrate-treated and the control group. That result is in accord with Adil et al. (2010), but not with Antogiovanni et al. (2007), who observed an increase in crypt depth in the jejunum feeding butyric acid glycerides at the same concentration (0.2%), while the villi were shorter but with longer microvilli (increased density). On the contrary, Adil et al. (2010) reported higher villi with the inclusion of 3 % butyric acid especially in the duodenum and jejunum. Except from butyric acid, Adil et al. (2010) studied the effect of fumaric and lactic acid on gut histomorphology as well, observing increased villus height with 3 % and 2% fumaric acid. However, that effect was not as great as that of 3% butyric acid. An interesting finding was that no significant differences in ileum histology were observed (Adil et al., 2010). That is in agreement with Owens (2008), but opposite to the findings of Pelicano et al. (2005) and Samanta et al. (2010) who reported higher villi in the ileum as well, following supplementation of an orthophosphoric, formic, propionic acid and calcium propionate blend. Senkoylu et al. (2007) made similar observations trying a combination of formic and propionic acid. The increased villus height and decreased width contributed to more extended surface area available for nutrient absorption, although the crypt depth was found decreased. This result is different from that of Garcia et al. (2007) who found increased crypt depth adding 10,000 ppm of formic acid in the feed. Trophic effects of formic acid on the intestinal epithelium are indicated but that requires further research to be confirmed. Unlike SCFAs, the effect of the rest of organic acidifiers is attributed to the inhibition on growth of many pathogenic and non-pathogenic bacteria that prevents inflammation at the intestinal mucosa and damage of epithelial cells. Therefore, nutrient absorption, functions of secretion and energy utilization are improved. However, the form and type of organic acids is believed to influence the effect on gut histology. This may be the reason why supplementation of citric acid in 3 concentrations (0, 20, 40 g kg^{-1}) had no effect on intestinal histomorphology (Esmaeilipour et al., 2012). Despite the generally accepted fact that organic acids enhance the integrity and effectiveness of intestinal mucosa, more research is needed to examine that effect under both viral and parasitic conditions, harming the intestinal cells. A summary of the organic acids and possible effects on the intestinal mucosa are in Table 1.

ORGANIC ACIDS AND THE IMMUNE SYSTEM

The intensive conditions established in the poultry industry demand an active and efficient immune system. There are several studies on the effect of

Table 1. Results of studies on the effects of organic acids on the intestinal mucosa.

Effect	Organic acids	References
Trophic effects	SCFAs	Frankel et al., 1994; Tappenden et al., 1994
Decreased permeability	butyric	van Immerseel et al., 2010; Van Deun et al., 2011
Increased villus height	butyric, fumaric, lactic, orthophosphoric, formic, propionic, calcium propionate	Pelicano et al., 2005; Senkoylu et al., 2007; Adil et al., 2010; Samanta et al., 2010
Reduced villus height	butyric glycerides	Antogiovanni et al., 2007
Deeper crypts	formic, butyric glycerides	Antongiovanni et al., 2007; Garcia et al., 2007
No effect	butyric, propionic, formic	Leeson et al., 2005; Owens et al., 2008; Esmaeilipour et al., 2012

Table 2. Effects of organic acids on the immune system of broiler chickens.

Effect	Organic acid	References
Non-specific immunity		
Enhanced mucin production, anti-inflammatory properties, stronger defense barrier	butyric	van Immerseel et al., 2010; Vieira et al., 2012
Enhanced host defense peptide gene expression	butyric	Sunkara et al., 2012
Specific immunity		
Promote humoral immunity	citric, acetic, lactic, butyric	Rahmani and Speer, 2005; Abdel-Fattah et al., 2008
Increased relative weight of bursa and thymus	acetic, citric, lactic	Abdel-Fattah et al., 2008
Increased density of immunocompetent cells	citric	Chowdhury et al., 2009

organic acids on immunological responses and immunocompetence of birds. Organic acids have been found to stimulate specific and non-specific gut immune functions (Friedman and Bar-Shira, 2005). Stimulation of humoral immunity has been measured by gamma globulin levels by Rahmani and Speer (2005), who found increased serum gammaglobulins adding 2% citric acid in broiler chickens' diet. These results are in accordance with those of Abdel-Fattah et al. (2008), who used acetic, lactic and citric acid in 1.5% and 3.0% concentrations and recorded significantly higher serum globulins. Citric acid though had lower effect compared to acetic and lactic acid, but still higher levels of γ-globulins compared to the control group. On a similar basis, antibodies were measured after vaccination against Newcastle Disease, Infectious Bronchitis and Gumboro. The supplementation of 0.25% butyric and citric acid improved antibody titres significantly, with butyric acid having the greatest effect specifically on Newcastle Disease antibodies 12 days post vaccination. These results are in agreement with the findings of Kazempour and Jahanian (2011) who found antibody titer against Newcastle disease virus markedly increased by dietary organic acid supplementation in laying hens.

