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REVIEW PAPER

PROSPECTS OF USING CITRIC ACID AS POULTRY FEED SUPPLEMENTS

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ABSTRACT

Growth promoters especially antibiotics have long been used in poultry, however, these antibiotics produce resistance in bacteria. Several alternatives to these antibiotics including organic acids have been suggested. These organic acids improve the digestibility of nutrients, lower the gastric pH, chelate the cations and serve as substrate in intermediary metabolism. Among the organic acids, citric acid has more potential as growth promoter. Being a strong chelater of calcium (Ca) and a proton donor, citric acid improves the phytate hydrolysis. By lowering the pH, it produces a favorable environment for the action of endogenous and exogenous microbial phytases. Its antimicrobial property and immune system stimulation activity is also well documented. Besides, it enhances the nutrient absorption and improves the phosphorus (P) utilization. Nevertheless, inconsistent results of citric acid effectiveness on growth performance were obtained. In this review, authors focus on applications of citric acid in poultry feed to improve nutrient digestibility, phosphorus utilization, immune status and phytase efficacy.

Keywords: Citric acid, poultry, phytase, growth performance, bone mineralization.

INTRODUCTION

Growth promoters have long been used in poultry and pig industry throughout the World (Charles and Duke, 1978). Antibiotics as growth promoters have been used since 1946. About 32 antibiotics have been used for therapeutic or prophylactic purposes and also as growth promoters in poultry up till 2003(Jones and Ricket, 2003). These antibiotics were known to improve the growth performance and reduce the mortality rate in poultry (Miles *et al*., 2006; Pfaller, 2006). However, a serious hazard associated with the use of these antibiotics was the resistance produced by bacteria against them. Salmonella samples isolated from poultry, poultry products and its surroundings were found resistant to erythromycin, lincomycin and penicillin (Roy *et al*., 2002). These resistant bacteria can create problems to human health by either directly transmitting to man themselves or indirectly transmitting their resistant genes to human biota (Greko, 2001). Due to these problems, European Union has announced ban on the use of antibiotic growth promoters in animal feed (Regulation 1831/2003). To replace these antibiotics, different alternatives have been suggested including organic acids, probiotics, herbs, enzymes and essential oils. Among them, organic acids are considered most promising alternatives in poultry nutrition (Gunal *et al*., 2006). Their inclusion has been found to enhance the nutrient utilization and feed efficiency resulting in improved growth performance (Jin *et al*., 1998; Denil *et al*., 2003).

Organic acids perform multifunctional role in physiology and biochemistry of nutrition. These organic acids enhance the nutrient digestibility especially protein digestion by improving the pancreatic secretion (Mellor, 2000), digestive enzyme activity and growth of gastrointestinal mucosa (Dibner and Buttin, 2002). It is well documented that organic acids lower the pH of the gastrointestinal tract which improves the health of the animal in many ways. Firstly, nutrient absorption increases at lower pH (Boling *et al*., 2001). Secondly, lower pH causes the death of majority of harmful micro-organisms which live in a pH close to 7 or slightly higher (Ford, 1974). These organic acids after penetrating the bacterial membranes, dissociate inside the bacterium (Ecklund, 1983). When internal pH of bacteria drops down which is normally neutral, bacteria uses ATP to actively transport excess protons from the cell to outside resulting in depletion of cellular energy which causes the death of the bacteria (Davidson, 2001). Another possible way of antimicrobial effects of organic acids includes the production of short-chain fatty acids inside bacteria. These compounds decrease the luminal pH and inhibit the growth of bacteria (Corrier *et al*., 1990). This reduction in colonization of pathogens on the intestinal wall reduces the damage to epithelial cells as well as the production of toxic compounds such as ammonia and amines by bacteria (Kirchgessner and Roth, 1988).The reduction in these microbes also reduces competition with host for available nutrients as these microbes use 6% of the net energy in pig diet (Vervaeke *et al*., 1979) and also use amino acids of the host diet resulting in reduction of N-utilization (Furuse and Okumura, 1994). In contrast, decreased pH enhances the growth of beneficial micro-organisms as they grow at acidic pH (5.8-6.2) (Ford, 1974). In addition, when intestinal pH decreases, solubility of phytate and P increases which result in increased P absorption in small intestine (Cross *et al*., 1990).

