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RESEARCH ARTICLE

EFFICACY OF CITRIC ACID AND MICROBIAL PHYTASE ON PROTEIN EFFICIENCY RATIO IN BROILER CHICKEN

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ABSTRACT

One hundred and ninety two day-old broiler chicks (Vencobb) were divided into four identical groups having four replicates in each group with 12 birds in each replicate and allotted randomly into four dietary treatments viz., T1, T2, T3 and T4 for eight weeks. The treatments consisted of a standard broiler ration (SBR) with 0.5 per cent available phosphorus (T1), low available phosphorus broiler ration having 0.3 per cent available phosphorus (LAPBR) and 3.0 per cent citric acid (T2), LAPBR supplemented with 700 U of microbial phytase/kg feed (T3) and LAPBR with 1.5 per cent citric acid and 350 U of microbial phytase /kg feed (T4). Protein intake (g) of the birds at replication wise was calculated at fortnightly intervals, based on the data on feed intake and protein content of feed. Protein efficiency ratio (PER; g body weight gain/g of protein consumed) was calculated based on the data on body weight gain and protein intake. Synergistic improvement ($P<0.01$) in cumulative protein intake was noticed in T4 both at sixth and eighth week of age. Regarding the cumulative PER, the birds belonging to various treatments T1, T2, T3 and T4 recorded similar PER ($P>0.05$) at eighth week and significantly higher PER ($P<0.05$) in T2 as well as T4 at sixth week. The values were 2.03, 2.14, 2.05 and 2.13 at sixth week and 1.89, 1.97, 1.94 and 1.96 at eighth week for T1, T2, T3 and T4, respectively. The results indicate that birds maintained on low phosphorus diets with additives (T2, T3 and T4) performed equally good compared to control group maintained on SBR, suggesting the favouring effect citric acid and/or microbial phytase on protein utilization.

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 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 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. To make phytate P biologically available, CA was used as a strong chelator of Ca, making phytate P less stable and more susceptible to endogenous and exogenous phytase (Boling *et al.*, 2000).

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So, 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 protein utilization in broiler chicken.

MATERIALS AND METHODS

Table 1 Ingredient and chemical composition of starter and finisher rations %

Ingredients	Starter ration (0-6 weeks)				Finisher ration (6-8 weeks)			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Yellow maize	50.0	46.5	50.0	48.0	61.8	58.3	61.8	59.8
Rice polish	5.7	6.2	6.2	6.2	2.1	2.5	2.5	2.5
Soybean meal	36.0	36.5	36.0	36.5	28.0	28.5	28.0	28.5
Unsalted fish	5.2	5.2	5.2	5.2	5.0	5.0	5.0	5.0
Shell grit	0.8	1.5	1.5	1.5	0.8	1.5	1.5	1.5
DCP	1.5	0.3	0.3	0.3	1.6	0.5	0.5	0.5
Citric acid	0.0	3.0	0.0	1.5	0.0	3.0	0.0	1.5
Phytase (U/Kg)[1]	0	0	700	350	0	0	700	350
Chemical Composition, % DM Basis								
Dry matter	91.1	91.6	91.3	91.6	90.9	90.3	90.7	90.4
Crude protein	23.6	23.1	23.6	23.4	20.2	20.4	20.2	20.4
Ether extract	4.6	4.4	4.7	4.5	4.8	4.9	5.3	4.8
Crude fibre	4.3	4.1	4.4	4.4	3.5	3.5	3.7	3.5
NFE	59.3	58.3	58.3	57.7	64.7	64.1	63.4	64.8
Total ash	8.2	10.0	9.00	10.1	6.8	7.1	7.2	6.5
AIA	2.5	2.4	2.5	2.6	1.5	1.5	1.5	1.4
Ca	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Total P	0.8	0.6	0.6	0.6	0.8	0.6	0.6	0.6
Mg	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Mn (mg/Kg)	126.6	126.1	123.7	123.7	123.3	124.73	128.1	129.5
Zn (mg/kg)	81.6	84.5	88.4	83.0	80.6	80.5	82.1	83.9
Calculated values								
ME (Kcal/Kg)	2804.6	2814.1	2818.6	2814.1	2894.3	2901.0	2905.5	2901.0
Lysine	1.4	1.4	1.4	1.4	1.1	1.1	1.1	1.1
Methionine	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4

