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Plasma Thyroid Hormone Concentrations and pH Values of Some GI-Tract Segments of Broilers Fed on Different Dietary Citric Acid and Microbial Phytase Levels

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Abstract: Problem statement: An experiment was conducted to study the effect of microbial phytase supplementation and citric acid on thyroid activity, relative weight of lymphoid organs and pH values of some GI-tract segments in broiler chickens fed corn-soybean meal based diets. **Approach:** The data was analyzed using a Randomized Complete Block Design (RCBD) with factorial arrangement of 3×3, three levels of citric acids (0, 3 and 6%) and three levels of phytase (0, 500 and 1000 IU kg^{-1}). There were three replicates (with ten chicks in each replicate) for each treatment. A total of 270 Ross 308 broiler chicks were used. **Results:** Addition of citric acid to diets caused significant decrease in pH values of crop, gizzard, duodenum, jejunum and ileum ($p<0.05$) and caused significant increase ($p<0.01$) in plasma triiodothyronine (T_3) concentration, T_3:T_4 ratio and relative weight of bursa and thymus, but had no significant effect on thyroxin (T_4) concentrations. Microbial phytase significantly increased relative weight of thymus ($p<0.01$), but had no significant effects on thyroid gland activity, relative weight of bursa and values of pH in different parts of the GI-tract. **Conclusion:** Broiler chicks fed on acidifiers diets had better immune response resistance that lead to immunological advances. Also, decreasing pH in GI-tract by CA caused a beneficial effect in the inhibition of intestinal bacteria competition.

Key words: Broilers fed, phytase supplementation, pH value, thyroid hormones, intestinal bacteria, plasma triiodothyronine, GI-tract segments, thyroid activity, Microbial Phytases (MP), broiler chicks, Citric acid (CA), Analysis Of Variance (ANOVA)

INTRODUCTION

Phytate and phytate-bound phosphorus (P) are present in all poultry diets and the partial availability of phytate-P has long been recognized (Lowe *et al*., 1939). Possibly, Warden and Schaible (1962) were the first to show that exogenous phytase enhances phytate-P utilization and bone mineralization in broiler chickens. Nevertheless, three decades elapsed before an *Aspergillus niger* derived phytase feed enzyme with the capacity to liberate phytate bound P to reduce P excretion that was commercially introduced in 1991. Then, the use of Microbial Phytases (MP) would be considered to areas where financial penalties on excessive P levels Production from intensive pig and poultry units were imposed (Chesson, 1993). In contrast, the inclusion of phytase feed enzymes in monogastric diets has been far more widely accepted and now exceeds that of NSP degrading enzymes. Phytase feed enzymes have more general application as their substrate is consistently present in pig and poultry diets and their dietary inclusion economically effect bioavailability of P and reduces the P load in the environment. Also, prohibition of animal origin protein meals accelerate P acceptance as phytase feed enzymes in certain countries. Phytase is naturally found in a number of seeds including cereals, legume, by-products, other feedstuffs and microbial sources (Viveros *et al*., 2000). Supplementation of diets with MP has known to increase availability of phytate P and Zn in chicks (Sebastian *et al*., 1996; Ravindran *et al*., 2000). Phytase increases availability and retention of Ca, improves absorption and retention of Mg, Cu and Fe (Sebastian *et al*., 1996).

Previous researches have shown that poultry digestive tract acidity is not desirable to complete hydrolyze or establish phytate by phytase (Brenes *et al*., 2003). Citric acid (CA) may change the intestinal pH

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and improve phytase enzyme activity, because the phytase efficiency is correlated with both acidity and concentration of other free cations (Nourmohammadi *et al.,* 2010a). Therefore, it might be used with chelated organic acids to enable intensification of phytase efficiency. Kemme *et al.* (1998) reported that phytase efficiency is related to plant or microbial sources, in hydrolyzed phytate and gut pH and time duration. Therefore, between CA and MP may have been synergistic effect. This study was carried out to investigate the effect of supplementing diet with both MP and CA and their interaction on plasma hormones concentration, relative weight of lymphoid organs and pH values of some gastrointestinal tract segments.