These organic acids are more important for young animals whose endogenous acid production in the gut is low (Cranwell and Titchen, 1974). Besides, organic acids chelate with various cations along the intestine and increased the absorption of minerals (Wood and Serfaty-Lacrosniere, 1992). The acidification of diet by adding these organic acids also reduces the rate of gastric emptying (Mayer, 1994). Furthermore, these organic acids have high gross energy values (Freitag, 2007) and are used in various metabolic processes for energy generation such as production of ATP in citric acid cycle (Diebold and Eidelsburger, 2006) and also serve as substrates in intermediary metabolism (Kirchgessner and Roth, 1988). They also reduce the subclinical infections and modify the immune system (Dibner and Buttin, 2002). Hence, organic acids are making a great contribution to the profitability in the poultry production and also providing people with the healthy and nutritious poultry products.

Citric acid (CA), one of the organic acids, is being used in several countries as alternative of antibiotic growth promoters (Estieve *et al*., 1997). Different studies have revealed that CA is a useful additive having more potential as a growth promoter as compared to antibiotics avilamycin (Chowdhury *et al*., 2009) and flavomycin (Haque *et al*., 2010). Liem *et al*. (2008) concluded that CA is more effective than other organic acids (malic and fumaric acid) with respect to phytate P retention, body weight gain and bone mineralization.

2-Hydroxy-1,2,3-Propanetricarboxylic acid is the chemical name of CA and its chemical formula is $COOHCH_2C(OH)(COOH)CH_2COOH$ (Dibner and Buttin, 2002). Its molecular mass is 192.14 g/mol and density is 1.67 g/ml. It is found in solid form having pKa-value of 3.13, 4.76 and 6.4(Foegeding and Busta, 1991). It has good water solubility and its gross energy is 2460 kcal/kg (Freitag, 2007). Like other tri- and dicarboxylic acids, its mode of absorption is Na^+-dependent transport mechanism across the intestinal brush border membrane (Wolffram *et al*., 1990, 1992).

History of CA use in feed to improve P utilization is traced back to 1937 when Shohl in Harvard University fed rachitogenic diet (deficient in Ca or P or both) to rat with supplementation of CA: sodium citrate mixture (1:1). This mixture improved phytate-P utilization and femur ash (61%) and prevented rickets in rats. Later on, these results were confirmed by Pileggi *et al*. (1956). They also fed the rats with rachitogenic diets limiting in P or Ca or both in the presence or absence of phytate. Femur ash was increased by 61% with the addition of 1:1 mixture of CA:sodium citrate in the diet while fecal P was decreased from 94 to 48%. Furthermore, it was observed that only phytate containing diets showed response to CA:sodium citrate mixture whereas no effect was observed in non-phytate or available P. In 1995, Zyla *et al*. reported that CA intensifies the dephosphorylation of phytate *in vitro*.

Citric acid improves phytate P (Rafacz-Livingston *et al*., 2005a,b) and other nutrients utilization by several ways. It may chelate Ca in competition of phytate and prevent the formation of insoluble phytate-Ca complex and result in dietary phytate which is more susceptible to endogenous phytases (PHYs) (Boling *et al*., 2000). Other possible mechanism of CA to improve phytate hydrolysis is decrease in pH due to CA inclusion. Phytate have one or more weak phosphate groups which have less affinity for $Ca^{++}$and Mg^{++} as compared to protons. At reduced pH CA might serveas proton donor to phytate and prevent the formation of insoluble Ca phytate complexes (Maenz, 2000). This reduced pH also enhances the dissociation of mineral from phytate (Maenz and Clansen, 1998).

Chowdhury *et al*. (2009) while reviewing the literature reported the antimicrobial effect of CA. It produces acidic environment (pH 3.5 to 4) in the digestive tract which inhibit the growth of *E. coli* and *Salmonella* and other gram negative bacteria while it enhances the *Lactobacilli*. It may also activate the proteolytic enzymes, stimulates feed consumption, depresses the microbial metabolites including ammonia, enhances the mineral absorption and decreases the chances of subclinical infections.

In the present article, authors review the effects of CA supplementation in poultry feed to improve the performance of birds. Focal point of this review is to develop the understanding of present status and future perspectives of CA applications in poultry nutrition.

