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Effect of Phytase and/or Citric Acid on Productive Performance and Blood Phosphorus Level in Broiler Chicks (part I.)

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Abstract
The present study was carried out to investigate the effect of phytase and/or citric acid (CA) on productive performance and blood phosphorus (P) in broiler chicks. For this purpose 120 male chicks 8 days old were divided into 4 groups with 3 replicate cages having 10 birds in each. The first group was fed on a basal diet without any additives and kept as a control, the second, third and forth groups were fed on a basal ration supplemented with phytase (0.1g/ kg), CA (10g/kg) or phytase (0.1g/kg) plus CA (10g/ kg), respectively. The results revealed that both feed additives produced a significant improvement in body weight (BW), body weight gain (BWG), feed conversion ratio (FCR) and feed efficiency (FE). All supplemented groups exhibited a significant decrease in FCR and a significant increase in FE at 24-40 days of age. There were no significant difference in the average feed intake (FI) between control group and groups supplemented with phytase or CA. However, FI in group supplemented with mixed additives was significantly lower than other groups at 24-40 days of age. Phytase supplemented group exhibited the highest level of serum P during the whole sampling periods. It was significantly higher than all experimental groups. Moreover, the overall mean of serum P was significantly higher in group supplemented with CA or mixed additives than control group. It is concluded that both phytase and CA improve growth performance of broilers and liberate the phytate-bound P, making P more available to birds.

Key words: broilers, growth performance, phosphorus, citric acid, phytase

Introduction

Growth promoters have been used extensively in animal feeds and water all over the world, especially in the poultry industry (Denli et al., 2003). Phytase, organic acids (citric acid, acetic acid, propionic acid, formic acid), multienzymes, absorption enhancers and probiotics are the most promising alternatives for poultry (Gunal et al., 2006; El-Samannoudy, 2013). Their supplementation in the diet of broilers enhanced nutrient utilization, growth, and feed efficiency (Jin et al., 1998; Denli et al., 2003; Bozkurt et al., 2009).

Phosphorus (P) is the second most abundant mineral in the animal body, approximately 80% of which is found in the bones and teeth. The 20% of P not present in the skeletal tissues is widely distributed in the fluids and soft tissues of the body, where it serves a range of essential functions (Underwood and Suttle, 1999). Approximately two-thirds of P in feedstuffs of plant origin is poorly digested by poultry because it bounds to phytic acid (PA), which is poorly hydrolyzed by endogenous enzymes of poultry (Ravindran, 1995; Punna and Roland, 1999; Viveros et al., 2000). This unavailability is due to the very low phytase activity found in the digestive tract. As a result, inorganic sources of P, which are expensive, are added to feeds to meet P requirements of poultry.In addition, the unabsorbed PA-bound P is discharged to environment, leading to environmental pollution (Selle and Ravindran, 2007). Moreover, phytic acid has been shown to complex with various nutrients, such as protein, starch, and minerals, which negatively affect the utilization of these nutrients by monogastric animals (Cosgrove, 1966). It was also reported to reduce solubility of protein at stomach pH (Kies et al., 2006), solubility of protein in the presence of Ca at small intestinal pH (Prattley et al., 1982), gastric pepsin activity in piglets (Woyengo et al., 2010b), trypsin activity in vitro (Singh and Krikorian, 1982), intestinal amylase activity in chickens (Liu et al., 2008), and intestinal lipase activity (Liu et al., 2010). The negative effects that are associated with PA can be alleviated, in part, by theuse of exogenous phytase, which degrades PA (Selle and Ravindran, 2007). Dietary supplementation with phytase is well established as an effective and practical method of improving phytate digestibility in production animals. Phytases are naturally found in a number of seeds including cereals, legume by-products, and other feed stuffs

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(Viveros et al., 2000) and in microbial sources (Wyss et al., 1999). Supplementation of diets with microbial phytase increases availability of phytate P and Zn in chicks (Ravindran et al., 2000) as well as to improve the utilization of other nutrients that are bound to plant phytate (Ravindran et al., 2006). Phytase has been shown to improve phytate P use based on decreased P excretion, increased P retention, and increased chick growth performance and bone ash (Denbow et al., 1995; Qian et al., 1997).

