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Organic acids as alternatives to antibiotic growth promoters in poultry

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Abstract
Organic acids are any carboxylic acid including fatty acids and amino acids, of the general structure R-COOH. Mainly short chain mono carboxylic acids such as citric, propionic, fumeric and formic acids etc. are used as potential alternative to antibiotic growth promoter. Organic acids and their salts are able to inhibit microbial growth in the food and consequently to preserve the microbial balance in the gastrointestinal tract. Modifications to the gastrointestinal microflora which reduce pathogen attachment may have a profound effect on the structure of the intestinal wall. Butyric acid is one such SCFA, which has higher bactericidal activity when the acid is undissociated. Butyrate also appears to play a role in development of the intestinal epithelium. However, the levels of SCFA are quite low in the intestine and caeca of young chicks and so the young chicks may be the best candidates for dietary supplementation.

Keywords: Birds, organic acids, production performance.

Introduction

India's poultry industry has transformed from a mere backyard activity into a major commercial activity in just four decades and now emerging as the world's 2nd largest poultry market with an annual growth of more than 14% [16]. Chicken meat production in India was ranked 6th and 3rd in egg production in the world using FAOSTAT [15] rankings. India's per capita consumption for chicken meat has gone up from 400 grams to 2.5 kg [8]. Poultry birds are raised for meat production due to high and faster growth rate but under the present farm practices in india they are always under stress. Antibiotics used in their diet pose a serious problem to health of the birds and in particular to the eventual consumers. So a most arising challenge in the poultry production is to exploit the use of specific dietary supplements to boost their intrinsic potential for optimal performance. Following the ban on the use of antibiotics as growth promoters by the European Union, the nutritionists and researchers attempted other alternatives to enhance the performance of birds. One such alternative was the use of organic acids as feed additives in the poultry production.

The gastrointestinal (GI) tract of newly hatched chicks is immature and sterile. It begins to develop function and its microflora when it starts to ingest feed. At this time, the chick is very susceptible to pathogenic microorganisms [2]. Under such circumstances, anti-microbial feed additives such as antibiotics are often used to suppress or eliminate harmful organisms in the intestine, and to improve growth and feed efficiency [29]. Organic acids and their salts are generally regarded as safe and have been approved by most member states of the EU to be used as feed additives.

Organic acids are considered to be any organic carboxylic acid including fatty acids and amino acids, of the general structure R-COOH. Not all of these acids have effects on gut microflora. Organic acids such as citric, propionic, fumeric and formic acids etc. mainly short chain monocarboxylic acids are used. Amongst the organic acids, short chain fatty acids (SCFA) are considered as potential alternative to antibiotic growth promoter [61]. Dietary organic acids and their salts are able to inhibit microbial growth in the feed, and consequently preserving the microbial balance in the gastrointestinal tract. In addition to modifying intestinal microflora, organic acids also improve the solubility, digestion and absorption of nutrients. Addition of 1.8% formic acid in diet reduced *E. coli* in the cecum and faeces [21]. Organic acids have made a great contribution to profitability in poultry production and also provided people with nutritious poultry products. Eggshell quality is one of the most important issues in the poultry industry, influencing the economic profitability of egg production and hatchability. Besides, high breaking strength of eggshell and absence of shell defects are essential for protection against the penetration of pathogenic bacteria such as *Salmonella sp*. into the eggs.

It has been estimated that eggs with damaged shells account for 6.0 to 10.0% of all eggs produced, which leads to great economic loss. Nollet *et al.* [44] and Mahdavi *et al.* [39] reported a positive effect of sodium butyrate and calcium propionate on laying performance.

Organic acids and their salts increased gastric proteolysis and improved digestibility of protein and amino acids. The advantage of salts over acids is that they are generally odorless and easier to handle in the feed manufacturing process owing to their solid and less volatile form [26]. Additionally, these acids have been shown to inhibit the growth of intestinal bacteria which compete with the host animal for availability of nutrients and reduced toxic bacterial metabolites such as ammonia and amines. As a result, feed efficiency and growth performance of the host improved. Health of the gut is one of the major factors governing the performance of birds and thus, the economics of poultry production [54] and the profile of intestinal microflora play an important role in gut health. The mechanism of action of organic acid is due to reduction of intestinal pH, which leads to an increase in the activity of digestive enzymes and solubility of minerals [59]. Organic acids and their salts associated with specific antimicrobial activity by entering into the bacterial cell. Only undissociated form of organic acids can penetrate the bacterial cell and dissociated form can't enter the bacterial cell. At low pH organic acids are more in undissociated form. Bacterial cell take up undissociated fatty acids and once these acids dissociate, there is change in the intracellular pH leading to death of bacterial cells as the formed organic anion inhibits the DNA replication and export of excess protons utilizes cellular ATP. Organic acids also improve the digestibility of proteins and amino acids and the absorption of minerals [43, 45], modulate endocrine and exocrine secretions and influence the mucosal morphology [48]. Therefore supplementation of organic acids in poultry has economic aspect by increasing efficiency, nutritional value of low-quality ingredients and by reducing feed losses caused by microbial spoilage.