Impact of CA acidification on broiler growth performance: Many studies have been conducted to investigate the effect of CA supplementation in diets on growth performance of broiler chicks. An improved growth performance has been observed in different types of broiler chicks with different levels of CA in different diets. However, growth performance responses to CA supplementation are somewhat inconsistent.

A comprehensive study was undertaken by Bolling *et al*. (2000) to evaluate the effect of supplementation of CA (0.5%), avilamycin (antibiotic growth promoter) and their combination on broiler chicks. A significantly higher body weight, total feed intake and improved feed conversion efficiency was attained in CA fed chicks (Bolling *et al*., 2000). Another possibility of increased feed intake might be the low level of pH created by CA inclusion in the feed and digesta of crop and gizzard. Citric acid may either itself or its resultant low pH stimulates the appetite and controls the nerve endings present in the animals (Atapattu and

Nelligaswatta, 2005). In the study of Islam *et al*. (2008), broiler fed 0.5% CA containing diets gained more weight, consumed more feed and achieved higher feed conversion ratio (FCR). Haque *et al*. (2010) also observed significantly increased weight gain, feed intake and feed efficiency with 0.5% CA level in chicks fed on standard corn-soybean based starter diet. Positive results in terms of growth responses of CA supplementation were also reported by Atapattu and Nelligaswatta (2005) and Ghazalah *et al*. (2011) (Table 1).

In contrast, no or negative responses to CA supplementation with respect to growth and feed performances were observed in other studies. Brenes *et al.* (2003) observed a significant decrease in weight gain and feed consumption (4% in each case) in chicks for the feed supplemented with 2% CA (Brenes *et al*., 2003). High concentration of CA in the diet might have some negative effect on palatability which causes decrease in feed intake (Al-Sharafat *et al*., 2009; Esmaeilipour *et al*., 2012). This decreased feed intake in turn may cause lower body weight (Al-Sharafat *et al*., 2009). Esmaeilopour *et al*. (2012) observed decreased feed intake (15.4%) and weight gain (11.8%) at higher level (4%) of CA supplementation while its lower dose (2%) had no effect on weight gain of broilers in wheat based diet at 24thday of age. Atapattu and Nelligaswatta (2005) found that inclusion of CA had no significant effect on growth performance and feed conversion ratio of broiler chicks fed on rice by product based diet. Higher concentration of phytates, trypsin inhibitor, fiber and low amino acid digestibility were the probable responsible for the poor growth in rice by product based diet. Similar observations regarding the negative effects of CA supplementation to the broiler diet were made by Biggs and Parsons (2008), Liem*et al*. (2008) and Al-Shrafat*et al*. (2009).

Effect of CA on bone mineralization: Phosphorus performs vital role in metabolism, growth and development of birds. Plant proteins are currently being used in poultry feed which contain phytate as P source which is poorly digested by mono-gastric animals. This undigested phytate P is discharged to environment which causes environmental pollution and to compensate this P loss, expensive in-organic sources of P are added (Selle and Ravindran, 2007). To overcome environmental problems and make a less expensive diet, several feed additives have been investigated. Different studies conducted on different types of broiler chicks have shown positive responses to CA supplementation in improving P utilization (Rafacz-Livingston *et al*., 2005; Chowdhury *et al*., 2009; Haque *et al*., 2010).

Increase in tibia ash in CA fed poultry birds has been reported by several authors and considered to be a good indicator of bone mineralization. Enhanced tibia ash was observed by addition of 0.5%(Chowdhury *et al*., 2009; Haque *et al*., 2010) and 3% CA (Rafacz-Livingston *et al*., 2005a; Rafacz-Livingston *et al*., 2005b) in a P-deficient corn-soybean meal (C-SBM) based diet. In another study, 2% CA addition to a P deficient rice by-product based diet increased toe ash percentage (Attapattu and Nelligaswatta, 2005). Rafacz-Livingston *et al*. (2005) reported a linear increase in tibia ash as CA concentration increased from 0% to 4% in C-SBM based diet.

Level of available phosphorus in the diet affects its contents in tibia. Several experiments were conducted by keeping available P at low levels (0.09 – 0.26%) to investigate its contents in tibia ash. Acidification of diet with CA at 3% level released 0.03% P from C-SBM based diet (Snow *et al*., 2004) while 0.049% P was released from DDGS (Dried Distillers Grains with Solubles) based diet (Martinez-Amezcua *et al*., 2006). Addition of CA in the poultry diet at the level of 2.5% improved the P digestibility (Nezhad *et al*., 2011) and its contents in tibia ash (Al-Sharafat *et al*., 2009; Nezhad *et al*., 2011). Improved P retention was also noticed in chicks fed C-SBM based diet (Liem *et al*., 2008) (Table 2).