The addition of organic acids, such as citric, formic, and fumaric acids, is known to lower diet acidity. Because the site of phytase activity is primarily the stomach (Yi et al., 1996), lowering the dietary pH might reduce the pH of the stomach digesta and thereby increase the effectiveness of microbial phytase. Supplementation of CA alone has been shown to improve performance and bone mineralization of broilers fed low-P diets (Boling et al., 2000), indicating improved utilization of P due to CA addition. The efficacy of phytase with regard to hydrolysis of PA is, however, limited, in part, by the presence of divalent cations like Ca in practical poultry diets, which form insoluble complexes with PA at pH found in the small intestine (Selle and Ravindran, 2007). Addition of organic acids to phytase supplemented diets was found to improve the efficacy of phytase because the organic acids chelate multivalent cations like Ca, thereby reducing the amount of the cations that are available for binding to PA (Boling et al., 2000). Furthermore, the organic acids reduce the pH of the digesta (Radcliffe et al., 1998), which can result in increased dissociation between PA and minerals (Maenz et al., 1999) and thus increase phytase efficacy due to more acidic pH (Simon and Igbasan, 2002). A proper combination of citric acid and phytase may represent a practical solution to improving phytate-P utilization and decreasing P levels in poultry excreta. Citric acid was found to be markedly efficacious for improving the utilization of phytate-phosphorus in broiler chicks (Boling et al., 2000). Snow et al. (2004) reported improved growth performance and bone mineralization of broilers due to addition of CA to a phytase supplemented low-P diet, whereas, (Brenes et al., 2003; Woyengo et al., 2010a) recorded that addition of CA to the phytase-supplemented diet did not result in a significant improvement in growth performance and bone mineralization in broilers. Considering the above statements, the present study was designed to evaluate the effect of phytase, citric acid and their combination on growth performance and blood P level in broiler chicks.

Materials And Methods

2.1 Birds

Two hundreds and thirty, one-day-old chick (Cobb strain) were used in this study. Chicks were kept on lighting regimen of 23 hrs light daily (Mousa, 2008) and temperature were kept at 31°C using air warmers during the first 2 weeks then decreased gradually till 29°C according to recommendations obtained from the providing company. The chicks were handled carefully to avoid any pain or harm. Chicks were fed on starter ration during the first two weeks, grower ration during the next two weeks and finisher ration during the rest of the 40 days experiment (table 1) At 8 days of age, it could be able to distinguish between male and female chicks. Females were excluded and the experiment was continued on males only.

2.2Feed Additives

2.2.1Phytase enzyme (Microtech 5000): A concentrated phytase enzyme was used (5000 U/gm). It was a gift from Dr.Hamed El-Banna, Delta Vet.Cennter, Cairo, Egypt.

2.2.2 Citric acid: Citric acid used in this study was obtained from El Gomhoria Company, Egypt.

2.3 Experimental Design

On the 8th day of age, 120 male chicks having approximately the same weight (500 - 633g) were chosen and randomly divided into 4 equal groups of 30 birds each with 3 replicate cages having 10 birds in each. They were treated as follows: The first group was kept as a control group and fed on a basal ration without any additives. The second group was fed on a basal ration to which phytase powder was added at a dose equal to 0.1g / kg as recommended by Delta Vet Company. The third group was fed on a basal ration to which 1% citric was added. The fourth group was fed on a basal ration to which phytase powder (0.1g / kg) and citric acid (10g / kg) were added.

2.4 Blood Sampling

Serum samples were collected from 10 chicks in each group at 16, 24 and 40 days of the experiment by slaughtering. Sera were obtained and stored at -20°C till assays were carried out.

Table 1: Composition of percentage and calculated nutrients profile of the basal diets.

Ingredients %	Starter (1-15 day)	Grower (16-30 day)	Finisher (31-40 day)
Yellow corn	51.70	56.15	61.15
Corn gluten meal	5.00	5.00	5.00
Soybean meal (44% CP)	37.30	31.50	25.90
Soy oil	2.20	3.50	4.00
Dicalcium phosphate	1.60	1.60	1.70
Limestone	1.40	1.45	1.44
Common salt	0.40	0.40	0.40
DL-Methionine	0.05	0.05	0.06
L-Lysin	0.05	0.05	0.05
Vitamin&mineral premix*	0.30	0.30	0.30
Calculated analysis:			
ME (Kcal/kg)	2951.80	3049.55	3124.07
Crude protein%	23.20	21.29	19.00
Crude fat%	6.00	6.92	8.00
Crude fiber%	4.50	4.80	5.20
Calcium%	1.00	1.00	1.00
Non-phytate phosphorus%	0.45	0.45	0.45

*Per kg premix: 1 200 000 IU-vit. A, 350 000 IU - vit.D_3, 4 000 mg - vit.E, 250 mg - vit.B_1,800 mg - vit.B_2, 600 mgvit. B_6, 3.2 mg-vit. B_{12}, 450 mg - vit. K_3, 4.5 g nicotinic acid, 1.5 g Ca-pantothenate, 120 mg folic acid, 5 mg biotin, 55 g choline chloride, 3 g Fe, 2 g Cu, 10 g Mn, 8 g Zn, 120 mg I, 40 mg Co.

