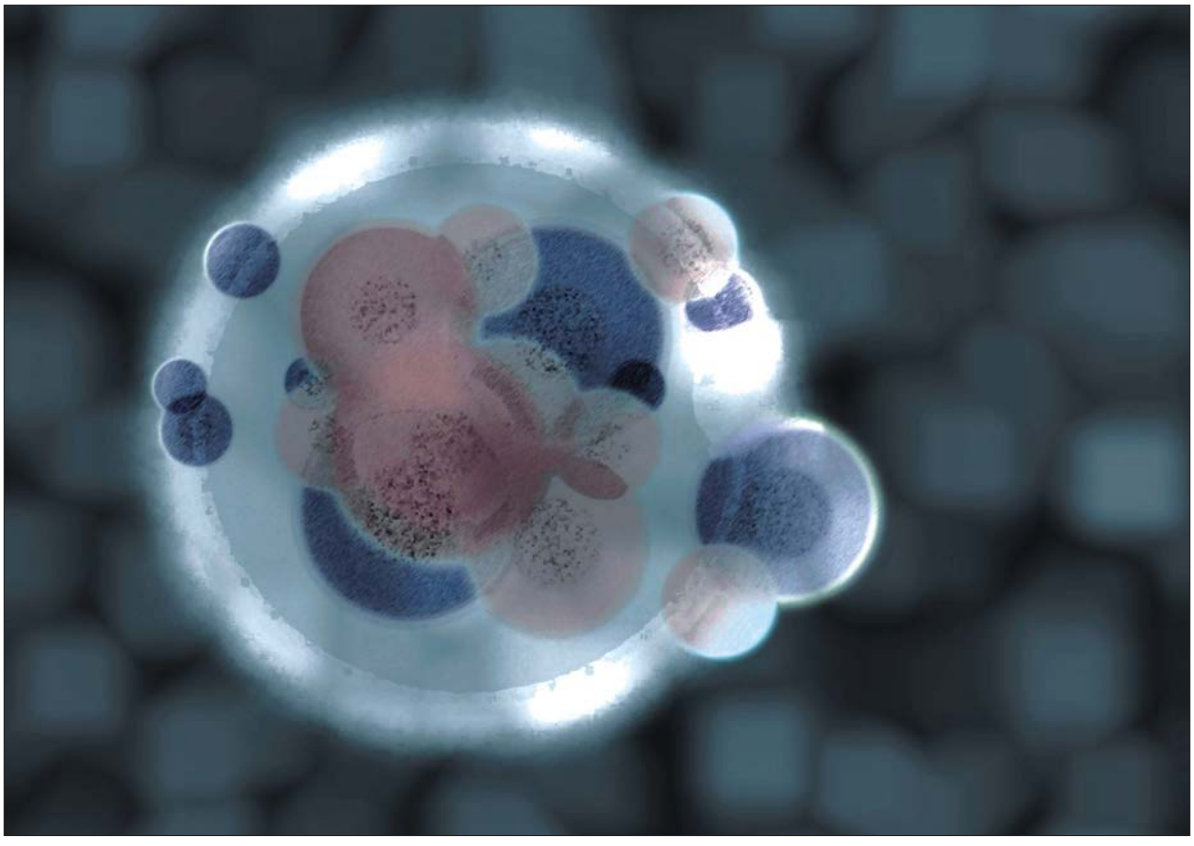

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Effects of Citric Acid Supplementation on Phytate Phosphorus Utilization and Efficiency of Microbial Phytase in Laying Hen

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Abstract: A synergistic effect between Citric Acid (CA) and Microbial Phytase (MP) in improving phytate Phosphorus (P) utilization have been reported in broiler previously. In order to evaluate such additive effect in laying hen, an experiment was conducted using 224 Hyline-W 36 laying hen. Experiment began at 53 w of age and lasted in 64 w of age. The experiment was carried out using a completely randomized design with factorial arrangement (0 and 300 IU MP and 0, 20 and 40 g citric acid per kg of diet). Four replicate of 8 hens per each were fed dietary treatments including 1) Positive Control diet (PC) which meet NRC recommended available P level (0.3% available P), 2) Negative Control diet (NC) that was similar to PC diet except that available P was reduced by 0.2 %, 3) NC+300 IU microbial phytase per kg of diet, 4) NC+20 g CA per kg of diet, 5) NC+20 g CA+300 IU microbial Phytase per kg of diet, 6) NC+40 g CA per kg of diet, 7) NC+40 g CA+300 IU microbial Phytase per kg of diet. Dietary supplementation of MP to low available P diets significantly improved egg production and restored it to the level similar to PC group, but CA supplemented diets failed to create such effects. NC and diets with only CA supplementation had significantly lower feed intake compared to PC and MP supplemented diets. MP supplemented diets were used as efficiently as the PC diet, but CA had any effects on feed efficiency. Results obtained in our study suggests that contrary to the effects of CA in broiler chicks, CA couldn't enhance phytase effectiveness in laying hen, probably due to high levels of Ca in laying hens diets.