Effect of citric acid on other nutrients: Phytic acid (anti-nutritional factor) has found to form complexes with protein (Gifford and Clydesdale, 1990) and it also inhibit the activities of trypsin and pepsin (Caldwell, 1992). Citric acid physically affects the chemical bonds of phytic acid with amino acids, protein and fiber which might make them more accessible to endogenous enzymes (Attapattu and Nelligaswatta, 2005).

Ghazalah *et al*. (2011) reported improved nutrient digestibility and metabolizable energy by feeding 2% CA in C-SBM based diet. In another trial, 2% CA increased crude protein digestibility in chicks fed rice by-products based diets. These organic acids might had reduced the gastric pH which in turn increased pepsin proteolysis (Kirchgessner and Roth, 1982) and resulted peptides triggering the release of hormones including gastrin and cholecystokinin, which finally regulated the digestion and absorption of proteins. Crude fiber digestibility also significantly increased by CA inclusion (Attapattu and Nelligaswatta, 2005) in chick diet.

Effect of citric acid on pH and gut microflora: Rahmani and Speer (2005) after reviewing the literature reported that gastrointestinal normal microflora plays an important role in health and well-being of poultry. Pathogenic microbes including *Escherichia coli* have been reported to reduce the growth of poultry by producing toxins, sharing the host nutrients and removing the beneficial microbes necessary to synthesize vitamins or growth factors essential for host. Like other vertebrates a tubular gastrointestinal system but with variations including crop (for feed storage), proventriculus (simple stomach), gizzard and paired ceca is found in poultry (Duke, 1986). Different sections of gut have different pH values such as crop have 4.5, proventriculus 4.4, gizzard 2.6, duodenum 5.7 to 6.0, jejunum 5.8, ileum and colon 6.3, ceca 5.7 and bile 5.9 (Farner, 1942). Different sections of gut, having different values of pH establishes a specific microbial population. A correlation is found between the pH profile of gastrointestinal system and growth performance of the chick. As pH lowers, body weight and FCR and ratio of *Lactobacillus* to *E. coli* are enhanced (Rahmani and Speer, 2005). This lowered pH prevent the growth of E. coli, make the absorptive area more effective (Dofing and Gottschal, 1997) and make the nutrients more available (Boling *et al*., 2001) which results in better performance.

Ghazalah *et al*. (2011) observed significantly lower pH in crop, gizzard, duodenum, jejunum and ileum in CA fed chicks. Reduced pH in crop by CA addition in poultry chicks has also been observed by Atapattu and Nelligaswatta (2005), Ao *et al*. (2009), and Esmaeilpour *et al*. (2012). However, no effect of CA on pH of next gut parts including gizzard, duodenum, jejunum, ileum and caecum contents was reported by Atapattu and Nelligaswatta (2005) and Esmaeilpour *et al*. (2012). Aydin *et al*. (2010) also reported no alteration in the pH of ileal contents by CA addition in the diet. Lack of effect on gut pH might be due to little appearance of organic acid in the lower parts of digestive tract (Hume *et al*., 1993; Thompson and Hinton, 1997).

A significantly higher number of *Lactobacillus* and colliforms were found in caecal contents at 2% CA while *E. coli* was significantly lower at all treatments (1%, 2% and 3%)(Ghazalah *et al*., 2011). Similarly, at different levels (0.2, 0.4, 0.6, and 0.8%) of CA, reduction in coliform bacteria and total bacterial count but slight increase in *Lactobacilli* was noticed (EL-Afifi *et al*. 2001). In another experiment conducted by Vogt *et al*. (1982) 2% CA increased coliform bacteria in small intestine of broiler. Acid-sensitive bacteria adapted well to moderately low pH and hence survived in moderate to high acidic conditions (Foster, 1995).