Additives added / 100 kg of feed

Vitamin Ad_3EK:
Vitamin A – 5156250 IU, Vitamin D_3 – 750000 IU, Vitamin E-2500 mg, Vitamin K-625 mg.
Vitamin B complex:
Vitamin B_1 – 250 mg, Vitamin B_2 – 3125 mg, Vitamin B_6 – 500 mg, Vitamin B_{12} – 2500 µg,
Niacin – 3750 mg, Ca pantothenate – 2500 mg.
Trace mineral mixture (ultra TM – 130 gm) contains:
Mn – 7.02g. Zn – 6.76g. Iron – 2.6g, Iodine – 260mg, Copper – 260 mg, Cobalt – 130mg.
Toxin Binder (Alusil Premix TM) : 200g
Coccidiostat : Maduramycin ammonium – 500 mg.
Common salt: 250 gram

[1]Each gram of phytase (Natuphos @ - 5000G M/s. BASF, Germany) contained phytase activity of 5000 U (one U is the quantity of enzyme that release one µmol of inorganic P per minute from 1.5mmol/L of sodium phytate at a pH of 5.5 and temperature of 37°C). One hundred and ninety two, day-old commercial broiler chicks (Vencobb) of similar mean body weight were randomly divided into 16 groups of 12 chicks each and were allotted randomly to four dietary treatments viz., T1, T2, T3 and T4 with four replicates in each treatment. The dietary treatments consisted of a standard broiler ration as per BIS with 0.5 % available P(T1), low available P broiler ration with 0.3% available P (LP) and 3.0% CA (T2), LP supplemented with 700 U of MP (Natuphos® 5000, BASF Corp., Germany)/kg diet (T3) and LP with 1.5% CA and 350U of MP/kg diet (T4). The composition and chemical analysis of different dietary treatments are presented in Table 1. The starter ration was fed up to six weeks and finisher ration from 7th to 8 weeks of age. The birds were provided *ad libitum* feed and water throughout the experimental period and were maintained under deep litter system of management.

The body weight of individual birds was recorded at fortnightly intervals from day old to eight weeks to study the pattern of growth rate under different dietary treatments. The fortnightly body weight gain was obtained by calculation. Feed intake of the birds was recorded replication wise at weekly intervals. From these data, the average feed intake per bird per day was calculated for various treatment groups. Feed conversion ratio (FCR- kg of feed consumed/kg body weight gain) was calculated based on the data on body weight gain and feed intake. Protein intake (g) of the birds at replication wise was calculated at fortnightly intervals based on the data on feed intake and protein content of feed. Protein efficiency ratio (g body weight gain /g of protein consumed) was calculated based on the data on body weight gain and protein intake.

The chemical compositions of experimental rations were determined as per the standard procedures (AOAC, 1990). The mortality of birds from different treatment groups was recorded and post mortem examination was conducted in each case to find out the cause of death.

Data collected on various parameters were statistically analyzed by Completely Randomised Design (CRD) method as described by Snedecor and Cochran (1985). Means were compared by Least Significant Difference (LSD) test using MSTATC. Results were expressed as mean ± S.E. The results were considered statistically significant if the ‘p’ value were 0.05 or less.

RESULTS AND DISCUSSION

From the results presented in Table 2, it can be seen that birds of all the four groups recorded almost similar PER during the various periods of the experiment, except at sixth week.

Regarding the cumulative protein efficiency ratio, the birds belonging to various treatments T1, T2, T3 and T4 recorded similar PER (P>0.05) at eighth week and significantly higher PER (P<0.05) in T2 as well as T4 at sixth week.

