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Influence of Citric Acid and Microbial Phytase on Growth Performance and Carcass Characteristics of Broiler Chickens

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Abstract: Problem statement: The aim of this study was to investigated the effects of adding citric acid and microbial phytase supplementation (Natuphos®) on growth performance and carcass characteristics of broiler chickens fed corn soybean meal base diets. **Approach:** The experiment included nine treatments with 10 birds in each replicate using a 3×3 factorial design for two main factors of citric acid (0, 3 and 6%) and three phytase enzyme (0, 500 and 1000 IU kg^{-1}). The diets were formulated based upon corn-soybean meal 7 to 21 and 22 to 42 day periods. **Results:** Using different levels of citric acid in diets had no effect on internal organs (except relative heart weight), whereas, diets containing 6% citric acid decreased feed intake, body weight gain and carcass yield ($p<0.05$) and improved feed conversion ratio and organs relative weight. Also, microbial phytase caused increase in feed intake, weight gain and relative neck weight ($p<0.05$). **Conclusion:** Depression of performance was differently affected by citric acid levels. Also, there was an additive effect between microbial phytase and citric acid.

Key words: Performance and broiler, Microbial Phytase (MP), Citric Acid (CA), Relative Economical Efficiency (REE), Randomized Complete Block Design (RCBD), Body Weight Gain (BWG), Feed Intake (FI), Phosphorus (P), phytase efficiency, Economical Efficiency (EE), Analysis Of Variance (ANOVA)

INTRODUCTION

Phosphorus (P) is an essential mineral for broilers metabolism and skeletal development. Also, with calcium it has a main role in the formation and maintenance of bone (Underwood and Suttle, 1999). However, 60 to 70% of the provided P in typical broiler diet ingredients such as corn and soybean is bound to phytic acid (Aguilar *et al*., 2008). Phytate-P is largely unavailable for utilization by monogastric animals, such as poultry, due to a lack of effective endogenous phytase enzyme that aids in digestion of the phytic acid complex (Waldroup *et al*., 2000). Phytic acid can also act as an anti-nutrient due to the ability of the compound to bind with starch, proteins and minerals, such as P, Zn, Fe, Ca and Mg. Because, diets of monogastric animals are often supplemented with inorganic P sources which increase the diets cost and contribute to environmental pollution and phytase is naturally found in a number of seeds including; cereals, legume and other feedstuffs, by-products and microbial sources. Exogenous phytase supplementation of broiler diets has been shown to effectively increase the availability of P to the bird and reducing P excretion by liberating phytate bounded P. Exogenous phytase can improve the retention of dietary P and the addition of exogenous phytases to poultry diets improves performance parameters other than those associated with improvement in P utilization (Hajati, 2010).

Recent researches have shown that the poultry gastrointestinal tract acidity is not desirable to complete hydrolyze or accepting of phytate by phytase. Given that Microbial Phytase (MP) is most active at 2.5 and 5.5 pH. Knowing that, some intestinal sections have different pH values, the effectiveness of phytase may be enhanced, at least in theory, by combining of feeds with an organic acid. In this respect, Afsharmanesh and Pourreza (2005) suggested that reduction in gastric pH occurs following organic acid feeding may increase pepsin activity. Moreover, peptides arising from pepsin proteolysis and triggers the release of hormones, including gastrin and cholecystokinin that regulate the

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digestion and absorption of protein. Citric Acid (CA) may change the intestinal pH and improve phytase enzyme activity, because the phytase efficiency is correlated with both acidity and concentration of other free cations. Therefore, it might be used with chelated organic acids to enable intensification of phytase efficiency. Also, it has been indicated that CA and MP may have synergistic effect. The main objective of the present study was to investigate the effect of supplementing diet with MP and CA and their combination on performance and carcass characteristics of broiler chickens.

MATERIALS AND METHODS

Experimental materials and procedures: This experiment took place at Poultry Research Station and nutrition laboratory in University of Birjand, Iran. A total of two hundred and seventy male Ross 308 broilers of similar mean body weight were randomly assigned to carried out an experiment with Randomized Complete Block Design (RCBD) and factorial arrangement of 3×3 including three levels of CA (0, 3 and 6%) and three levels of phytase (0, 500 and 1000 IU kg^{-1}). There were 9 experimental diets, 3 replicates with ten chicks in each replicate. All chicks were fed a typical commercial broiler starter ration for the first 6 days. On 7 day, after an overnight fast, the chicks were weighed and allocated to treatments.