Mechanism of action of organic acids

In general, potential bacterial targets of biocidal compounds include the cell wall, cytoplasmic membrane, and specific metabolic functions in the cytoplasm associated with replication, protein synthesis, and function [12]. It is known that their activity is related to the reduction of pH, as well as the ability to dissociate. The lowering of dietary pH alone, however, failed to show any nutritive efficacy. It has been assumed that undissociated forms of organic acids penetrate the lipid membrane of the bacterial cell and dissociate within the cell. As bacteria maintain a neutral pH of the cytoplasm, the export of excess protons consumes cellular ATP and results in depletion of energy [51]. Unlike antibiotics, the antimicrobial activity of organic acids is thus pH dependent. At low pH, more of the organic acid will be in the undissociated form. Consequently, antimicrobial activity of organic acids is undisputable at low pH, but uncertain at pH above 6. In cultures of *E. coli* treated with caprylic acid at pH 5.2 the number of viable cells decreased to ≈ 10^2 per ml. A reduction of between 0.94 and 1.96 log10 colony forming units was observed at pH 6.5 or 6.6 [40]. Similarly, incubation of Salmonella sp. with caprylic acid at pH between 5.2 and 5.3 led to a reduction in the concentration of viable cells below the detection limit, but between 2 and 6 percent of *Salmonella sp.* cells survived at pH between 6.3 and 6.6 [57].

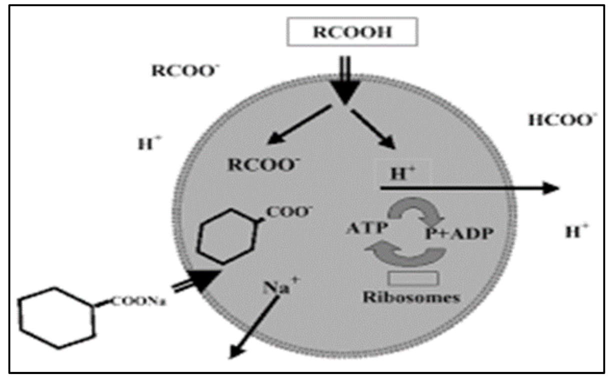

Fig 1: Schematic representation of the mode of action of organic acids and their salts.

Effect of organic acids on feed intake, body weight gain, FCR and nutrient digestibility

High levels of production and efficient feed conversion are the need of the modern broiler industry which to a certain extent could be achieved by the use of dietary organic acids as feed additives. Acetic acid as dietary acidifier was also used by Kral *et al.* [35] and Islam *et al.* [28]. In the experiment conducted by Kral *et al.* [35] broiler chickens in control group were fed with standard feed mixture and experimental groups with 5% acetic acid used in drinking water. Body weight, FCR were recorded. The body weight of broiler chickens was significantly increased ($P<0.05$) in weeks 5, and 6 of age. Feeding of citric acid, acetic acid and their combination significantly increased the body weight gain ($P<0.05$) and FCR in broiler chicks [28].

Partially protected sodium butyrate had significant effect on performance, digestive organs, intestinal villi and *E. coli* development in broilers chickens [11]. Another work on butyric acid was done by Adil *et al.* [4] and Therpour *et al.* [60] and they observed that the birds fed diets supplemented with organic acids showed significantly ($p<0.05$) higher body weight gains and feed conversion ratio [4]. Basal diet supplemented with organic acid (citric acid and tartaric acid @ 0.5% and 1%) showed significantly higher body weight gains [37]. Sodium butyrate and calcium propionate supplementation @ 0.5 and 1% also improved the weight gain and feed efficiency of broilers [62]. Organic acids also improve the digestibility of proteins and amino acids and the absorption of minerals [45, 43]), modulate endocrine and exocrine secretions and influence the mucosal morphology [48]. Organic acids exert several additional effects and these include reduction in digesta pH, increased pancreatic secretion and atrophic effects on the gastrointestinal mucosa. Acidification has the potential of controlling all enteric bacteria, both pathogenic and non-pathogenic [41]. Various organic acids including formic, fumaric, propionic and sorbic acid have been added to broiler feed resulting in positive response [42]. Thus these acids enhance growth and feed efficiency by eliminating organisms that compete with the bird for nutrients.