MATERIALS AND METHODS

Diets, birds and experimental design: This experiment took place in Poultry Research Unit and nutrition laboratory at University of Birjand, Iran. Two hundred and seventy day-old male chicks (Ross-308) were obtained from a commercial hatchery, weighed on arrival and randomly assigned to 27 pens of 10 birds each. The experiment was carried out using a randomized complete block design (RCBD) with factorial arrangement of 3×3, three levels of CA (0, 30 and 6%) and three levels of phytase (0, 500 and 1000 IU kg^{-1} MP enzyme). There were 9 experimental treatments, 3 replicates with ten chicks in each replicate. Feed and water were provided *ad libitum* and a continuous lighting schedule were used throughout the experimental period. A basal diet (without phytase enzyme or CA), was formulated with corn-soybean meal from 7 to 21 and 22 to 42 day periods according to National Research Council recommendations. Diets were provided in the mash form. Broiler chicks were fed the following diets with equal energy and protein levels: T_1) basal diet, 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 MP IU kg^{-1}, 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 and T_9) basal diet+6% CA+1000 IU kg^{-1} MP. CA was supplied as monohydrate with 99.5% purity and phytase (Natuphos® 500, BASF Corp., Mt. Live, Nj), diet source also had 10,000 active phytase unit per gram.

Samples collection and analysis: At the end of the experimental period (42 d of age), two birds per replicate (6 birds per treatment) were selected randomly and killed by cervical dislocation. Blood samples (approx. 10 ml) were collected in heparinized vacuitainer tubes for measuring plasma hormones concentration (triiodothyronine and thyroxin). Immediately after collection, tubes were placed in an ice bath and transferred to the laboratory. Plasma was harvested subsequently after centrifuging the whole blood samples at 3000 rpm for 15 min. The heparinized plasma samples were stored at -20°C in Eppendorf tubes and analyzed subsequently. The triiodothyronine (T_3) and thyroxin (T_4) concentrations in the plasma samples were determined by radioimmunoassay (RIA) using the procedure described by Darras *et al.* (1992).

At the end of the experimental period, two birds per replicate (6 birds per treatment) were taken randomly, weighed and killed by cervical dislocation, then scalded and defeathered. Thymus (all lobes of both sides) and bursa were removed and their relative percentages of live body weights were calculated.

PH Values for different segments of the gastrointestinal tract (GI-tract) were measured immediately by using a digital pH meter. 10 g of contents from crop, gizzard, duodenum, jejunum and ileum were collected aseptically in 90 ml sterilized physiological saline (1: 10 dilution) and their pHs were determined (Al-Natour and Alshawabkeh, 2005).

Statistical analysis: The data were subjected to an Analysis Of Variance (ANOVA) through fitting general linear model (GLM) using SAS® (SAS Institute, 2000) software and the corresponding means were compared by Tukey-Kramer test. The statistical model was as follows:

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

Where

Y_{ijkl} = The individual observation
μ = The experimental mean
CA_i = The Citric Acid effect
MP_j = The Microbial Phytase effect
B_k = The block effect
e_{ijkl} = The error term with mean 0 and variance σ_e^2.

RESULTS

The main effects results of CA and MP on plasma hormones and relative weight of lymphoid organs are shown in Table 1. The results indicated that CA caused significant increase in T_3, T_3:T_4 ratio, bursa and thymus ($p<0.01$), but had no significant effect on plasma T_4 concentration. Also, MP caused an increase in relative weight of thymus ($p<0.01$) although MP main effect was not significant for T_3, T_4, T_3: T_4 ratio and relative weight of bursa.

Table 1: The main effects of CA and MP on plasma thyroid hormones and relative weight of lymphoid organs (g kg^{-1} of live weight)

	Thyroid gland activity			Lymphoid organs	
Main effects	T_3 (ng mL^{-1})	T_4 (ng mL^{-1})	T_3:T_4	Bursa	Thymus
MP (IU kg^{-1})					
0	1.702	16.133	0.105	0.27	0.33^{b}
500	1.704	16.251	0.105	0.28	0.35^{a}
1000	1.701	16.245	0.105	0.28	0.35^{a}
CA (%)					
0	1.571^{c}	16.234	0.097^{c}	0.25^{c}	0.28^{c}
3	1.718^{b}	16.141	0.106^{b}	0.27^{b}	0.34^{b}
6	1.818^{a}	16.255	0.112^{a}	0.31^{a}	0.41^{a}
SEM	0.0020	0.0643	0.0005	0.003	0.005
Probabilities					
MP	NS	NS	NS	NS	0.01
CA	0.01	NS	0.01	0.01	0.01
MP×CA	NS	NS	NS	NS	NS

Mean values within a column with no common superscript differ significantly from each other (P <0.05),T_3= triiodothyronine, T_4= thyroxin, MP= microbial phytase, CA= citric acid, SEM= standard error of mean, NS= not significant

