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Effect of Enzyme Supplementation to Normal and Low Density Broiler Diets Based on Corn-soybean Meal

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ABSTRACT

This study was performed to evaluate the effect of feeding Tomoko, a commercial enzyme supplement that contains an acidic protease, α-amylase, pectinase, phytase, glucoamylase, cellulase and *Aspergillus Awamori* cells in a standard corn-soy ration for broiler chicken from 1 to 42 day of age. A total of 960 Cobb 500 chicks were randomly distributed in a randomized complete block design among 16 floor pens with 4 replicate pens/treatment. Two levels of diet density (normal and low) and two levels of enzyme (without and with) in a factorial arrangement resulted in four dietary treatments: T_1 = normal density diet; T_2 = T_1+0.05% enzyme; T_3 = low density (low energy, low protein diet); T_4 = T_3+0.05% enzyme. Body weight was significantly affected by diet density and enzyme at 42 d ($p<0.001$). Enzyme supplementation improved eviscerated, breast and total meat percentages ($p<0.01$, 0.01 and 0.001, respectively) while diet density had a significant effect on all parts yield measured. Ileal protein retention showed a significant density × enzyme interaction ($p<0.005$). Serum total protein, calcium and phosphorus were improved as a result of enzyme supplementation. Enzyme was able to restore the nutritional value in the low density diet. These findings suggesting that increased muscle mass is partially responsible for the observed increased in body weight on use of enzyme preparation.

Key words: Tomoko, broiler, performance, retention, serum metabolite

INTRODUCTION

Broiler feed is based primarily on corn and Soy Bean Meal (SBM). Corn is the predominant source of energy in feed because of its abundance and economy while SBM is a valuable protein source in broiler diets because of its high protein content and well balanced amino acid profile. However, bioavailability of nutrients from corn and SBM is not ideal and recent data indicate that there is room for improvement. Corn and SBM have shown a degree of variability in terms of feeding values as measured by ME (Iji *et al*., 2003). It was reported that they are incompletely digested by poultry due to the presence of Non-Starch Polysaccharides (NSPs) which is considered as anti-nutritional factor (Cone *et al*., 1994; Pack and Bedford, 1997). Corn contains 9.7% NSP which comprise 70-90% of the cell wall (Bach Knudsen, 2001). While, SBM contains 30.3% NSPs which constitutes 70-90% of the plant cell wall (Smits and Annison, 1996; Bach Knudsen, 2001). Water soluble NSPs fed to young chicks interfere with the digestion and absorption of other nutrients by increasing the viscosity of digest in the gut (Ward and Marquardt, 1983).

Several attempts have been made to increase the nutritional value of corn and SBM by adding protease and carbohydrases either before or after processing (Abudabos, 2010; Bedford, 1993;

Gracia *et al*., 2003; Ghazi *et al*., 1997; Rostango *et al*., 2000; Scheideler *et al*., 2005). It was suggested that enzymes reduce the negative effects of NSPs and improve the digestion of nutrients in poultry diets. Hydrolysis of NSPs reduces the viscous properties of β-glucan and pentosans, release some available monosaccharides and in part, eliminate the nutrient encapsulating effect of the cell wall (Wyatt, 1992).

There are two main ways when considering incorporation of enzymes into corn-SBM diets. The simplest is "over the top" addition to an existing formulation so as to achieve economic improvements in performance without changing its nutrients density (Costa *et al*., 2008). The second approach is to change the nutrient density of the feed to reduce the cost per ton of feed and then, by adding enzymes, to restore the nutritional value of the feed. This results in performance better or at least similar to a normal feed density (Pack and Bedford, 1997).

The objective of the present study was to evaluate the efficacy of Tomoko in corn-SBM rations on performance, nutrient retention and carcass yield of broiler chicken from 1 to 42 days of age in diets that differ in energy and protein content.