Table 1: Effect of different organic acids on production performance, nutrient digestibility

S. No.	Organic acid	Inclusion rate	Results	References
1.	Ammonium formate	3 gm/kg diet	Increased the live weight and live weight gain and FCR at day 21 in broilers	Paul *et al*., 2007 [47]
2.	Citric acid and acetic acid	0.5% of the diet	Showed significant improvement the body weight gain (P<0.05) and FCR in chicks	Islam *et al*., 2008 [28]
	Citric acid	2% of the diet	increased the retention of DM, CP and neutral detergent fibre	Ao *et al*., 2009 [7]
3.	Butyric acid	0.4% of the diet	Resulted in superior FCR	Panda *et al*., 2009 [46]
4.	organic acid blend	1 g and 2 g/kg diet	improved the gastric proteolysis and digestibility of protein and amino acids	Samanta *et al*., 2010 [53]
5.	Fumaric acid	3% of the diet	highest weight gains	Adil *et al*., 2011 [4]
6.	Formic acid and acetic acid	0.5% and 0.75% respectively	improved both ME and nutrient digestibility	Ghazala *et al*., 2011 [22]
7.	Citric acid	20 and 40 mg/kg diet	Increased dry matter and protein retention	Esmaeilipour *et al*., 2011 [14]
8.	Organic acids mixture	comprising 30.0% lactic acid, 25.5% benzoic acid, 7% formic acid, 8% citric acid and 6.5% acetic acid	Improved broiler's performance at 42 days of age as compared to control and provided better carcass characteristics.	Fascina *et al*., 2012 [17]
9.	Citric acid and tartaric acid	0.5% and 1%	Improve the body weight gain significantly	Laxman *et al*., 2013 [37]
10.	Acidifier mixture (formic, phosphoric, lactic, tartaric, citric and malic acids)	0.15% of the diet	Increase in body weight gain was observed in acidifier mixture supplemented group.	Hashemi *et al*.,2014 [25]

Effect on meat composition

Organic acid salts supplementation improved the meat poly unsaturated fatty acids and increase in the PUFA content may be due the reason that the gut microorganisms are able to hydogenise unsaturated fatty acids to more saturated ones. So by controlling gut microbial population organic acids controls the process of hydrogenation. Similarly butyric acid glycerides supplementation reduced the serum LDL cholesterol [32] and triglycerides [55] in broiler chicken. Lactobacillus, which has a high bile salt hydrolytic activity, is responsible for deconjugation of bile salts [56]. Deconjugated bile acid are less soluble at low pH and less absorb in the intestine and is more likely to be excreted in feces [33]. On the other hand, Salma *et al*. [52] have shown that cholesterol concentration in thigh and breast muscle of the broilers had a positive correlation with the change of the cholesterol contents in serum. Thus, it is expected that with decreasing of serum cholesterol, the amount of meat cholesterol is tending to decrease too.

Table 2: Effect of different organic acids on serum constituents and meat yield

S. No.	Organic acid	Inclusion rate	Results	References
1.	Formic acid	0.5% and 1.0% of diet	Not showed any significant effect on breast and thigh yields of broilers at 49 days of age.	Garcia *et al*., 2007 [20]
2.	Butyric acid glycerides	2 and 3 kg/ton	Lowered the level of serum cholesterol	Taherpour *et al*., 2009 [60]
3.	Butyric acid, fumaric acid and lactic acid	2% and 3% each acid	Serum calcium and phosphorus concentrations were increased (P<.05)	Adil *et al*., 2010 [3]
4.	Fumaric Acid	0.5% of diet	Higher breast meat yield (BMY) percentage and no significant effect on serum AST, ALT, uric acid, and creatinine.	Ragaa *et al*., 2016 [49]

Effect on gut morphology and flora

Addition of organic acids cause the reduction of total bacterial counts in different parts of the digestive tract and also agreed by Al-Kassie and Abd-AL-Aljaleel [6] who used Gallic acid and obtained a reduction in the total bacterial count in the crop, small intestine and caecae. The addition of organic acids cause a reduction of the bacteria in the colon but have no such decreasing effects on the lactic acid bacteria. Most of the pathogens grow in a pH close to 7 or slightly higher. In contrast, beneficial microorganisms live in an acidic pH (5.8-6.2) and compete with pathogens [18]. In addition, lowering the pH by organic acids reduce the pathogenic microbes from GIT and improves nutrient absorption [10]. Propionic acid effectively inhibits the growth of *E. coli* bacteria in the animal's gastrointestinal tract. At the same time it does not inhibit the growth of *Lactobacillus* bacteria [24].