Following Katanbaf et al. (1989), who reported that increase of spleen, bursa and thymus relative weight is an indicator of immunological advances, acetic, citric and butyric acid were studied on this respect. Supplementation of all three organic acids was found to increase primary lymphoid organs relative weight (thymus and bursa) compared to the controls, but this effect was not attained for spleen relative weight among all groups (Abdel-Fattah et al., 2008). Chowdhury et al. (2009) added 0.5 % citric acid in a basal diet and found an improvement on immune status, detected by densely populated immunocompetent cells in the lamina propria and submucosa of caecal tonsils and ileum and also in the cortex and medulla of bursa-follicles. A summary of organic acids and possible effects on the immune system are in Table 2.

As for non-specific immunity, it has been proposed that organic acids, especially butyric acid, reinforce the intestinal defense barrier by increasing the production of mucins and antimicrobial peptides. Furthermore, it has been well proven that organic acids have anti-inflammatory properties (van Immerseel et al, 2010; Vieira et al., 2012). As for butyrate, the finding that it can enhance disease resistance of chickens by inducing antimicrobial host defense peptide gene expression has been a whole novel approach to control bacteria, protozoa, enveloped viruses and fungi through immune stimulation (Sunkara et al., 2012).

ORGANIC ACIDS AND POULTRY PERFORMANCE

The reduction of the gastrointestinal pH caused by dietary supplementation of organic acids increases gastric proteolysis, protein and amino acid digestibility. Pancreatic secretions, appetite, palatability of the feed and mineral utilization are also influenced by dietary organic acids (Cave, 1982). These factors along with the properties mentioned above affect zootechnical parameters and performance of poultry.

A positive effect on either feed conversion ratio (FCR) or growth performance has been reported for fumaric, propionic, sorbic and tartaric acid (Vogt et al., 1981). FCR was significantly improved by the addition of 1.5% fumaric acid, with lower feed intake compared to the control group. However, body weight gain was not significantly different (Pirgozliev et al., 2008). By contrast, Adil et al. (2010) found significantly higher weight gain following 3 % fumaric acid supplementation, whereas De Arruda Campos et al. (2004) did not find beneficial effect of fumaric acid additive on 21 and 49 days old broiler chickens. Pirgozliev et al. (2008) tried sorbic acid as well reaching the same conclusions as with fumaric acid, but with both acids a decrease of endogenous losses measured by sialic acid was reported. Similarly, Garcia et al. (2007) reported improved FCR with no significant body weight difference feeding 5,000 and 10,000 ppm formic acid, unlike Hernandez et al. (2006) and Acikgoz et al. (2011) who failed to observe any positive effect on performance of broiler chickens when formic acid was added to the feed or the drinking water respectively. A combination of formic and propionic acid, though, as well as their ammonium salts were found to increase body weight gain and improve FCR. (Spais et al., 2002; Senkoylu et al., 2007). Organic acid salts, particularly ammonium formate and calcium propionate, increased live weight and weight gain of broilers until day 21, but no significant differences compared to controls were observed on day 42, although FCR was improved (Paul et al., 2007). Esmaeilipour et al. (2012) studied the performance of broilers fed 0, 20 or 40 g kg^{-1} citric acid for 24 days. Addition of 40 g kg^{-1} decreased feed intake and body weight gain. This negative effect was also found by Brenes et al. (2003), but not by Chowdhury et al. (2009) who discerned significant improvement not only on FCR but on body weight as well. Antogiovanni et al. (2007) observed higher average body weight and better feed efficiency at 35 days by the use of butyric acid glycerides, results that were

Table 3. Conflicting performance results observed in published studies on supplementary organic acids in broilers feed.

Effect	Organic acid	References
Improved feed conversion ratio with no difference in weight gain	fumaric, sorbic, formic, ammonium formate, calcium propionate.	Paul et al,. 2007; Garcia et al., 2008; Pirgozliev et al. 2008
Improved feed conversion ratio and increased weight gain	butyric, fumaric, lactic, citric, formic, propionic	Leeson et al., 2005; Senkoylu et al., 2007; Chowdhury et al., 2009; Adil et al., 2010; Jang et al., 2011
No effect on performance	formic, fumaric	De Arruda Campos et al., 2004; Hernandez et al., 2006; Acikgoz et al., 2011
Decreased weight gain	citric	Brenes et al., 2003; Esmaeilipour et al., 2012

confirmed by Leeson et al. (2005) and Jang (2011). In a comparative study, where various forms and levels of butyric acid glycerides were tried, 0.2 % powdery butyric acid glyceride had the best effect on broilers performance, while 0.3% oily form caused the lowest feed intake (Mansoub et al., 2011). The conflicting opinions regarding effects in poultry performance are in Table 3.