Key words: Critic acid, laying hens, microbialphytase, phytate P, corn soy

INTRODUCTION

The major ingredients used in poultry feeds are of plant origin. About two third of the Phosphorous (P) in these feedstuff is present as phytate P, which is poorly utilized by poultry. The inability of poultry to utilize phytate P causes both economic and environmental problems. Physical methods such as soaking, drying, germination (Jongbloed *et al*., 1991), supplementation of diets with exogenous microbial Phytase (Kornegey, 2001) and Vitamin D (Mitchel and Edwards, 1996) have found to be effective in increasing phytate hydrolysis. Although positive effects of citric acid on utilization of phytate phosphorus in broiler chicks are well documented (Bolling *et al*., 1998, 2000a, 2001; Brenes *et al*., 2003) but little are known about its effects in laying hen. Bolling *et al*. (2000b) reported that citric acid didn't improve Phosphorus utilization in laying hens Fed a Corn-Soybean Meal Diet. It is hypothesized that citric acid complex with Ca and reduces the formation of more stable Ca-phytate complexes. Alternatively, citric acid may change the intestinal pH for better Phytase activity. In theory, these supplements could have synergistic or additive effect. Synergism between microbial Phytase and Vit D analogs (Biehl *et al*., 1995; Mitchell and Edwards, 1996) and phytase and citric acid (Boling *et al*., 2000a) have been assessed in broiler chicks. The objective of this study was to investigate the synergistic effects of citric acid and phytase on the performance of laying hen, activity of alkaline phosphatase and utilization of phytate P in corn soy meal based diet.

MATERIALS AND METHODS

A total of 224 HyLine-W 36 laying hens were used in this study. Experiment began at 53 w of age and lasted in 64 w of age. The experiment was carried out using a completely randomized design with factorial arrangement

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Table 1: Ingredients and nutrient composition (g kg^{-1}) of experimental diets

	Treatments						
Ingredients	1	2	3	4	5	6	7
Corn	656.0	664.4	663.8	632.7	632.1	601.1	600.5
Soybean meal (44%)	212.8	211.2	211.3	211.7	217.3	223.4	223.5
Soybean oil	14.6	12.1	12.3	15.7	19.9	19.4	19.6
Oyster shell	20	20	20	20	20	20	20
Dicalcium phosphate	10	-	-	-	-	-	-
Calcium carbonate	75.4	81.2	81.2	81.2	81.2	81.2	81.2
Salt	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sodium bicarbonate	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Premix[a]	5	5	5	5	5	5	5
DL-Methionine	1	1	1	1	1	1	1
Citric acid (92%)	-	-	-	21.9	21.9	43.8	43.8
Phytase[b]	-	-	0.3	-	0.3	-	0.3
Calculated Nutrient							
ME (MJ kg^{-1})	11.78	11.78	11.78	11.78	11.78	11.78	11.78
Crude protein (g kg^{-1})	150	150	150	150	150	150	150
Available P (g kg^{-1})	3	1	1	1	1	1	1
Total P (g kg^{-1})	5.1	3.2	3.2	3.2	3.2	3.2	3.2
Calcium (g kg^{-1})	38	38	38	38	38	38	38
Methionine+cystine (g kg^{-1})	6	6	6	6	6	6	6
Lysine (g kg^{-1})	7.4	7.4	7.4	7.4	7.4	7.4	7.4

[a]Vitamin and mineral mix supplied/kg diet: vitamin A, 11000 IU; vitamin D3, 1800 IU; vitamin E, 11 mg; vitamin K3, 2 mg; Vitamin B2, 5.7 mg; Vitamin B6, 2mg; vitamin B12, 0.024 mg; Nicotinic acid, 28 mg; folic acid, 0.5 mg; pantothenic acid, 12 mg; choline chloride, 250 mg; Mn, 100 mg; Zn, 65 mg; cu, 5 mg; Se, 0.22 mg; I, 0.5 mg; Co, 0.5 mg [b]Natuphos® (BASF Crop., Mt. Olive, NJ) was used to supply 300 U microbial phytase per kilogram of diet