A basal mash diet was formulated based on corn-soybean meal for 7-21 and 22-42 day periods according to NRC (1994) recommendations. Broiler chicks were fed the following diets with equal energy and protein levels: T_1) basal diet (control), T_2) basal diet+500 IU kg^{-1} MP, T_3) basal diet+1000 IU kg^{-1} MP, T_4) basal diet +3% CA, T_5) basal diet+3% CA+500 IU kg^{-1} MP, T_6) basal diet+3% CA+1000 IU kg^{-1} MP, T_7) basal diet+6% CA, T_8) basal diet+6% CA+500 IU kg^{-1} MP, T_9) basal diet+6% CA+1000 IU kg^{-1} MP. CA was supplied as monohydrate with 99.5% purity and phytase source (Natuphos® 500, BASF Corp., Mt. Live, Nj) also had 10,000 active phytase unit per gram. The composition and chemical analysis of the control and basal diets are presented in Table 1.

Feed and water were provided *ad libitum*. Body weight and Feed Intake (FI) were recorded on pen basis weekly. Feed Conversion Ratio (FCR) was adjusted according to the FI of the live broilers. Body weight was recorded before offering feed. Body Weight Gain (BWG) was obtained by calculation.

Table 1: Percentage inclusion and calculated composition of basal diet during grower and finisher period

Ingredient (%)	Grower (7-21 day)	Finisher (22-42 day)
Corn	57.00	58.60
Soybean meal	33.10	30.00
Fish meal	3.40	3.50
Soybean oil	2.00	3.50
Dicalcium phosphate	1.55	1.10
Oyster shell	1.03	1.18
DL-Methoinine	0.01	0.01
Common salt	0.26	0.26
Sand	0.65	0.85
Trace minerals mix[1]	0.50	0.50
Vitamins mix[2]	0.50	0.50
Total	100.00	100.00
Calculated composition		
ME (Kcal kg^{-1})	2910.00	3030.00
Crude Protein	20.10	19.00
Calcium	0.95	0.90
Total Phosphorus	1.23	1.06
Non phytate phosphorus	0.45	0.36
Methionine	0.50	0.38
Lysine	1.10	1.00
Methionine + cystine	0.83	0.71

[1]Mineral mix supplied kg^{-1} diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg. [2]Vitamins mix supplied kg^{-1} diet: vitamin A, 18000 IU; vitamin D_3, 4000 IU; vitamin E, 36 mg; vitamin K_3, 4 mg; vitamin B_{12}, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg

Analysis: At the end of the experimental period (day 42), two birds per replicate (6 chicks per each treatment) were taken randomly, weighed and killed by cervical dislocation, then scalded and de-feathered. Carcasses were manually eviscerated and weighed. Carcass factors (breast, thighs, wings, back and neck) and internal organs (liver, heart, spleen and abdominal fat pad) were removed and their relative percentages to live body weight were calculated. The Economical Efficiency (EE) was calculated according to the equation EE= ((A-B)/B)×100, where A is the selling cost of the obtained weight gain and B is the feeding cost of this gain. Performance Index (PI) was estimated according to North (1981): Performance index = (LBW/FCR) × 100, where LBW is the live body weight. Economical efficiency of the basal diet was assumed 100 for Relative Economical Efficiency (REE) in experimental diets and the main effects were calculated based on basal diet.

Statistical analysis: The data was subjected to Analysis Of Variance (ANOVA) using the General Linear Models (GLM) procedures of SAS software (SAS Institute, 2000) and the corresponding means were compared by Tukey-Krammer test at P <0.05. The used statistical model was:

$$Y_{ijklm} = \mu + CA_i + MP_j + (CA \times MP)_{ij} + R_k + e_{ijklm}$$

Where:

Y_{ijklm}	=	The individual observation
μ	=	The experimental mean
CA_i	=	The CA effect
MP_j	=	The microbial phytase effect
$(CA\times MP)_{ij}$	=	The interaction between CA and microbial phytase effect
R_k	=	The replication effect
e_{ijklm}	=	The error term

The percentages of slaughter parts were divided by 100 and subjected to arc-sin transformation of the square root before analysis