Many researchers have studied the carcass characteristics of broilers fed organic acids, resulting in varying results, like higher breast percentage (Leeson et al., 2005; Jang, 2011) . Antogiovanni et al. (2005) and Garcia et al. (2007) reported that organic acids did not affect meat yield. Generally, benefits of exceeding the dose of supplementary organic acids more than 1g kg^{-1} are not always conspicuous. Marcos et al. (2004) reported that broilers fed a mixture of formic and propionic acid at 0.25% and 0.5% concentration had better performance than chickens fed higher levels of the mixture (1%, 2%). That is in contrast with the findings of Adil et al. (2010) who claimed that addition of 3% lactic, fumaric or butyric acid improved performance more than 2% inclusion levels. When compared with avilamycin or bacitracin, addition of 0.5% citric acid and 2% organic acid blend respectively were found more efficient, suggesting an excellent candidate for total replacement of AGPs (Chowdhury et al., 2009; Samanta et al., 2010). On the contrary, in an experiment under commercial conditions, inclusion of flavomycin in broilers caused greater FCR reduction than a mixture of formic, propionic acid, their ammonium salts, essential oils and plant extracts. (Spais et al., 2002). Still, broiler chickens fed the product at issue presented a significantly better performance in comparison to the chickens fed the control diet. There is a suggestion that as with AGPs, growth enhancing effect of organic acids becomes apparent under suboptimal conditions. This could explain the better performance of broiler chickens after 0.4% inclusion of the mixture of organic acids in the above described commercial experiment compared to the same experiment performed under ideal conditions, where no effect was observed (Florou-Paneri et al., 2001; Spais et al., 2002; Giannenas, 2006).

As for mineral utilization, it has been found greater due to the complex of the acid anion with calcium, phosphorus, magnesium and zinc, resulting in higher levels of these minerals in the blood. Increased egg specific gravity and femur strength in laying hens fed diet with ascorbic acid was attributed to higher calcium blood concentration (Orban et al., 1993). Apart from ascorbic acid, caproic, capric and short chain fatty acids as well improved eggshell characteristics (Swiatkiewicz et al., 2010). Chowdhury et al. (2009) reported increased tibia ash in broilers fed 0.5 % citric acid, being in agreement with Snow et al. (2004) and Liem et al. (2008) who tried citric, malic and fumaric acid in phosphorus deficient diets. Tibia ash was significantly increased only in the citric acid group, while phosphorus utilization was significantly affected by citric acid and less by malic acid. The reason why some organic acids are more efficient than others needs to be further studied. Similarly, Houshmand et al. (2011) tried an organic acid mixture in a low-calcium level diet and observed significant improvement of tibia characteristics that helped chickens overcome tibial dyschondroplasia. The results mentioned above consolidate the suggestion that feed additives may be more efficient when nutrient content is less than optimum level (Torres-Rodriguez et al., 2005).

CONCLUDING REMARKS

Summarizing the published data presented in this review article, it can be concluded that organic acids have valuable properties affecting the gut ecosystem and the performance of poultry. If used correctly along with management and biosecurity measures, they can even serve as growth promoters, although there is not always agreement on the proper concentrations, the specific age or duration of feeding organic acids and the safety levels.

These special properties can be further applied in field in order to control subclinical pathological conditions, diet deficiencies, or even immunosupression, but more research is needed on this regard. It seems that each organic acid affects the gut ecosystem to a different degree, but the reason why some organic acids have, for example, better effects on the morphology of the intestinal epithelium while others induce stronger immune responses or better performance remains unclear. Apart from the SCFAs, where studies have shown their ability to induce immune and mucosal cell gene expression, it is not known whether other organic acids share the same trait. The role of each organic acid, the form and the concentration chosen needs to be further clarified, not only on a growth-promoting basis, but under challenge as well. The potential benefits of adding organic acids in the diet when the intestinal cell integrity is challenged by common intracellular pathogens such as *Eimeria* spp. should be considered. Given the fact that coccidia, both under clinical and subclinical conditions, as well as live anticoccidial vaccination affect the gut ecosystem in numerous ways, the impact of dietary organic acids should be further studied.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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