2.5 Measured parameters

2.5.1 Growth parameter: The initial body weights of all birds on the 8th day of age were measured. The weekly changes in the live body weight and the daily feed consumption were calculated to compute the following: average weekly body weight gain (g/week), average daily feed intake (g/day), feed efficiency and feed conversion ratio.

2.5.2 Blood phosphorus level: Phosphorus was estimated by the enzymatic colorimetric method of (Fossati, 1985) using Randox kit's reagents (Spain) obtained from Middle East Medical Company, Egypt.

2.6Statistical analysis

All Data were presented as Mean ± Standard error. They were subjected to one way analysis of variance test (ANOVA), followed by the Tukey-Kramer multiple comparison test using statistical analysis system program (Instat-3).

Results and Discussion

No health problems were associated with the use of the feed additives throughout the experiment.

3.1 Effect on growth performance

3.1.1 Average live body weight (BW) and body weight gain (BWG): Tables (2&3) represent the effect of Phytase and/or citric acid on the average live BW and BWG of broiler chicks. No significant differences were observed in the average BW of all groups at 8 (start of the experiment) and 16 days of age. No significant differences were also recorded between control group and group supplemented with mixed additives during the whole sampling periods. Meanwhile, there was a significant increase in the average BW of both phytase and citric acid supplemented groups vs control group ($P \leq 0.001$) starting from 24 days of age up to the end of the experiment (40 days of age). The lowest BW was achieved in birds fed mixed additives. It was significantly lower than groups supplemented with phytase or citric acid at 24 ($P \leq 0.01$ and $p \leq 0.001$, respectively), 32 ($P \leq 0.01$) and 40 days of age ($P \leq 0.05$ and $P \leq 0.01$, respectively).

It appears from table (3) that there was no significant difference in the BWG in all groups at 16 days of age. However, groups supplemented with phytase or citric acid showed a significant gain in BWvs control at 24 ($P \leq 0.001$), 32 ($P \leq 0.01$and $P \leq 0.05$, respectively) and 40 days of age ($P \leq 0.01$ and 0.001, respectively). Meanwhile, there was a significant increase in BWG of group supplemented with mixed additives only at 24and 40 days of age. The overall means of groups supplemented with phytase and citric acid were significantly higher ($P \leq 0.05$) than that of the control group. The combination between phytase and citric acid did not add any further improvement to body weight gain than phytase or citric acid alone.

The phytase-induced improvement in BW and BWG may be attributed to its ability to release phytate –bound nutrients and improve their utilization. Addition of phytase to the diet of poultry has been shown to improve the utilization of other nutrients that are bound to plant phytate (Ravindran et al., 2006) such as protein, starch and minerals, which negatively affect the utilization of these nutrients. Phytic acid has been shown to reduce digestibility of energy yielding nutrients by: 1) binding to proteins in the stomach and small intestine (Maenz, 2001) and (Selle et al., 2009), 2) binding to carbohydrates and lipids in the small intestine (Selle and Ravindran, 2007) and binding to endogenous enzymes and metal cofactors of enzymes involved in hydrolysis of energy yielding molecules. Phytic acid was also reported to reduce pepsin activity in piglets (Woyengo et al., 2010a), trypsin activity in vitro (Singh and Krikorian, 1982), intestinal amylase activity in chicken (Liu et al., 2008) and intestinal lipase activity (Liu et al., 2010).

The observed improvement in BW and BWG induced by CA could be possibly due to better utilization and improvement of the digestibility of nutrients. CA was reported to acidify the gastro intestinal contents leading to increased activity of gastric enzymes (Biggs and Parsons, 2008). Also the reduction of the pH of digesta with various organic acids has been reported to reduce the production of toxic compounds by the bacteria and colonization of the pathogens on the intestinal wall, thus preventing the damage of epithelial cells (Langhout, 2000). Moreover, the use of organic acid has been reported to protect young chicks by competitive exclusion (La Ragione and Woodward, 2003), enhancement of nutrient utilization and growth (Denil, 2003), and decreasing the total bacteria and gram negative bacterial count in broilers (Andrys, 2003). Furthermore, organic acids were reported to improve villus height in the small intestine (Adil et al., 2010) and also have a direct effect on the gastrointestinal cell proliferation (Tappenden and Mcburney, 1998). These histopathological changes in small intestine probably had increased the intestinal surface area, facilitating the nutrient absorption to greater extent and thus boosted the growth promoting effect of CA supplementation.