Effect of citric acid on immune status: Organic acid supplementation to the chick diet has been found to improve the immune status of fish. Chowdhury *et al*. (2009) reported increased number of immunocompetent cells in chicks fed on 5% CA supplemented diet which indicates strong immune status and health to fight against infectious diseases and enteric pathogens. Haque *et al*. (2010) observed an improvement in the density of lymphocyte in lymphoid organs and tissues in chick birds at 0.5% CA supplementation level in a standard C-SBM based basal starter diet which clearly suggested stronger immune status to fight against pathogens (Khan *et al*., 2008).

Combined effect of citric acid and phytase: In poultry industry, cereals and oilseed meals are commonly used in feed as protein sources. However, these plant proteins have anti-nutritional factors such as phytate (*myo*-inositol-1,2,3,4,5,6-hexakisphosphates) which contains up to 80% of the total P in plants which is practically not available to mono-gastric animals (National Research Council, 1993). It also reduces the availability of cations (Sohail and Roland, 1999), carbohydrates, amino acids (Ravindran *et al*., 1999), enzymes (Sebastian *et al*., 1998) and also lowers the fat digestibility (Ravindran and Bryden, 1999). The enzyme system responsible for the hydrolysis of this phytate is lacking in most of the animal groups.

Phytases (PHYs), is a group of enzymes, specific to hydrolyze phytate present in plants. It is pH dependent enzyme and performs optimally at pH 2.5 and 5.5 (Simons *et al*., 1990). Brenes *et al*. (2003) has the opinion that gut acidity of chicken is not suitable for complete hydrolysis of phytate by PHYs. Inclusion of CA in PHY treated diet may lower the intestinal pH and may help to enhance the PHY efficiency (Nourmohammadi *et al*., 2011). Many studies have been conducted to investigate any possible synergistic effect of CA with PHY on performance and mineral utilization in broiler (Boling *et al*., 2000), pig (Radcliffe *et al*., 1998) and fish (Phromkunthong *et al*., 2010). Deepa *et al*. (2011) observed a synergistic effect between CA and PHY for the growth performance of broilers. By using 2% CA and 800 U of PHY, improved weight gain, feed intake and feed conversion ratio were recorded. In another study, Boling *et al*. (2001) also observed synergism between CA and PHY for growth performance of chick.

It is also indicated that microbial PHY is more efficient in diets where no or low levels of supplemented inorganic P is present (Qian *et al*., 1996). Nezhad *et al*. (2011) conducted an experiment investigate the interaction between CA (2.5 and 5%) and microbial PHY (500 FTU/kg) on the left tibia bone Ca content in broilers fed C-SBM based diet deficient in P. At 500 FTU/kg PHY level, supplementing 2.5% CA improved the left tibia bone Ca but at 5% CA, Ca content was decreased probably due to intense decline in the intestinal pH ultimately exerting negative effect on microbial PHY and phosphatases activity. A combined effect of PHY, CA and 1α-(OH) D_3 was also investigated and resulted in 0.13% P release in C-SBM based diet (Snow *et al*., 2004).

Improved tibia ash (Boling *et al*., 2000; Boling *et al*., 2001), enhanced P, Ca and N retention (Deepa *et al*., 2011), increased digestibility of total and phytate P (Woyengo *et al*., 2010), improved protein and P deposition (Sukria and Liebert, 2004) and significant interaction between CA and PHY on *Enterococcus* counts in the ileal digesta (Aydin *et al*., 2010) was found in different studies with different combinations of CA and PHY.

In contrast, no synergistic effect of microbial PHY and CA was observed on amino acid digestibility (Centeno *et al*., 2007). The reason might be that CA complexes with Ca and prevent its binding with phytate hence hydrolysis of phytate increases which results in additional P release that maydecreases the effectiveness of PHY (Ballam *et al*., 1984; Yi *et al*., 1996; Ravindran *et al*., 2000). While in the study of Brenes *et al*. (2003) growth response to PHY was negatively affected by CA addition. A possible explanation of negative growth response to PHY with CA supplementation mainly in highest enzyme concentration might be that CA makes complex with Ca and decreases its binding to phytate, makes the phytate hydrolysis easy and result in liberation of P which imbalance the Ca: AP (available P) ratio that causes negative effect on performance. A negative response of CA and microbial PHY interaction to P in tibia ash was also observed by Al-Sharafat *et al*. (2009) where P was reduced as compared to PHY alone. The review of literature indicates that a proper combination of CA and PHY may present a practical solution to improve growth performance, phytate P and other mineral utilization (Deepa *et al*., 2011) (Table 3).