Length and width of intestinal villi are of histomorphometrical indices and any increase in the values enhances the absorptive surface of intestine. Researchers have shown that organic acids can reduce the intestinal lumen pH and increase antibacterial enzymes produced by some bacteria, so increasing villi height. Moreover, organic acids reduce amount of pathogenic bacteria in the small intestine wall and decreases production of toxic compounds which cause changes in the morphology of the intestine of birds and in consequence prevent destruction and damage to intestinal epithelial cells [20]. Longer villus could be considered as an indicator of an active functioning of intestinal villi. Increased villi height provides more surface area for nutrients absorption [9].

Table 3: Effect of different organic acids on gut morphology

S. No.	Organic acid	Inclusion rate	Results	References
1.	Butyric acid	0.2%, 0.4% and 0.6% in the broiler's diet	Improved the villus length and crypt depth in the duodenum.	Leeson *et al.*, 2005 [38] and Panda *et al.*, 2009 [46]
2.	Formic acid	1.0% of the diet	Reported the longest villi and deeper crypts on acid supplementation	Garcia *et al.*, 2007 [20]
3.	Ammonium formate	3 gm/kg diet	Lowered E. coli count in the gut	Paul *et al.*, 2007 [47]
4.	Sodium butyrate (in both partially protected with vegetable fats and unprotected forms)	0.92 g/kg diet	Prevent *Salmonella* colonization in the crop and caeca of broilers	Fernandez-Rubio *et al.*, 2009 [19]
5.	Sorbic acid and citric acid	1.0% and 0.2% respectively	Significantly increased the villus width, height and area of the duodenum, jejunum and ileum of broiler chicks at 14 days	Kum *et al.*, 2010 [36]
6.	Propoinic acid	4% of the diet	Significant reduction in the number of Salmonella	Koyuncu *et al.*, 2013 [34]

Effect on Egg production

There was significant positive effect on percent hen day egg production by the supplementation of different levels (0.5%, 1.0% and 1.5%) of salts of organic acids in the diet of layers and Hen–day egg production was highest in 1.5% sodium butyrate supplemented group as compared to others [12]. Dietary supplementation of salts of organic acids had accelerated the laying capacity in 24-28 weeks old laying hens and extended the period of egg production in 36-38 weeks old hens than hens of control group [65] and egg weight was significantly improved by different dietary treatments of organic acids [23, 66]. Addition of organic acids to the diet causes diet acidity and lowering the dietary pH may increase the solubility of minerals, thereby increasing the effectiveness of calcium which ultimately improve the egg shell quality.

Table 4: Effect of organic acid on egg production

S. No.	Organic acid	Inclusion rate	Results	References
1.	Organic acid mixture,	0.5%, 1.0% and 1.5% of the diet	Average egg production increased	Yesilbag and Çolpan (2006) [65]
2.	Organic acid mixture (formic acid and salt of butyric, propionic and lactic acids	0.078% of diet	Percent average egg production significantly increased by about 5.77% to 9.84% of laying hens	Soltan 2008; [58] Rahman *et al.*, 2008 [50].
3.	Sodium butyrate and calcium propionate	0.5%, 1.0% and 1.5% of the diet	Highest hen–day egg production in 1.5% sodium butyrate	Dahiya *et al.,* 2016 [12].

Table 5: Effect of organic acid on egg quality

1.	Lactic acid	1% of diet	Significant improvement in the albumen index ($P < .05$) and yolk index ($P < .05$) in layers.	Yalcin *et al.*, 2000 [64]
2.	Acetic acid	600, 400 and 200 ppm in drinking water	Linear increase in external egg qualities such as egg weight, egg length, egg diameter and egg shell colour	Kadim *et al.*, 2008 [31]
3.	Phenyllactic acid	0.2% of diet	Egg shell strength improved	Wang *et al.,* 2009 [63]
4.	Formic acid	0.1%, 0.2%, and 0.3% in drinking water	Egg shell thickness and egg grading significantly greater	Abbas *et al.*, 2013 [1]

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