RESULTS

The main effects of MP and CA on growth performance of the broiler are summarized in Table 2. The data main effects indicated that the addition of 6% CA caused significant decrease ($p<0.01$) in BWG during all experimental periods, however, addition of 3% CA showed a tendency to increase BWG. Diets supplemented with 1000 IU kg^{-1} MP improved BWG significantly ($p<0.01$) compared to diets without enzyme and that of supplemented with 500 IU kg^{-1} MP. Furthermore, BWG was just numerically increased as the levels of MP diets combined with CA were increased. The addition of 6% CA to diets caused significant reduction in FI ($p<0.01$), but diets having 3% CA just numerically increased FI during the periods of 7 to 42 and 22 to 42 day. During these periods there were no significant differences between diets having 0 and 6% CA. The inclusion of 1000 IU kg^{-1} MP to diet significantly increased FI ($p<0.05$) during the periods of 7 to 21 and 7 to 42 day. Although the data also showed that FI was not significantly affected at 22 to 42 day by addition of MP, FI was just numerically increased during this period. The addition of MP to each CA levels influenced FI during 7 to 21 day. FCR was significantly ($p<0.01$) increased by CA diets during 7 to 21 day (Table 2). However, CA effect on FCR was not significant during 22 to 42 and 7 to 42 day periods, but just numerically lower FCR was seen during these periods. The results also showed that MP had no significant affect on FCR during any experimental periods. The addition of various levels of MP to each CA level did not affect FCR either.

Eviscerated carcass and carcass organs weights (carcass without; head, feed and gut) to live body weight is presented in Table 3. The data main effects indicated that CA caused significant decrease in carcass yield ($p<0.01$). However, MP had no significant effect on carcass yield, but addition of MP to the diets showed a tendency to increase carcass yield. CA numerically decreased relative weight of breast, thighs and back but did not significantly increased relative weight of wings and neck. Whereas, MP caused significant increase in relative weight of neck ($p<0.01$), but had no significant effect on other edible organs weights. Also, interaction between CA and MP was not observed for carcass yield and relative edible organs weight.

The main effects of CA and MP and their interaction effect on relative internal weights of organs are shown in Table 4. The results indicated that addition of CA caused significantly decrease in relative heart weight ($p<0.05$), but had no significant effect on other internal organs. Supplementation of diets with 1000 IU kg^{-1} MP caused an increase in abdominal fat pad ($p<0.05$) but the effects on other relative internal organs weights were not significant. According to the obtained results, the addition of 500 IU kg^{-1} MP to each CA level caused significant increases in relative spleen weights ($p<0.05$).

Table 2: Effects of CA and MP on growth performance of broiler chicks

	BWG (g)			FI (g)			FCR (g g^{-1})		
Main affects	7-21 day	22-42 day	7-42 day	7-21 day	22-42 day	7-42 day	7-21 day	22-42 day	7-42 day
MP (IU kg^{-1})									
0	360.3^{b}	1042.1^{b}	1409.7^{b}	669.46^{b}	2208.1	2877.5^{b}	1.87	2.11	2.04
500	370.9^{b}	1068.5^{b}	1449.1^{ab}	671.1^{a}	2244.0	2915.1^{ab}	1.82	2.08	2.01
100	393.0^{a}	1108.7^{a}	1511.8^{a}	711.9^{a}	2284.2	2996.2^{a}	1.81	2.08	2.01
CA (%)									
0	363.5^{b}	1146.6^{a}	1510.8^{a}	666.4^{b}	2414.4^{a}	3080.8^{a}	1.83^{ab}	2.11	2.04
3	427.5^{a}	1135.2^{a}	1572.7^{a}	752.8^{a}	2425.3^{a}	3178.1^{a}	1.76^{b}	2.13	2.03
6	333.2^{c}	937.6^{b}	1287.2^{b}	633.4^{c}	1896.6^{b}	2530.0^{b}	1.91^{a}	2.04	2.00
SEM	6.62	21.87	25.59	8.30	27.01	31.88	0.05	0.07	0.05
Probabilities									
MP	0.01	0.05	0.05	0.01	NS	NS	NS	NS	NS
CA	0.01	0.01	0.01	0.01	0.01	0.01	0.01	NS	NS
MP×CA	0.01	NS	NS	0.01	NS	NS	NS	NS	NS

Means in columns with different superscripts differ significantly ($p<0.05$). BWG: Body Weight Gain, FI: Feed intake, FCR: Feed Conversion ratio, SEM: Standard Error of Mean, MP: Microbial Phytase, CA: Citric Acid, NS: Not Significant

Table 3: Effect of CA and MP on carcass yield and relative edible organs weight

		Relative edible organs weight (%)				
Main effects	Carcass (%)	Breast	Thighs	Wings	Back	Neck
MP (IU kg^{-1})						
0	70.90	22.40	20.40	7.80	15.40	4.80[b]
500	70.70	22.20	20.50	8.10	14.40	5.20[ab]
1000	71.30	22.00	20.80	7.80	14.40	5.60[a]
CA (%)						
0	71.00[ab]	22.80	20.70	7.60	15.20	5.00
3	71.80[a]	22.30	20.80	8.10	14.40	5.30
6	70.10[b]	21.50	20.30	7.90	14.60	5.30
SEM	0.28	0.52	0.64	0.21	0.43	0.18
Probabilities						
MP	NS	NS	NS	NS	NS	0.01
CA	0.01	NS	NS	NS	NS	NS
MP×CA	NS	NS	NS	NS	NS	NS

