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Effect of organic acid supplementation on performance of poultry

J.V. Ramana*, R. Vikram Reddy, D. Srinivasa Rao***, S. Shakila****, J. Suresh*******

*Controller of Examinations, Sri venkateswara Veterinary University, Tirupati. **Research Scholar ***Professor and Head, Department of Animal Nutrition ****Professor and Head, Department of Poultry Science *****Professor and Head, Department of Livestock and Production Management, College of Veterinary Science, Tirupati-517 502.

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Abstract

In recent times, the poultry industry has paid more attention for addressing the public concerns for food and environmental safety. In an attempt to promote exports of poultry products, antibiotics are being withdrawn from the poultry diets around the world. Organic acids are studied as potential alternatives to antibiotic growth promoters. Their action is related to the pH reduction of the intestinal digesta and 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 microorganisms, due to exclusive competition. Antibacterial activity has been widely reported for many poultry pathogens, such as *Salmonella* spp., *Escherichia coli*, *Clostridium perfringens*, *Campylobacter* sps., both *in vitro* and *in vivo*. 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 immune-stimulating 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.

Introduction

The removal of antibiotic growth promoters (AGPs) from poultry diets in the countries of the European Union in 2006 and similar demands in India, 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 alternatives, widely studied are the organic acids. The organic compounds having carboxylic groups including fatty acids with the general structure of R-COOH are termed as acidifiers or organic acids.

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).

However, there are substantial differences in the effect of different organic acid additives. Acetic acid

Corresponding Author: J.V. Ramana, Professor and Head, Department of Animal Nutrition, College of Veterinary Science, Tirupati – 517 502, AP, India.
E-mail: jvenkataramana@rediffmail.com
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and propionic acid show moderate effect on feed pH, while citric acid, formic acid, fumaric acid, lactic acid lower the feed pH substantially. Moreover due to different chemical structures, different acids have different chemical properties. For example, formic acid is active at low concentration against *E.coli* and effectively eliminates salmonellae from the feeds, whereas lactic acid producing bacteria and moulds are relatively resistant to its effect. Propionic acid is more effective in its action against yeasts and moulds compared to other organic acids. Hence research seeks to optimize the additive effects of a combination of buffered organic acids (Hyden, 2000).

The dietary acidification in swine was found to resemble the effect of AGPs in the gastrointestinal tract of farm animals (Senkoylu *et al.*, 2007). 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.

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 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. 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).

- *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 acidsin the intestine of young chickens.

Effect of Organic Acids on Production Performance in Poultry

Feed Conversion Ratio (FCR) was found to be better with organic acid supplementation due to decreased feed intake and higher weight gain coupled with improved conditions in the intestines leading to improved digestion, absorption and utilization of nutrients (Parks *et al.*, 2001). 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, 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 as well as their ammonium salts were found to increase body weight gain and improve FCR. (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 citric acid for 24days. Addition of 40 g/kg 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. Effect of organic acids on production performance of broilers is summarized in Table 1

Table 1: Conflicting results on production performance of broilers with supplementation of organic acids

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; Prigozliev *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; Zhang *et al.*, 2011
No effect on performance	Formic, fumaric	Hernandez *et al.*, 2006; Acikgoz *et al.*, 2011
Decreased weight gain	Citric	Bernes *et al.*,2003; Esmaeillipour *et al.*,2012

Vikram Reddy *et al.*, (2017) studied the effect of dietary supplementation of organic acids in combination on performance and carcass traits of broiler chicken. Six experimental diets, viz. T1 (Basal diet), T2 (Basal diet+ Antibiotic @ 50 gm/100 kg feed), T3 (Basal diet+20:40:40 combination of citric, formic and propionic acids @ 1.5gm/100 g of feed), T4 (Basal diet+30:40:30 combination of citric, formic and propionic acids @1gm/100g feed), T5 (Basal diet+ 30:30:40 combination of citric, formic and propionic acids @1gm/100 g feed), T6 (Basal diet+ 10:45:45 combination of citric, formic and propionic acids @ 1.5g/100g feed) were prepared. Two hundred and seventy day old, straight run commercial broiler chicks were distributed randomly to six treatments with three replicates of fifteen birds each and fed with the experimental diets from 0 to 42 days of age. Body weight gains and feed efficiency were significantly ($P<0.05$) improved during all phases of the experiment. Feed intake in organic acid supplemented groups (T3 to T6) was significantly ($P<0.05$) reduced during the pre-starter and starter phases. Organic acid supplementation revealed no significant ($P>0.05$) difference in the ready-to-cook-yields among the groups but significantly ($P<0.05$) increased giblet yields on % live weight basis, liver weights, intestinal length and intestinal weight. The organic acid combination of citric, formic and propionic at 20:40:40 combination could be safely incorporated at 1.5% level in broiler diets for better performance.

Effect of Organic Acids on Nutrient Digestibility

Dietary acidification was found to increase the gastric proteolysis, protein and amino acid digestibility and in addition serving as substrates in intermediary metabolism .The improvement in protein digestibility with citric and ascorbic acid was seen to be suggestive of reduction in pH and an increase in the pepsin activity (Kirchgessener and Roth, 1982).