MATERIALS AND METHODS

Birds and management: The study was conducted under a protocol approved by King Saud University and complies with the current laws of Saudi Arabia. A total of 960 one day old Cobb 500 (Oyester house, severalls lane, colchester ,essex Co_{49} PD, UK) broiler chicks were obtained from a commercial hatchery, weight sorted and randomly distributed, 60 birds per pen, into one of 16 pens which were bedded with wood shavings. Stocking density was 10-birds/m^2 at 6 weeks of age. The allocation was such that all pens had a similar average body weights. The birds were reared in a conventional poultry house with raised side windows. The birds were maintained on a 24 h light schedule.

Diets and experimental design: In order to test the effectiveness of Tomoko (Biogenkoji research institute, 876-15, Kagoshima, Japan) enzyme on the performance of broiler chicken, two corn-SBM rations were formulated, one to produce a normal levels of nutrient density (ME and crude protein), the other with subnormal or low levels of density. Each diet was supplemented with or without the enzyme in a factorial arrangement (2 levels of nutrient density and 2 levels of enzyme), resulting in a total of 4 experimental treatments: T_1 = normal density diet (control); $T_2 = T_1$+0.05% enzyme; T_3 = low density (low energy, low protein diet); $T_4 = T_3$ + enzyme. Isocaloric and isonitrogenous corn-SBM meal diets were formulated except for the low density diets while lysine and sulfur containing amino acids were maintained at the same levels for all diets (Table 1). The starter diet was fed from 1 to 10 d and contained 21% CP and 3000 kcal of ME kg^{-1} for diet 1 and 2; 20% CP and 2820 kcal of ME kg^{-1} for diet 3 and 4. The grower diet was fed from 11 to 22 day, and contained 19% CP and 3080 kcal of ME kg^{-1} for diets 1 and 2; 18% CP and 2930 kcal of ME kg^{-1} for diets 3 and 4. In the finisher diet (23 to 42 day), CP and ME were 18% and 3170 kcal kg^{-1} for diets 1 and 2; 17% and 3000 kcal kg^{-1} for diets 3 and 4. All nutrients were calculated on an as fed basis. The experimental diets were formulated to meet the nutrient requirements for Cobb 500 strain. Feed and water were provided *ad libitum*. Tomoko is a commercial multi-enzyme feed additive which is produced by fermentation using Koji-feed (*Aspergillus awamori*), produced from wheat bran and distillery by-product from rice, sweet potato or barley. The enzyme used in the study was authenticated by the supplier to have minimum level

Table 1: Composition of experimental diets fed to broilers from 1 to 42 days

	Starter		Grower		Finisher	
Item	Normal (T_1)[1]	Low (T_3)[2]	Normal (T_1)[1]	Low (T_3)[2]	Normal (T_1)[1]	Low (T_3)[2]
Ingredients (g kg^{-1})						
Corn	603.50	608.50	645.20	658.80	663.00	667.90
Soybean meal (48% CP)	330.00	290.00	283.00	235.00	258.00	218.00
Wheat bran	--	50.00	--	50.00	--	50.00
Corn oil	25.00	9.00	32.00	14.00	42.00	26.00
Salt	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	9.00	0.90	8.00	9.00	8.00	8.00
Di calcium phosphate	21.00	21.00	22.00	21.00	19.50	19.50
DL-methionine	2.00	2.00	1.50	1.80	1.50	1.60
L-Lysine	1.50	2.50	1.80	3.90	2.00	3.00
Vitamin-mineral premix[3]	3.00	3.00	3.00	3.00	3.00	3.00
Coccidiostat	0.50	0.50	0.50	0.50	0.00	0.00
Choline chloride premix, 60%	1.50	1.50	0.00	0.00	0.00	0.00
Calculated analysis						
ME (kcal kg^{-1})	3000.00	2820.00	3080.00	2930.00	3170.00	3000.00
CP (%)	21.00	20.00	19.00	18.00	18.00	17.00
Methionine (%)	0.50	0.50	0.47	0.47	0.44	0.44
Methionine+Cystine (%)	0.85	0.85	0.78	0.78	0.73	0.73
Lysine	1.20	1.20	1.10	1.10	1.05	1.05
Linoleic acid	1.90	1.80	2.10	1.80	2.40	2.10
Calcium	1.00	1.00	0.96	0.96	0.90	0.90
Available phosphorns	0.50	0.50	0.48	0.48	0.45	0.45