The pattern of the results obtained during the current study clarified that the combination between phytase and CA did not produce any significant improvement in the average BW than control group and was lower than groups supplemented with phytase and CA individually during most of the sampling periods. Regarding the BWG, the group supplemented with both additives showed a significant increase than control only at 24 and 40 days of age. (Brenes et al., 2003) did not observe improved growth performance and bone mineralization in broilers due to the addition of CA to a phytase supplemented low-P diet, whereas, (Snow et al., 2004) reported improved growth performance and bone mineralization of broilers due to addition of CA to phytase supplemented low P diet only when vitamin D was added to the diet. (Jin et al., 1998) also found that there was no effect of adding CA to the phytase supplemented group on BWG. The authors attributed the lack of effect of adding CA to phytase supplemented diets on broiler performance to the fact that the dietary Ca was not adequate enough to allow for utilization of P that was made available by CA. The lack of a beneficial effect of combining CA and phytase reported in the present study is not clear. It may be due to the reduction in feed intake observed in the group supplemented with mixed additives; however, this point needs further investigation.

3.1.2 Average daily feed intake: As shown from table (4) there were no significant differences in the average daily feed intake between control group and groups supplemented with phytase and CA throughout the sampling periods. However, the average daily feed intake in group supplemented with mixed additives was significantly lower than control group and groups supplemented with phytase or CA at 24 and 32 ($P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.001$, respectively) days of age. It was also significantly lower than control group and group supplemented with phytase ($P \leq 0.05$) at 40 days of age.

Table (2): Effect of phytase and / or citric acid on average body weight (g) in broiler chicks

Age/days	Control	Phytase	Citric acid	Mixed additives
8	221.2±2.6	220.1±2.2	226.1±2.4	221.0±2.2
16	591.6±6.0	606.8±7.4	608.0±6.4	592.3±10.0
24	1100.3±24.1^{ab}	1223.7±14.4^{ac}	1256.0±13.7^{bd}	1144.0±16.0^{cd}
32	1674.8±26.7^{ab}	1831.8±33.0^{ac}	1831.1±29.5^{bd}	1681.7±23.6^{cd}
40	1995.6±40.5^{ab}	2216.4±38.8^{ac}	2223.6±32.9^{bd}	2055.2±32.3^{cd}

- Each value is expressed as Mean ± Standard Error
- Means having the same letter in the same raw are significantly different.

Table (3): Effect of Phytase and/or Citric Acid on Average Live Weight Gain (g) in Chicks

Age/days	Control	Phytase	Citric acid	Mixed additives
16	370.43±3.6	386.68±5.3	381.95±4.4	371.13±8.0
24	500.0±19.0^{abc}	611.5±7.4^{ad}	648.0±8.1^{be}	545.2±7.2^{cde}
32	521.4±11.3^{ab}	596.0±18.8^{ac}	581.6±14.8^{b}	535.7±8.5^{c}
40	252.7±17.9^{abc}	305.4±11.0^{a}	324.9±6.9^{b}	309.8±14.5^{c}
Overall mean	384.3±8.6^{ab}	421.6±10.2^{a}	419.2±8.8^{b}	399.1±8.1

- Each value is expressed as Mean ± Standard Error
- Means having the same letter in the same raw are significantly different.

These results are matching with those of (Assuena et al., 2009; Junqueira et al., 2011) who found that feed intake in broiler chicks was not affected by adding different levels of phytase to broilers diet. Moreover, (Hernández et al., 2006; Nezhad et al., 2007) could not detect any significant effect on feed intake in broilr chicks fed a diet supplemented with CA. In the same concern (Nourmohammadi et al., 2012) reported that Addition of 6% CA to broilers diets significantly decreased feed intake compared with diets without acid and the diets having 3% CA. On the other hand some investigators found that addition of phytase (Dilger et al., 2004; Deepa et al., 2011) and CA (Andrys, 2003) to broilers feed increased fed consumption. Moreover this study was also not in accordance with those of (Andrys, 2003; Islam, 2012) who reported that supplementation of phytase and CA either alone or in combination in the basal diet clearly increased feed intake in broiler chicks. These contradictory results may be attributed to the differences in the doses of phytase and CA and/or the difference in the type of phytase used. The decrease in feed intake reported in the group supplemented with mixed additives in this study may explain the reason for the improvement of FCR and FE although the body weight was not changed.