Means in columns with different superscripts differ significantly ($p<0.05$). SEM: Standard error of mean, MP: microbial phytase, CA: Citric acid, NS: Not significant

Table 4: Effect of CA and MP on relative internal organs weight (g kg^{-1} of LW) of broiler chicks

Main effects	Abdominal fat pad	Liver	Spleen	Heart
MP (IU kg^{-1})				
0	11.3[b]	19.4	1.2	5.60
500	11.9[b]	20.1	1.4	6.30
1000	15.8[a]	20.2	1.3	5.70
CA (%)				
0	12.3	19.9	1.3	6.41[a]
3	12.2	20.0	1.4	5.45[b]
6	14.6	19.7	1.2	5.71[ab]
SEM	1.05	0.86	0.05	0.24
Probabilities				
MP	0.05	NS	NS	NS
CA	NS	NS	NS	0.05
MP×CA	NS	NS	0.01	NS

Means in columns with different superscripts differ significantly ($p<0.05$). LW = Live weight, SEM: Standard error of mean, MP: microbial phytase, CA: Citric acid, NS: Not significant

Table 5: Effect of CA and MP on economic indexes

Main effects	PI	EE	REE
MP (IU kg^{-1})			
0	68.59[b]	86.50	96.97
500	72.07[a]	88.53	99.25
1000	73.71[a]	89.20	100.00
CA (%)			
0	73.85[a]	92.47[b]	103.66
3	77.28[a]	97.93[a]	109.79
6	63.23[b]	73.83[c]	82.77
SEM	0.72	0.73	0.82
Probabilities			
MP	0.05	NS	-
CA	0.01	0.01	-
MP×CA	0.01	NS	-

Means in columns with different superscripts differ significantly ($p<0.05$). PI: Performance Index, EE: Economic Efficiency, REE: Relative Economic Efficiency, SEM: Standard Error of Mean, MP: Microbial Phytase, CA: Citric Acid, NS: Not Significant.

The results also indicated that addition of MP caused improvements in performance index, economical efficiency and relative economic efficiency (Table 5). According to the obtained results, 6% CA caused a decrease in economic indexes. Birds fed diets containing CA at level of 3% had the best values of either economic or relative economic efficiency compared with the other levels of CA used in the present experiment.

DISCUSSION

Results of performance factors in our study were in agreement with reports of Viveros *et al.* (2002) and Martinez-Amezcua *et al.* (2006). In contrast, some reports showed that addition of MP to diets did not influence broiler performance factors (Centeno *et al.*, 2007). The phytase mechanism as growth promoter may be due to increase in bioavailability of minerals such as; P and Ca, that increases myo-inositol concentration and also, the release of minerals and trace elements which are conjuncted to phytic acid. The other reason for improvement in growth rate in present of MP is inositol utilization by chicks, after phytic acid is being hydrolyzed to inositol and inorganic phosphate. The mechanism thought to be involved in the improvement of growth performance of phytase by chicks that includes; liberation of P from phytate salt (Qian *et al.*, 1996), enhance digestibility of starch (Knuokles and Betschart, 1987) or availability of protein and amino acids (Selle *et al.*, 2000) and increase efficacy utilization of myo-inositol (final product of phytate dephosphorylation) and other material which are liberated from phytate compounds (Simons *et al.*, 1990). Phytase supplementation of adequate amount of P in broiler diets has been shown to generate equivocal growth performance responses that might be mediated by dietary nutrient specifications. For example, in a report by Selle *et al.* (1999), standard and modified sorghum-based diets offered to broilers from 7 to 25 days of age, without and with 600 IU kg^{-1} phytase, the modified diets reduced role of P, Ca, protein/amino acids and energy density of diet. Phytase did not influence growth performance of broilers on standard diets but significantly increased weight gain (7.6%) in modified diets. Moreover, there was a significant interaction between diet type and phytase addition in feed efficiency. Generally, responses to phytase in FI and BWG are more robust and consistent than feed efficiency responses alone. Also, Rosen (2003) disagrees that feed efficiency responses to phytase have been declining with time which he attributes to concurrent improvements in broiler strains, feeds and management techniques. Martinez-Amezcua *et al.* (2006) and Hassanabadi *et al.* (2007) showed that adding phytase enzyme caused an increase in FI that ia in agreement with our finding. The formers observed positive effects of phytase supplementation on FI compared with diets without enzyme. Essentially, FI by itself alone is not responsible for performance evaluation effect of phytase and should include parameters such as; average weight gain and FCR. Afsharmanesh and Pourreza (2005), Ebrahimnezhad *et al.* (2008) and Nourmohammadi *et al.* (2010) reported positive effects by utilization of CA in diets. On the other hand, CA provided suitable pH in GI tract for proteolytic enzymes activities and also, increased feed digestion by reduction in GI tract micro flora of birds. Therefore, the addition of CA to diets should promote growth. This was also found by Jang *et al.* (2004) who reported that a blend of organic acid with essential oils showed increase in pancreatic and intestinal mucosa digestive enzyme activities, resulting an increase in growth. Moreover, downfall in pH caused reduction in GI tract digesta transmission speed that was justifiable by resulting in reduction of FI in diets having 6% CA. This suggestion was partially in agreement with the earlier findings of Cave (1984) who reported that addition of high levels of organic acid would strongly decrease feed palatability reflected by reduction in FI. The reason for not seeing significant effect on FCR in our findings was probably due to both increase in BWG and FI simultaneously. This result is in agreement with some studies (Ebrahimnezhad *et al*., 2008; Nourmohammadi *et al*., 2010).