Table 1. Impact of citric acid supplemented feed on growth and feed performance of broilers.

Rf.[a] No.	Contributors	CA[b] (g/kg)	Period (Days)	Age (Days)	Strain	Diet	Diet Proximate Composition: ME[c] (kcal/g)	CP[d] (g/kg)	Ca[e] (g/kg)	Total P[f] (g/kg)	AP[g] (g/kg)	Observations
1	Brenes *et al*., 2003	20	21	1	Cobb	M-SBM	3.104	230	-	-	-	4% decrease in weight gain and feed consumption
2	Attapattu and Nelligaswatta, 2005	20	21	20	-	RBPBD[j]	3.175	200	7.4	9	3	No significant effect on daily feed intake, growth performance and feed conversion ratio while 2% dietary CA increased feed intake
3	Biggs and Parsons, 2008	30 & 40	21	1	New Hampshire × Columbian	C-SBM	-	230	10	-	4.5	4% showed reduction in growth, 3% had no significant effect on growth
4	Islam *et al*., 2008	5	35	1	Hubbard classic	M-SBM[i]	3.241	222.1	-	-	-	Gained more weight, consumed more feed and had higher feed conversion.
5	Liem *et al*., 2008	32.3	16	1	Cobb × Cobb 500	C-SBM	3.13	232.7	10	5	2.6	No increase in body weight and feed gain:
6	Chowdhury *et al*., 2009	5	35	1	Hubbard classic	C-SBM[h]	3.278	227.1	9.8	7.4	-	Improved body weight and total feed intake and feed conversion efficiency
7	Al-Sharafat *et al*., 2009	25	105	161	Lowman Brown-Classic laying hens	C-SBM	2.761	164	39.2	3.29	-	Decreased feed intake, reduced hen body weight, improved feed conversion ratio
8	Haque *et al*., 2010	5	35	1	Hubbard classic	C-SBM	3.28	227.1	9.5	7.1	-	Increased weight gain, feed intake and feed efficiency
9	Ghazalah *et al*., 2011	10, 20 & 30	42	1	Arbor-Acres	C-SBM	Diet 1- Starter diet (1-14 d)					Increased body weight, European production efficiency index and improved FCR
							3.096	220	10	-	4.99	
							Diet 2- Grower diet (15-28 d)					
							3.159	200.7	9	-	4.5	
							Diet 3- Finisher diet (29-42 d)					
							3.192	180.2	8.48	-	4.2	
10	Esmaeilipour *et al*., 2012	20 & 40	24	1	Ross	W-SBM[k]	Diet 1- Starter diet (1-10 d)					4% CA decreased the feed intake and body weight gain while 2% concentration decreased feed intake but have no affect on the body weight; gain: feed ratio also increased at both levels.
							2.913	226	10.1	-	4.8	
							Diet 2- Grower diet (11-24 d)					
							3.009	209.5	8.6	-	4.3	

Only concerning diets are mentioned. [a] Reference No. [b] Citric acid [c] Metabolizable energy, [d]Crude protein, [e] Calcium, [f] Total Phosphorus, [g] Available Phosphorus, [h] Corn-soybean meal based diet, [i] Maize-Soybean meal based diet, [j] Rice by-product based diet, [k] Wheat-meal based diet

Table 2: Impact of citric acid supplemented feed on bone mineralization of broilers.