Table (4): Effect of Phytase and/or Citric Acid on Average Daily Feed Intake (g) in Chicks

Age/days	Control	Phytase	Citric acid	Phyt.+Cit.acid
16	77.8±6.7	79.0±6.4	82.3±6.5	75.9±5.8
24	116.2±3.8^{a}	104.7±2.6^{b}	108.3±1.7^{c}	88.4±3.5^{abc}
32	142.2±2.1^{a}	133.9±1.2^{b}	142.0±2.3^{c}	120.0±3.3^{abc}
40	169.4±2.7^{a}	169.0±7.9^{b}	165.3±4.8	143.5±8.6^{ab}

- Each value is expressed as Mean ± Standard Error
- Means having the same letter in the same raw are significantly different.

3.1.3 Feed conversion ratio: The effect of phytase and/or citric acid on feed conversion ratio of broiler chicks is presented in table (5). There was a significant decrease in feed conversion ratio at 16 days of age in phytase and phytase + citric acid supplemented groups as compared to control group ($P\leq0.05$ and 0.01, respectively). Meanwhile at 24, 32 and 40 days of age the feed conversion ratio was significantly lower in all supplemented groups than that of control group ($P\leq0.001$). These results are in agreement with (Deepa et al., 2011) who reported that supplementation of phytase and CA independently in the basal diet showed lower FCR in broiler chicks. Moreover, (Cowieson et al., 2006) observer better FCR with phytase supplementation. Meanwhile, the results on the beneficial effect of CA on FCR have been reported by several researchers

(Andrys, 2003; Abdel-Fattah et al., 2008; Adil et al., 2010)

3.1.4 Feed efficiency: It is clear from table (6) that Supplementation of broiler chicks with phytase and/or citric acid significantly increased feed efficiency vs control group in group supplemented with mixed additives at 16 days of age and in all supplemented groups starting from 24 days of age up to the end of the sampling periods. These results are in agreement with some investigators who reported an increase in FE in chicks fed diets with added phytase (Dilger et al., 2004). Moreover some other studies support the present study concerning the beneficial effect of CA on improving FE in broiler chicks. (Denli et al., 2003; Nezhad et al., 2007; Abdel-Fattah et al., 2008; Chowdhury et al., 2009). Contradictory to the present results (Viveros et al., 2002) stated that FE was not affected at any stage by addition of phytase to broilers feed. The recorded increase in FCR and FE reported in the current study in the group supplemented with mixed additives is due to the increase in BWG, which was accompanied by a decrease in the quantity of feed intake.

Table (5): Effect of Phytase and/or Citric Acid on Feed Conversion Ratio in Broiler Chick

Age/days	Control	Phytase	Citric acid	Mixed additives
16	2.0±0.02^{ab}	1.9±0.03^{a}	2.0±0.02	1.9±0.03^{b}
24	2.4±0.1^{abc}	1.6±0.02^{a}	1.6±0.02^{b}	1.5±0.02^{c}
32	2.2±0.05^{abc}	1.8±0.06^{ad}	2.0±0.05^{bd}	1.8±0.03^{c}
40	6.1±0.5^{abc}	4.6±0.2^{a}	4.1±0.1^{b}	3.8±0.2^{c}

Table (6): Effect of Phytase and/or Citric Acid on Feed Efficiency in Broiler Chick

Age/days	Control	Phytase	Citric acid	Mixed additives
16	0.506 ± 0.005^{a}	0.534 ± 0.007	0.516 ±0.006	0.543±0.012^{a}
24	0.445 ± 0.017^{abc}	0.610 ± 0.007^{ad}	0.615 ± 0.008^{b}	0.655 ± 0.009^{cd}
32	0.458 ± 0.010^{abc}	0.543 ± 0.018^{ad}	0.512 ± 0.013^{bd}	0.549 ± 0.009^{c}
40	0.187 ± 0.013^{abc}	0.226 ± 0.008^{ad}	0.246 ± 0.005^{b}	0.274 ± 0.012^{cd}

- Each value is expressed as Mean ± Standard Error.
- Means having the same letter in the same raw are significantly different.