Table 2: Interaction effects between CA and MP on plasma thyroid hormones and relative weight of lymphoid organs (g kg^{-1} of live weight)

Treatments		Thyroid gland activity			Lymphoid organs	
CA (%)	MP (IU kg^{-1})	T_3 (ng mL^{-1})	T_4 (ng mL^{-1})	T_3:T_4	Bursa	Thymus
0	0	1.571^{c}	16.228	0.097^{c}	0.24^{b}	0.27^{e}
0	500	1575^{c}	16.247	0.097^{c}	0.26^{b}	0.28^{e}
0	1000	1.569^{c}	16.228	0.097^{c}	0.26^{b}	0.29^{de}
3	0	1.719^{b}	15.918	0.108^{ab}	0.27^{b}	0.33^{cd}
3	500	1.718^{b}	16.251	0.106^{b}	0.27^{b}	0.35^{bc}
3	1000	1.717^{b}	16.254	0.106^{b}	0.27^{b}	0.35^{bc}
6	0	1.817^{a}	16.254	0.112^{a}	0.31^{a}	0.38^{ab}
6	500	1.821^{a}	16.256	0.112^{a}	0.32^{a}	0.42^{a}
6	1000	1.817^{a}	16.255	0.112^{a}	0.30^{a}	0.42^{a}
SEM		0.0034	0.1113	0.0005	0.006	0.009

Mean values within a column with no common superscript differ significantly from each other (P <0.05),T_3= triiodothyronine, T_4= thyroxin, MP= microbial phytase, CA= citric acid, SEM= standard error of mean, NS= not significant

Table 3:The main effects of CA and MP on pH values of some gastrointestinal tract segments in broiler chicks

Main effects	Crop	Gizzard	Duodenum	Jejunum	Ileum
MP (IU kg^{-1})					
0	5.00	3.16	5.77	6.45	7.20
500	5.02	3.16	5.77	6.45	7.19
1000	5.03	3.17	5.77	6.45	7.19
CA (%)					
0	5.17^{a}	3.21^{a}	5.80^{a}	6.63^{a}	7.22^{a}
3	5.00^{b}	3.19^{a}	5.79^{a}	6.49^{b}	7.21^{a}
6	4.89^{c}	3.09^{b}	5.71^{b}	6.23^{c}	7.16^{b}
SEM	0.012	0.006	0.003	0.007	0.004
Probabilities					
MP	NS	NS	NS	NS	NS
CA	0.01	0.01	0.01	0.01	0.01
MP×CA	NS	NS	NS	NS	NS

Mean values within a column with no common superscript differ significantly from each other (P <0.05),SEM= standard error of mean, MP= microbial phytase, CA= citric acid, NS= not significant

Table 4: Interaction effect between CA and MP on pH values of some gastrointestinal tract segments in broiler chicks

Treatments						
CA (%)	MP (IU kg^{-1})	Crop	Gizzard	Duodenum	Jejunum	Ileum
0	0	5.17^{a}	3.21^{a}	5.81^{a}	6.64^{a}	7.22^{a}
0	500	5.17^{a}	3.20^{a}	5.80^{a}	6.63^{a}	7.22^{a}
0	1000	5.17^{a}	3.21^{a}	5.80^{a}	6.63^{a}	7.22^{a}
3	0	4.99^{b}	3.20^{a}	5.80^{a}	6.48^{b}	7.21^{a}
3	500	5.00^{b}	3.20^{a}	5.79^{a}	6.50^{b}	7.20^{ab}
3	1000	5.00^{b}	3.18^{a}	5.79^{a}	6.49^{b}	7.21^{a}
6	0	4.85^{c}	3.08^{b}	5.72^{b}	6.23^{c}	7.17^{bc}
6	500	4.90^{bc}	3.09^{b}	5.72^{b}	6.23^{c}	7.16^{c}
6	1000	4.92^{bc}	3.11^{b}	5.71^{b}	6.23^{c}	7.15^{c}
SEM		0.022	0.010	0.004	0.012	0.007

Mean values within a column with no common superscript differ significantly from each other (P <0.05),SEM= standard error of mean, MP= microbial phytase, CA= citric acid, NS= not significant

Moreover, the present findings showed that there were significant differences between T_3, T_3:T_4 ratio and relative weight of bursa and thymus ($p<0.05$), (Table 2). Current study data indicated that CA significantly decreased pH value of GI-tract segments ($p<0.01$), but MP had no significant effect on pH values (Table 3). Also, the results showed that there was significant effect between treatments for pH values of GI-tract parts ($p<0.05$) (Table 4).