Ferguson *et al.* (1998); Preston *et al.* (2000) and Hassanabadi *et al.* (2007) reported that adding different levels of phytase (500 and 1000 IU kg^{-1}) caused a tendency to increase carcass yield. Increasing of carcass yield in chicks having 1000 IU kg^{-1} phytase in their diets, probably, was a response to nutritional effects of enzyme. The discussed hypothesis is based on improvement causes of phytase in performance by liberating of lysine and other essential and non-essential amino acids from the phytate-protein compound (Ravindran *et al*., 2001). In this regard, Ravindran *et al.* (2001) showed that dietary phytase increased amino acids availability. Lysine is one of most important amino acids to accretion of breast muscle. This respond to phytase may be responsible for increasing breast muscle yield that was also seen in the present study. Ferguson *et al.* (1998) and Preston *et al.* (2000) in this regard reported increasing in carcass yield by addition of phytase enzyme. Dressed weight is a function of live weight. A positive correlation of dressed weight with live body weight or age coincided with the findings of some earlier workers (Howlider and Rose, 1989). Hassanabadi *et al.* (2007) and Nourmohammadi *et al.* (2010) also, reported that relative carcass organs weight (breast, neck, back and thighs) were numerically affected by diets having phytase which is in agreement with our findings. Luckstadt *et al.* (2004) supported the use of CA to preserve and protect feed from microbial and fungal destruction. The later concluded that organic acids showed variable effects on the performance of broilers. With the same purpose, researchers (Viola and Vieira, 2007) have reported the beneficial effects of organic acids such as CA as alternatives to some feed additives and antibiotics on performance and carcass quality of broilers. Similar results with present study were found by Ebrahimnezhad *et al.* (2008) and Nourmohammadi *et al.* (2010) indicating that addition of CA caused significant increase in carcass yield of broiler chickens.

Ebrahimnezhad *et al.* (2008) and Nourmohammadi *et al.* (2010) found that dietary CA and phytase supplementation had affected the relative weight of liver, abdominal fat pad and heart weight of broiler chickens at 42 d. On the other hand, Abdel-Azeem *et al.* (2000) noted that addition of CA to the diet was associated with higher and lower dressing and liver percentages, respectively. The lack of significance in the relative liver weights between the acidified and control treatment chicks may be responsible for the more storage of glycogen and lower lipid repletion that was induced by dietary organic acid. This supposition may emphasize the hypothesis of Fushimi *et al.* (2001) who stated that dietary acidification might stimulate glycogenesis by increasing the influx of glucose 6-phosphate (G-6-P) into the glycogen synthesis pathway through the inhibition of glycolysis due to an increase in citrate concentration.

CONCLUSION

According to the results of present study, MP and 3% CA supplementation of diet improved performance of broiler chickens. Under the condition of this experiment, also, no further benefits were achieved as a result of increasing the dietary CA level to 6%. As well, the diets containing 3% CA and 1000 IU kg^{-1} MP was more beneficial to birds. Clearly, the effective role of organic acids with/without MP in poultry diets has been emphasized by many researchers. However, there is still a need to conduct more research in order to establish the suitability of adding such combinations to obtain satisfactory results in enhancing feed utilization of broiler diets respond on productive performance of broilers.

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