Reduction in gastric pH follows organic acid feeding which may increase the pepsin activity and the peptides arising from pepsin proteolysis trigger the release of hormones including gastrin and cholecystokinin, which regulate the digestion and absorption of nutrients (Hersey 1987). Furuse and Okumura (1989) found that protein, fat and energy retention were linearly lower as dietary acetic acid was increased from 12.7 to 63.5g/kg diet.

Birds raised on acidified diets exhibited jejunal villi in a zigzag fashion resembling a wave, which facilitated nutrient absorption more efficiently than when they are positioned parallel (Yamauchi and Isshiki, 1991). Abdel-Azeem *et al.,* (2000) concluded that the dietary organic acidification increased the protein utilization and improved its digestibility coefficient. Dibner and Buttin (2002) suggested that organic acids enhanced protein and energy digestibility by reducing microbial competition with the host nutrients, endogenous nitrogen losses, production of ammonia and other growth depressing microbial metabolites. They also minimized the incidence of sub-clinical infections and secretion of immune mediators leading to better performance in the birds.

Thirumeignanam *et al.,* (2006) reported that ileal digestibility of DM, CP, EE and GE were significantly (P<0.01) higher in groups supplemented with mixture of organic acid salts at the rate of 1, 1.5 kg/ton in broiler diets compared to unsupplemented and antibiotic supplemented groups. Ghazalah *et al*., (2011) observed that the digestibility of EE, NFE, as well as the ME were significantly (P<0.05) improved with organic acids supplementation, while there were no significant differences for digestibility of CP and CF compared to control group.

Ramigani *et al*., 2015 studied the effect of dietary supplementation of organic acids on digestibility of nutrients and serum biochemical profile of broiler chicken. Six experimental diets, T1 (basal diet), T2 (basal diet + furazolidone @ 50g/100 kg feed), T3 (basal diet + 20: 40: 40 combination of citric, formic and propionic acids @ 1.5g/100 g of feed), T4 (basal diet+30: 40: 30 combination of citric, formic and propionic acids @ 1g/100 g feed), T5 (basal diet + 30: 30: 40 combination of citric, formic and propionic acids @1g/100g feed), T6 (basal diet + 10: 45: 45 combination of citric, formic and propionic acids @ 1.5 g/100g feed) were prepared. Digestibility of nutrients and serum biochemical parameters were studied during two phases i.e. at the end of 4th and 6th week. Digestibility of DM, CP and EE increased significantly (P<0.05) in organic acid supplemented groups (T3 to T6) compared to T1 and T2 during both stages of the experiment but no significant difference was observed in ME values and CF digestibility. Significantly (P<0.01) higher serum total protein, albumin, globulin and calcium levels were recorded in organic acid supplemented groups in both the phases. Serum cholesterol levels decreased (P<0.01) in organic acid supplemented groups (T3 to T6) compared to T1 and T2.

Effect of Organic Acids on 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. *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. 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 flavors 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* be controlled, but the results were not that encouraging, especially when compared to those of antibiotics.

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.

Ramigani *et al.*, 2015 studied the effect of dietary supplementation of organic acid combinations on gut pH and *E. coli* count of intestinal contents in broilers. Six experimental diets T1 (Basal diet), T2 (Basal diet+ Antibiotic @ 50 g/100 kg feed), T3 (Basal diet+20: 40: 40 combination of citric, formic and propionic acids @ 1.5 g/100 g of feed), T4 (Basal diet+30: 40: 30 combination of citric, formic and propionic acids @ 1 g/100 g feed), T5 (Basal diet+ 30: 30: 40 combination of citric, formic and propionic acids @ 1 g/100 g feed), T6 (Basal diet+ 10: 45: 45 combination of citric, formic and propionic acids @1.5 g/100 g feed) were prepared. The pH values of crop, duodenum and *E.coli* counts of intestinal contents decreased (P<0.01) in organic acid supplemented groups (T_3 to T_6) compared to T_2 and T_1 during both the phases but no significant difference was found in ceacum pH among treatments.

Organic acid combinations supplementation to broiler diets resulted in improvement of acidic environment in the gut of broilers and decrease of microbial load especially during the starter phase. The organic acid combination of citric, formic and propionic at 20: 40: 40 combination can be safely incorporated at 1.5% level in broiler diets for better productive performance.

Effect of Organic Acids on Intestinal Mucosa

Short Chain Fatty Acids (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 up regulate 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).

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).

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 nonpathogenic 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

Table 2: Effect of Supplementation of Organic acids on Intestinal Mucosa

Effect	Organic acid	References
Trophic effects	Short Chain Fatty Acids	Frankel *et al*.,1994; Tappendan *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
Decreased villus height	Butyric glycerides	Antogiovanni *et al*., 2007
Deeper crypts	Formic, butyric glycerides	Antogiovanni *et al*., 2007; Garcia *et al*., 2007
No effect	Butyric, propionic, formic	Leeson *et al*., 2005; Owens *et al*., 2008; Esmaeilipour *et al*.,2012

the effect on gut histology. This may be the reason why supplementation of citric acid in 3 concentrations (0, 20, 40g 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 2.

Effect of Organic acids on 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 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 gamma globulins 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 ã globulins compared to the control group.

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.

Table 3: Effect of Organic acids on Immune System

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	Citric, acetic, lactic,	Abdel-Fattah *et al*., 2008
Increased density of immunocompetent cells	Citric	Chowdhry *et al*., 2009

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 3.

Conclusion

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 bio-security 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.

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