3.2 Effect on blood phosphorus

Table (7) clarifies that the overall mean of serum P was significantly higher in group supplemented with phytase than control group ($P \leq 0.001$), CA-supplemented group ($P \leq 0.001$) and group supplemented with mixed additives ($P \leq 0.05$). Meanwhile, phytase-supplemented group exhibited the highest level of serum P during the whole sampling periods. It was significantly higher than control ($P \leq 0.001$) and other supplemented groups. Moreover, the overall mean of P was significantly higher in CA-supplemented group and group supplemented with mixed additives than control group ($P \leq 0.05$ and $P \leq 0.001$, respectively). Results from several studies have shown increased P digestibility and utilization and hence reduced P excretion into the environment due to phytase addition to poultry diets (Applegate et al., 2003; Penn et al., 2004; Angel et al., 2006) Phytase supplementation was also reported to increase plasma P level (Underwood and Suttle, 1999; Viveros et al., 2002) and improved P (Adil et al., 2010) in broiler chicks. Moreover, (Denil, 2003; Dilger et al., 2004) further added that phytase supplementation improved ilial phytate and total P digestibilies and retention of total P due to liberation of P from phytic acid molecule.(Zhou et al., 2008) attributed the phytase-induced increase in blood P level to its ability to act on the phosphate group associated with the inositol ring of the phytic acid backbone and libratesphytate-bound P, making more P available to the bird. Moreover adding different levels of CA was reported to induce an increase in P level in blood serum (Abdel-Fattah et al., 2008; Adil et al., 2010).CA supplementation to broilers feedwas also reported to increase significantly tibial ash indicating improved utilization of P (Mousa, 2008; Kalafova et al., 2014). This CA-induced increase in serum P level may be attributed to the lowering gastrointestinal pH, which increases its absorption from the gut into blood stream (Abdel-Fattah et al., 2008).

Table (7): Effect of phytase and/or citric acid on phosphorus level (mg %) in the serum of broiler chicks

Age/days	Control	Phytase	Citric acid	Mixed additives
16	5.5 ± 0.2^{ab}	9.1 ± 0.6^{acd}	6.5 ± 0.3^{ce}	10.9 ± 0.4^{bde}
24	4.5 ± 0.2^{abc}	7.8 ± 0.4^{a}	9.0 ± 0.5^{b}	7.4 ± 0.6^{c}
32	6.0 ± 0.4^{a}	10.3 ± 0.7^{abc}	6.0 ± 0.3^{b}	7.1 ± 0.2^{c}
40	6.6 ± 0.2^{a}	10.5 ± 0.5^{abc}	6.7 ± 0.4^{b}	6.7 ± 0.7^{c}
Overall mean	5.6 ± 0.2^{abc}	9.5 ± 0.4^{ade}	7.0 ± 0.3^{bd}	8.0 ± 0.4^{ce}

- Each value is expressed as Mean ± Standard Error
- Means having the same letter in the same raw are significantly different

References

Abdel-Fattah S, El-Sanhoury M, El-Mednay N and Abdel-Azeem F (2008) Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int J Poult Sci 7:215-222.

Adil S, Banday T, Bhat GA, Mir MS and Rehman M (2010) Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Veterinary medicine international**2010**:1-7.

Andrys R, D. Klecker, L. Zeman, and E. Marecek (2003) The effect of changed pH values of feed in isophosphoric diets on chicken broiler performance. Czech Journal of Animal Science**48**:197-206.

Angel R, Saylor W, Mitchell A, Powers W and Applegate T (2006) Effect of dietary phosphorus, phytase, and 25-hydroxycholecalciferol on broiler chicken bone mineralization, litter phosphorus, and processing yields. Poultry science**85**:1200-1211.

Applegate T, Joern B, Nussbaum-Wagler D and Angel R (2003) Water-soluble phosphorus in fresh broiler litter is dependent upon phosphorus concentration fed but not on fungal phytase supplementation. Poultry science**82**:1024-1029.

Assuena V, Junqueira O, Duarte K, Laurentiz A, Filardi R and Sgavioli S (2009) Effect of dietary phytase suplementation on the performance, bone densitometry, and phosphorus and nitrogen excretion of broilers. Revista Brasileira de Ciência Avícola**11**:25-30.

Biggs P and Parsons C (2008) The effects of several organic acids on growth performance, nutrient digestibilities, and cecal microbial populations in young chicks. Poultry science**87**:2581-2589.

Boling SD, Webel DM, Mavromichalis I, Parsons CM and Baker DH (2000) The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs. Journal of animal science**78**:682-689.

Bozkurt M, Küçükyılmaz K, Çatlı A and Çınar M (2009) The effect of single or combined dietary supplementation of prebiotics, organic acid and probiotics on performance and slaughter characteristics of broilers. South African Journal of Animal Science**39**:197-205.