DISCUSSION

It is well known that bursa and thymus are considered as parts of the immunity system (Sturkie, 1999) and this system is responsible for producing cells that protect the birds from the invaded microorganism. From Table 1, it is clearly observed that supplemental MP and CA significantly increased the relative weight of both primary lymphoid organs (bursa and thymus). Increasing the weight of thymus may be due to the impact of MP on the functional activities of the immune system responses that led to increase in the number of lymphocytes in the primary lymphoid organs. These results may imply that broilers fed acidifiers diets obtained higher immune response and disease resistance. In this respect, Katanbaf *et al.* (1989) reported that increases in the relative lymphoid organs weights are considered as an indication of the immunological advances. The fact of thyroid hormones as a major role in regulating oxidative metabolism of birds has been established (Sturkie, 1999). Triiodothyronine (T_3) level, as the metabolic activity of thyroid hormone, plays an active role in energy metabolism and metabolic rate. Any pronounced alteration in thyroid function (hyperthyroidism or hypothyroidism) is reflected in alteration of metabolic rate. Our results pointed out superior metabolic and growth rate due to the addition of acidifiers into broiler chickens diet. The hyperthyroidism and peripheral conversion of T_4-T_3 was signified better. The concentration of thyroid hormones circulating in chicken blood plasma was found to be around 1.2 μL/100 mL (Davison, 1976), showing daily variations due to an extremely short half-life and showing T_3-T_4 ratio to be 60:40, in favor of T_4 (Mehner and Hartfiel, 1983).

Similar results with the present study were found by Abdel-Fattah *et al.* (2008). In contrast, other studies, in which ascorbic acid and citric acid (Brown and Southern, 1985) were added to broiler diets, indicated that experimental treatments of intestinal content pH levels were not different compared to control that did not support our results. Decreasing pH in GI-tract had a beneficial effect in the inhibition of intestinal bacteria competition with the host for available nutrients and the possibility of reducing bacterial toxicity, e.g., ammonia and amines, thus improving weight gain of the host animals. Furthermore, the growth inhibition of potential pathogen bacteria, e.g. *E. coli and Salmonella*, in the feed and GI-tract is beneficial in respect to animal state of health (Thompson and Hinton, 1997). Organic acids are not antibiotics but, if used correctly along with proper nutritional, managerial and biosecurity measures, they can be a powerful tools in maintaining the GI-tract poultry state of health, thus improving their zootechnical performances. If applied correctly, organic acids function in poultry, not only as a growth promoter but also as a meaningful mechanism for controlling both pathogenic and non-pathogenic bacteria (Wolfenden *et al*., 2007). Moreover, feeding organic acids are believed to have several beneficial effects such as improving feed conversion ratio, growth performance, enhancing mineral absorption and speeding recovery from fatigue (Zeinb, 2004; Nourmohammadi *et al*., 2010b). The antibacterial activity of organic acids is related to reduction of pH, as well as their ability to dissociate that is determined by the pKa-value of the respective acid and pH of the surrounding environment, because the antibacterial activity increases with decreasing pH value. Several investigations have shown a strong bactericidal effect of organic acid without significantly decreasing the pH value in the GI-tract. Generally lactic acid bacteria are able to grow at relatively low pH which means that they are more resistant to organic acids than other bacterial species, e.g., E. coli (Russell and Diez-Gonzalez, 1998). In poultry, pathogenic bacteria e.g. *Salmonella* enters the GI-tract via crop. The crop environment with respect to microbial composition and pH seems to be very important in relation to the resistance to pathogens. High amounts of *Lactobacilli* and low pH in the crop have shown to decrease the occurrence of *Salmonella* in the crop (Hinton *et al*., 2000). Also the antibacterial effect of dietary organic acids in chickens is believed to take place mainly in the upper part of the digestive tract (crop and gizzard). Therefore, following combination addition of formic and propionic acid (Bio-Add) in high concentrations could only be affected by crop and gizzard (Thompson and Hinton, 1997).

CONCLUSION

Using organic acid and phytase supplementation as physiological additives might be useful to promote the immune response of broilers through their physiological action effect on the growth activities of some endogenous mechanisms responsible for better growth performance. As well, under the condition of this experiment, depression of pH values of GI-tract parts

by CA that can be a powerful tool in maintaining the GI-tract poultry state of health, thus improving weight gain of the broilers.

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