(0 and 300 IU MP and 0, 20 and 40 g citric acid per kg of diet). Four replicates of 8 hens per each were fed dietary treatments including 1) Positive Control (PC) with NRC (1994) recommended available P level (0.3%), 2) Negative Control (NC) that was similar to PC diet except that available P was reduced by 0.2 %, 3) NC+300 IU microbial phytase per kg of diet, 4) NC+20g CA per kg of diet, 5) NC+20 g CA+300 IU microbial Phytase per kg of diet, 6) NC+40 g CA per kg of diet, 7) NC+40 g CA+300 IU microbial Phytase per kg of diet (Table 1). All the diets were kept isocaloric and isonitrogenous and formulated to meet or exceed the NRC (1994) recommendations. Citric acid was supplied as monohydrate citric acid with 92% purity and the Phytase source had 10000 unit active Phytase per gram. Hens were fed *ad libitum* and were exposed to a 16L: 8D lighting schedule. Daily egg production (based on hen day production) and eggs weight were measured for each experimental unit and then eggs mass were calculated. Feed intake was measured every 2 weeks and then feed conversion ratio was calculated. All the hens were weighted at the end of every month individually and body weight changes were calculated. At the termination of the experiment 2 birds were chosen from each replicate randomly and 3 mL blood sample were taken from their wing vein. Serum concentration of Alkaline Phosphatase (ALP) was measured using commercial kits (Diagnostic. Cat No.10-508, 525) and Ca and P concentration were assessed by calorimetric method. Data were analyzed using GLM procedure of SAS software (SAS Institute, 1990) and significant differences were separated using Duncan's multiple range test.

RESULTS

Daily egg production, egg weight, egg mass, feed intake and feed efficiency are presented in Table 2. Dietray supplementation of MP to low available P diets significantly improved egg production ($p<0.001$) and restored it to the level similar to PC group, but CA supplemented diets failed to create such effects. NC and diets with only CA supplementation had significantly lower feed intake compared to PC and MP supplemented diets ($p<0.005$). MP supplemented diets were used as efficiently as the PC diet, but CA had no significant effects on feed efficiency. Hens received PC diet and diets with MP supplementation produced heavier eggs and higher ($p<0.013$) egg mass ($p<0.003$). PC diet and MP supplemented diets had significantly lower concentration of ALP than NC and CA supplemented diets ($p<0.001$). Dietary treatments didn't affect Ca concentration, but serum P concentration significantly affected by MP ($p<0.001$) (Table 3). Hens received MP supplemented diets had significantly ($p<0.001$) higher body weight at the termination of experimental period. Neither MP nor CA had any significant effects on mortality rate. No significant interactions were observed between MP and CA on any of measured parameter.

Table 2: The Effect of citric acid and microbial phytase on egg production, feed intake, feed conversion ratio, egg weight and daily egg mass in laying hens

Treatments						
Citric acid (g kg^{-1})	(U kg^{-1}) Phytase	Egg production (%)	Feed intake (g/hen/day)	Feed conversion ratio (kg kg^{-1})	Egg weight (g)	Egg mass (g)
0	(PC) 0[1]	80.36^{a}	101.12^{a}	2.84^{b}	60.63^{ab}	48.69^{a}
0	(NC) 0[2]	32.86^{c}	81.43^{b}	5.70^{a}	51.38^{d}	19.45^{c}
0	300	77.00^{a}	100.37^{a}	2.21^{b}	59.77^{ab}	46.10^{a}
20	0	35.76^{c}	82.10^{b}	5.85^{a}	54.87^{c}	21.16^{c}
20	300	81.39^{a}	99.11^{a}	2.07^{b}	58.91^{b}	48.01^{a}
40	0	48.01^{b}	86.99^{b}	5.17^{a}	56.48^{c}	27.71^{b}
40	300	70.79^{a}	96.59^{a}	2.27^{b}	61.05^{a}	43.27^{a}
SEM Pooled		9.06	6.88	1.06	1.46	5.55
Main effect						
Citric acid	0	54.93^{b}	90.90^{b}	3.95^{a}	55.57^{b}	32.77^{b}
	20	58.51^{b}	90.06^{b}	3.96^{a}	56.89^{b}	34.58^{b}
	40	59.4^{b}	91.77^{b}	3.72^{a}	58.76^{b}	35.49^{b}
Phytase	0	38.78^{bc}	83.50^{b}	5.57^{a}	54.24^{b}	27.77^{bc}
	300	76.39^{a}	98.69^{a}	2.18^{b}	59.91^{a}	45.79^{a}
Probabilities						
Citric acid		NS	NS	NS	NS	NS
Phytase		0.001	0.005	0.001	0.013	0.003
Citric acid phytase		NS	NS	NS	NS	NS

$^{a-c}$Means in columns with no common superscript differ significantly ($p<0.05$), [1]PC = Positive control group, [2]NC = Negative control group, NS = Not Significant

Table 3: The Effect of citric acid and microbial phytase on alkaline phosphatase, calcium and phosphorus concentration of serum and body weight changes and mortality in laying hens