Brenes An, Viveros An, Arija I, Centeno C, Pizarro M and Bravo C (2003) The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Animal Feed Science and Technology**110**:201-219.

Chowdhury R, Islam K, Khan M, Karim M, Haque M, Khatun M and Pesti G (2009) Effect of citric acid, avilamycin, and their combination on the performance, tibia ash, and immune status of broilers. Poultry science **88**:1616-1622.

Cosgrove D (1966) The chemistry and biochemistry of inositol phosphates. Rev Pure Appl Chem**16**:209- 224.

Cowieson A, Acamovic T and Bedford M (2006) Phytic acid and phytase: implications for protein utilization by poultry. Poultry Science**85**:878-885.

Deepa C, G.P. J and D. C (2011) Effect of Phytase and Citric Acid Supplementation on the Growth Performance, Phosphorus, Calcium and Nitrogen Retention on Broiler Chicks Fed with Low Level of Available Phosphorus Asian Journal of Poultry Science**5**:28-34.

Denbow DM, Ravindran V, Kornegay ET, Yi Z and Hulet RM (1995) Improving phosphorus availability in soybean meal for broilers by supplemental phytase. Poultry science**74**:1831-1842.

Denil M, F. Okan, and K. Celik (2003) Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. Pak J Nutr**2**:89-91.

Dilger R, Onyango E, Sands J and Adeola O (2004) Evaluation of microbial phytase in broiler diets. Poultry science**83**:962-970.

El-Samannoudy (2013) Physiological studies on the effect of exogenous digestive enzymes and some absorption enhancers on grpwth performance and some metabolic parameters in broilers. MVSC, Fac of Vet Med, Cairo university.

Fossati P (1985) Phosphate determination by enzymatic colorimetric assay. Analytical biochemistry**149**:62-65.

Gunal M, Yayli G, Kaya O, Karahan N and Sulak O (2006) The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. International Journal of Poultry Science**5**:149-155.

Hernández F, García V, Madrid J, Orengo J, Catalá P and Megias M (2006) Effect of formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite levels of broiler chickens. British Poultry Science**47**:50-56.

Islam K (2012) Use of citric acid in broiler diets. World's Poultry Science Journal**68**:104-118.

Jin LZ, Y. W. Ho, N. Abdulrahim and . SJ (1998) Growth performance, intestinal microbial populations and serum cholesterol of broilers fed diet containing Lactobacillus cultures. Poult Sci 77:1259–126577:1259-1265.

Junqueira OM, Duarte KF, Assuena V, Filardi RdS, de Laurentiz AC and Praes MFFM (2011) Effect of phytase supplementation on performance, bone densitometry and carcass yield in broilers chicks. Acta Scientiarum: Animal Sciences**33**:307-311.

Kalafova A, Capcarova M, Hrncar C, Petruska P, Tusimova E, Kopecky J and Weis J (2014) Metabolic effect of citric acid in broiler chickens. JournalJournal of Microbiology, Biotechnology and Food Science**3**:110-112.

Kies AK, De Jonge LH, Kemme PA and Jongbloed AW (2006) Interaction between protein, phytate, and microbial phytase. In vitro studies. Journal of agricultural and food chemistry**54**:1753-1758.

La Ragione RM and Woodward MJ (2003) Bacillus subtilis spores of Salmonella enterica serotype Enteritidis and Clostridium perfringens in young chickens. Veterinary microbiology**94**:245-256.

Langhout P (2000) New additives for broiler chickens. Feed Mix:24-27.

Liu N, Ru Y, Li F and Cowieson A (2008) Effect of diet containing phytate and phytase on the activity and messenger ribonucleic acid expression of carbohydrase and transporter in chickens. Journal of animal science**86**:3432-3439.

Liu N, Ru Y, Wang J and Xu T (2010) Effect of dietary sodium phytate and microbial phytase on the lipase activity and lipid metabolism of broiler chickens. British journal of nutrition**103**:862-868.

Maenz D (2001) Enzymatic characteristics of phytases as they relate to their use in animal feeds. Enzymes in farm animal nutrition:61-84.

Maenz DD, Engele-Schaan CM, Newkirk RW and Classen HL (1999) The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in a slurry of canola meal. Animal Feed Science and Technology**81**:177-192.

Mousa AS (2008) Physiological studies on the effect of some feed additives on some metabolic and hemostatic parameters in broilers. PhD thesis,Physiology Dept, Fac of VetMed, Cairo University.