Rf.[a] No.	Contributors	CA[b] (g/kg)	Period (Days)	Age (Days)	Strain	Diet	Calculated analyses ME[c] (kcal/g)	CP[d] (g/kg)	Ca[e] (g/kg)	Total P[f] (g/kg)	AP[g] (g/kg)	Observation
1	Snow *et al*., 2004	30	13	8	New Hampshire × Columbian	C-SBM	3.260	237	6.2	3.9	1.3	Released 0.03% P from a corn soybean meal based diet
2	Attapattu and Nelligaswatta, 2005	20	21	20	-	RBPBD[i]	3.175	200	7.4	9	3	Increased toe ash percentage
3	Rafacz-Livingston *et al*., 2005a	30	14	8	New Hampshire × Columbian	C-SBM	3.190	230	7.5	4.5	1.6	Increased tibia ash
4	Rafacz-Livingston *et al*., 2005b	30	14	8	Ross × Ross	C-SBM	3.190	230	8	4.6	1.8	Increased tibia ash
5	Martinez-Amezcua *et al*., 2006	30	13	8	New Hampshire × Columbian	DDGS[j]	3.200	190	8.5	2	1.2	Increased tibia ash
6	Liem *et al*., 2008	32.3	16	1	Cobb × Cobb 500	C-SBM	3.130	232.7	10	5	2.6	Increased bone ash
7	Al-Sharafat *et al*., 2009	25	15	23	Lowman Brown-Classic laying hens	C-SBM	2.761	165.7	27.6	3.3	-	Increased P content in tibia ash
8	Chowdhury *et al*., 2009	5	35	1	Hubbard classic	C-SBM[h]	3.278	227.1	9.8	7.4	-	Increased tibia ash
9	Haque *et al*., 2010	5	35	1	Hubbard classic	C-SBM	3.280	227.1	9.5	7.1	-	Increased tibia ash
10	Nezhad *et al*., 2011	25 & 50	49	1	Ross 308	C-SBM	2.875	202.5	9	4.5	2	Improved P contents in tibia bone

Only concerning diets are mentioned. [a] Reference No. [b] Citric acid [c] Metabolizable energy, [d]Crude protein, [e] Calcium, [f] Total Phosphorus, [g] Available Phosphorus, [h] Corn-soybean meal based diet, [i] Rice by-products based diet, [j] Dried distillers grains with solubles

Table 3. Synergistic effect of citric acid and phytase on growth performance and mineral metabolism in feed of broilers.

Rf[a]No.	Contributors	CA[b] (g/kg)	PHY[c] (FTU/kg)	PHY company	Period (Days)	Age (Days)	Strain	Diet	Diet Proximate Analysis ME[d] (kcal/g)	CP[e] (g/kg)	Ca[f] (g/kg)	Total P[g] (g/kg)	AP[h] (g/kg)	Observation
1	Snow *et al*., 2004	30 & 40	300	-	13	8	New Hampshire × Columbian	C-SBM	32.6	237	6.2	3.9	1.3	0.13% P released from C-SBM based diet
2	Sukria and Liebert, 2004	30	500	SP-1002 ct	35	1	-	C-SBM	32	218	-	4.6	1.6	Improved protein and P deposition significantly; Improved feed intake and growth, FCR was not enhanced
3	Centeno *et al*., 2007	20 & 50	750	Natuphos	3	1	Cobb	M-SBM[j]	30.4	230	8	6	2.5	No synergistic effect of microbial phytase and CA was observed for amino acids digestibility
4	Al-Sharafat *et al*., 2009	25	300	Ronozyme	15	161	Lowman Brown-Classic laying hens	C-SBM	27.61	164	3.92	3.3	-	Reduced Pin tibia ash as compared to phytase alone
5	Aydin *et al*., 2010	30	750	-	29	13	Ross 308	C-SBM	-	-	-	-	-	Increased *Enterococcus* counts in the ileal digesta
6	Woyengo *et al*., 2010	5	600	Ronozyme	21	1	Ross strain	C-SBM	29.59	231	8.1	5.7	2	No significantly improved tibia ash but increased digestibility of total and phytate P and enhanced gross energy
7	Deepa *et al*., 2011	20	800	-	42	1	-	C-SBM	Diet 1- Starter diet					A significantly improved weight gain, feed intake and feed conversion ratio. Also improved P, Ca and N retention significantly
									30.7	215	9.5	5.4	3	
									Diet 2- Finisher diet					
									32	190	8.9	5.3	3	
8	Nezhad *et al*., 2011	25	500	Natuphos	49	1	Ross 308	C-SBM[i]	28.75	202.5	9	4.5	2.	Improved left tibia bone Ca and Ca digestibility

Only concerning diets are mentioned. [a] Reference No., [b] Citric acid, [c] Phytase, [d] Metabolizable energy, [e] Crude protein, [f] Calcium, [g] Total phosphorus, [h] Available phosphorus, [I] Corn-soybean meal,Maize-soybean meal

Conclusion: On the basis of reviewed literature, it is concluded that CA is a promising supplement to improve growth and feed performance, bone mineralization and gut health of poultry birds. Hence, it is suggested that CA supplementation can be used in poultry feed to improve production.

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