Treatments						
Citric acid (g kg^{-1})	Phytase (IU kg^{-1})	Alkaline phosphatase (U L^{-1})	Calcium (mg dL^{-1})	Phosphorus (mg dL^{-1})	Body weight changes (g)	Mortality (%)
0	(PC) 0[1]	152.50^{b}	19.37^{a}	4.84^{a}	67.71^{a}	1.30^{b}
0	(NC) 0[2]	213.25^{a}	15.25^{ab}	1.51^{b}	-15.33^{b}	1.65^{a}
0	300	115.75^{b}	18.30^{ab}	4.90^{a}	42.30^{ab}	1.38^{ab}
20	0	196.50^{a}	15.68^{ab}	2.08^{b}	-6.71^{b}	1.52^{ab}
20	300	172.75^{ab}	19.22^{a}	4.57^{a}	45.48^{ab}	1.38^{ab}
40	0	221.50^{a}	14.29^{b}	1.48^{b}	2.5^{ab}	1.56^{ab}
40	300	150.50^{b}	18.61^{ab}	5.14^{a}	43.72^{ab}	1.47^{ab}
SEM Pooled		28.01	1.38	0.496	36.53	0.086
Main effect						
Citric acid	0	164.50^{a}	16.77	3.20^{b}	13.48^{b}	1.51
	20	184.62^{a}	17.75	3.32^{b}	19.38^{b}	1.45
	40	186.00^{a}	16.45	3.31^{b}	23.10^{b}	1.51
Phytase	0	210.41^{a}	15.07	1.69^{bc}	-19.54^{c}	1.57
	300	146.33^{b}	18.71	4.87^{a}	43.83^{a}	1.41
Probabilities						
Citric acid		NS	NS	NS	NS	NS
Phytase		0.001	NS	0.001	0.001	NS
Citric acid phytase		NS	NS	NS	NS	NS

$^{a-c}$Means in columns with no common superscript differ significantly ($p<0.05$), [1]PC = Positive control group., [2]NC = Negative control group, NS: Not Significant

DISCUSSION

MP supplementation of low available P diets restored their egg production to the level equal to PC diet. Positive effects of MP on egg production of laying hens fed low available P diets are well documented (Boling *et al*., 2000b; Gordon and Roland 1997; Li *et al*., 1998; UM and Paik 1999). Although CA have shown to facilitate phytate phosphorus hydrolysis in broiler chicks, but results obtained in our study and the results reported by Boling *et al*. (2000b) suggests that CA may have no effect on phytate phosphorus liberation in laying hens. These findings with laying hens are in contrast to previous studies with rats (Shohl, 1937; Pileggi *et al*., 1956) and chicks (Boling *et al*., 2000a; Snow *et al*., 2004; Rafacz-Livingston *et al*., 2005). The reason for the lack of effect in laying hens is unknown, but it is hypothesized that it may be associated with the dietary Ca level. Laying hen diet contained a much higher level of dietary Ca (3.8%) than do rat diets (available proximately 0.5%) and chick diets (available proximately 1%). It has been proposed that the ability of citric acid to improve phytate-P utilization is associated with its Ca-complexing property (Hamilton and Dewar, 1937;

Day, 1940; Pileggi *et al*., 1956). Erdman (1979) in a review of the literature, reported that phytate binds minerals such as Ca. Perhavailable Ps citric acid, a strong chelator of Ca, removes Ca from, or decreases Ca binding to, the phytate molecule, thus making it less stable and more susceptible to endogenous phytase. If the latter is correct, or at least partially true, it is possible that the very high dietary Ca level in our basal laying hen diet resulted in the supplemental citric acid being bound to nonphytate Ca. Consequently, there was still ample Ca available for binding to phytate and the citric acid would not have been available to bind to the Ca in the Ca-phytate complex. Lowe and Steenbock (1936) reported that the presence of excess Ca carbonate reduces the availability of phytin P in the intestinal tract of rats.

Decreased feed consumption and impaired feed efficiency in low available P diets with CA supplementation have been previously reported by Boling *et al*. (2000b) in laying hens. It seems that inability of CA in liberation of phytate P in low available P diets will result in marginal P deficiency and subsequently impaired feed efficiency.

Higher egg weight and egg mass observed in our study in low available P diets with MP supplementation are in agreement with those reported by Jalal and Scheideler (2001), Keshavarz (2003) and UM and Paik (1999). As CA had no significant effect on both egg weight and egg mass in the study of Boling *et al*. 2000b, no significant effect was observed in this study.

ALP is Zn^{++} containing metaloenzyme that has a key role in bone mineralization. Decreased blood available P level (decreased serum P concentration in NC and CA supplemented diets in the current study) by any reason, will increase ALP activity. Viveros *et al*. (2002) and Brenes *et al*. (2003) reported that decreasing available P level of diet increased ALP activity.

Results obtained in our study suggest that contrary to the effects of CA in broiler chicks, CA couldn't enhance phytase effectiveness in laying hen, probably due to high levels of Ca in laying hens diets.

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