Table 2 Fortnightly and cumulative mean protein efficiency ratio of birds maintained on different dietary treatments

Treatments	Fortnightly mean protein efficiency ratio				Cumulative mean protein efficiency ratio	
	Age in weeks				Age in weeks	
	2	4	6	8	0-6	0-8
T1	3.12±0.07	2.07±0.04	1.74^{b}±0.01	1.60±0.03	2.03^{b}±0.03	1.89±0.03
T2	2.96±0.04	2.24±0.05	1.92^{a}±0.04	1.66±0.07	2.14^{a}±0.01	1.97±0.02
T3	3.06±0.10	2.12±0.05	1.75^{b}±0.04	1.69±0.04	2.05^{b}±0.04	1.94±0.03
T4	3.24±0.02	2.24±0.03	1.81^{ab}±0.05	1.59±0.10	2.13^{a}±0.02	1.96±0.04

Figures with different superscripts in a row differed significantly, P<0.05

The second week PER values showed that T4 has registered the highest value of 3.24 and T2 has the lowest value of 2.96 and no significant difference exits between treatments. The fourth week PER values for T1, T2, T3 and T4 were 2.07, 2.24, 2.12 and 2.24 respectively (P>0.05).

In the sixth week, the PER values differed significantly (P<0.05) and values were 1.74, 1.92, 1.75 and 1.81 for T1, T2, T3 and T4, respectively. There is no significant difference in eighth week PER values and the values were 1.60, 1.66, 1.69 and 1.59 for T1, T2, T3 and T4, respectively.

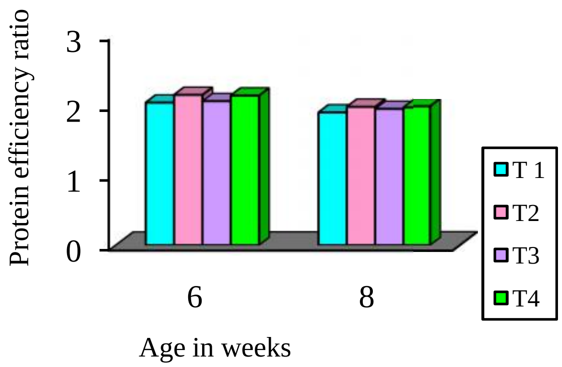

Fig. 1. Cumulative mean protein efficiency ratio of birds maintained on different dietary treatments

The trend in cumulative PER at sixth and eighth week of age was also similar to fortnightly PER at sixth and eighth week of age (Fig. 1). The values were 2.03, 2.14, 2.05 and 2.13 at sixth week and 1.89, 1.97, 1.94 and 1.96 at eighth week for T1, T2, T3 and T4, respectively.

Maheswari and Kadirvel (1996) obtained nearly same PER values at fourth week ranging from 2.43 to 2.44 by supplementation of malic acid, when compared to unsupplemented groups and are in agreement with the present study. However, at sixth week, significantly higher (P<0.05) PER value for T2 and T4 in the present study might be due to increased nutrient utilization on citric acid supplementation in diet and it correlate well with increase in body weight gain.

Boling-Frankenbach et al., (2001) and Peter and Baker (2001) also observed no significant effect of phytase (1200 U/kg diet) on PER values and agree well with the present study.

The results indicate that birds maintained on low P diets with additives (T2, T3 and T4) performed equally good compared to control group maintained on SBR, suggesting the favouring effect on protein utilization.

On scrutiny of the results of the present study, it could be concluded that inclusion of citric acid (3.0%) or phytase (700 U/kg diet) or its combination (1.5% citric acid +350 U Phytase/kg diet) as additives in low available P diets (0.3%) of broiler chicks from day-old to eight weeks of age resulted in better protein utilization in chicks than chicks maintained on standard broiler ration with 0.5% available P; tending to suggest that inclusion of citric acid or phytase or the combination of both could improve protein efficiency in low available P diets in broiler chicken.

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