Nezhad Y, Shivazad M, Nazeeradl M and Babak M (2007) Influence of citric acid and microbial phytase on performance and phytate utilization in broiler chicks fed a corn-soybean meal diet. J Fac Vet Med Univ Tehran**61**:407-413.

Nourmohammadi R, Hosseini SM, Farhangfar H and Bashtani M (2012) Effect of citric acid and microbial phytase enzyme on ileal digestibility of some nutrients in broiler chicks fed corn-soybean meal diets. Italian Journal of Animal Science**11**:1-6.

Penn C, Mullins G, Zelazny L, Warren J and McGrath J (2004) Surface runoff losses of phosphorus from Virginia soils amended with turkey manure using phytase and high available phosphorus corn diets. Journal of environmental quality**33**:1431-1439.

Prattley CA, D. W. Stanley and Voort FRvd (1982) Protein-phytate interactions in soybeans. II. Mechanism of protein-phytate binding as affected by calcium. J Food Biochem **6**:255-271.

Punna S and Roland D (1999) Variation in phytate phosphorus utilization within the same broiler strain. The Journal of Applied Poultry Research**8**:10-15.

Qian H, Kornegay ET and Denbow DM (1997) Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium: total phosphorus ratio in broiler diets. Poultry science**76**:37-46.

Radcliffe JS, Zhang Z and Kornegay ET (1998) The effects of microbial phytase, citric acid, and their interaction in a corn-soybean meal-based diet for weanling pigs. Journal of animal science**76**:1880-1886.

Ravindran V, Cabahug S, Ravindran G, Selle P and Bryden W (2000) Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. British poultry science **41**:193-200.

Ravindran V, Morel P, Partridge G, Hruby M and Sands J (2006) Influence of an Escherichia coli-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poultry science**85**:82-89.

Ravindran V, W.L. Bryden and Kornegay (1995) Phytases: Occurrence, bioavailability and implications in poultry nutrition. Poult Avian Biol Rev**6**:125-143.

Selle P and Ravindran V (2007) Microbial phytase in poultry nutrition. Animal Feed Science and Technology**135**:1-41.

Selle PH, Cowieson AJ and Ravindran V (2009) Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livestock science**124**:126-141.

Simon O and Igbasan F (2002) In vitro properties of phytases from various microbial origins. International journal of food science & technology**37**:813-822.

Singh M and Krikorian A (1982) Inhibition of trypsin activity in vitro by phytate. Journal of Agricultural and Food Chemistry**30**:799-800.

Snow J, Baker D and Parsons C (2004) Phytase, citric acid, and 1alpha-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. Poultry Science**83**:1187-1192.

Tappenden KA and Mcburney MI (1998) Systemic short-chain fatty acids rapidly alter gastrointestinal structure, function, and expression of early response genes. Digestive diseases and sciences**43**:1526-1536.

Underwood E and Suttle N (1999) The Mineral Nutrition of Livestock 3rd ed. CABI, Wallingford, UK. 105–148

Viveros A, Brenes A, Arija I and Centeno C (2002) Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poultry Science**81**:1172-1183.

Viveros A, Centeno C, Brenes A, Canales R and Lozano A (2000) Phytase and acid phosphatase activities in plant feedstuffs. Journal of agricultural and food chemistry**48**:4009-4013.

Woyengo T, Adeola O, Udenigwe C and Nyachoti C (2010a) Gastro-intestinal digesta pH, pepsin activity and soluble mineral concentration responses to supplemental phytic acid and phytase in piglets. Livestock science**134**:91-93.

Woyengo T, Slominski B and Jones R (2010b) Growth performance and nutrient utilization of broiler chickens fed diets supplemented with phytase alone or in combination with citric acid and multicarbohydrase. Poultry science**89**:2221-2229.

Wyss M, Brugger R, Kronenberger A, Remy R, Fimbel R, Oesterhelt G, Lehmann M and van Loon AP (1999) Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): catalytic properties. Applied and environmental microbiology**65**:367-373.

Yi Z, Kornegay E and Denbow D (1996) Effect of microbial phytase on nitrogen and amino acid digestibility and nitrogen retention of turkey poults fed corn-soybean meal diets. Poultry science**75**:979-990.

Zhou J, Yang Z, Yang W, Wang X, Jiang S and Zhang G (2008) Effects of a new recombinant phytase on the performance and mineral utilization of broilers fed phosphorus-deficient diets. The Journal of Applied Poultry Research**17**:331-339.