[1]Diet 2 had 0.05% Tomoko enzyme added to diet 1. [2]Diet 4 had 0.05% Tomoko enzyme added to diet 3. [3]Vitamin-mineral mix is supplied in the following per kg of diet: Retinyl acetate, 3.41 mg; cholecalciferol, 0.07 mg; DL-a-tocopheryl acetate, 27.5 mg; menadione sodium bisulphate, 6 mg; riboflavin, 7.7 mg; niacin, 44 mg; pantothenic acid, cyanocobalamin, 0.02; choline, 496 mg; folic acid, 1.32 mg; pyridoxine HCl, 4.82 mg; thiamine mononitrate, 2.16 mg; D-biotin, 0.11 mg; manganese, 67 mg; zinc, 54 mg; copper, 2 mg; iodine, 0.5 mg; iron, 75 mg; selenium, 0.2 mg

of acidic protease (10,000 U g^{-1}), alpha-amylase (40 U g^{-1}), pectinase (30 U g^{-1}), phytase (10 U g^{-1}), glucoamylase (5 U g^{-1}), cellulase (4 U g^{-1}) and *Aspergillus awamori* cells (10 mg g^{-1}).

Measurements: Body weight and feed consumption were recorded weekly by pen and feed conversion was computed at 22 and 42 d of age. At 42 d of age, 3 males from each pen were selected and kept without food for 3 h then bled from the cutaneous ulnar vein. Blood samples were centrifuged for 15 min at 2, 500x g and the serum was harvested and stored at -80°C. Protein (Biuret Method), triglyceride (Sulfo-Phosph Vanillin Method), calcium (End Point Method) and phosphorus (Molybdate U.V. Method) concentrations were analyzed using enzymatic colorimetric kits (Biolabo Reagents); all analyses were carried out in duplicate. After euthanasia, feather, heads, neck and shanks were removed and the remaining carcasses were dissected to breast, thigh, drumstick and abdominal fat and weighed. The percentages of eviscerated and yield of each part was calculated on the basis of live weight. The breast, thigh and drumstick were deboned and total meat percentage was calculated.

A digestion trial was performed at the end of the experiment on separate group of birds. Thirty six birds (4 replications per diet) were housed (2 birds/cage) in 16 cages with wire bottoms. All

finisher diets were supplemented with 3 g kg^{-1} chromic oxide as an analytical marker for the retention trials. At 37 day of age, birds were fed chromium oxide mashed feed for 5 days adaptation period and on the 5th day approximately 200 g of clean excreta (free of feed and visible feather contaminants) was collected to determine the feces moisture. At 42 days of age, all birds were euthanized by cervical dislocation to determine ileal retention of protein and ether extract. The intestinal tract was removed and the contents of the tract from Meckel's diverticulum to the ileal-cecal-colon junction were collected from two birds and pooled to determine ileal retention. Approximately 3 g of homogenized chyme sample was collected. Samples were dried and dry matter was determined by oven-drying at 105°C for 16 h. Diets and ileal digest samples were ground to pass through a 1.0 mm screen. Crude protein (N×6.25) and ether extract were analyzed according to the procedures established by the Association of Official Analytical Chemists (AOAC, 1980). All analyses were carried out in duplicate. Chromic oxide was analyzed according to the procedure described by Williams *et al.* (1962). The following equation was used for calculation of percent retention (Scott *et al.*, 1976):

$$\text{Retention (\%)} = 100-((\text{Diet } Cr_2O_3/\text{Digest } Cr_2O_3 \times \text{Digest nutrient/Diet nutrient}))\times 100$$

Statistical analysis: Analysis of variance was performed using General Linear Model procedure of the Statistical Analysis System (SAS Institute, 1996) for randomized complete block design with 2×2 factorial arrangements of treatments, in which each experimental diet was fed to 4 replicate pens. The data were tested for main effects of nutrient density, enzyme and for interaction effect for density×enzyme. The experimental unit was the pen mean. Statistical significance was assessed at $p<0.05$.

RESULTS

Feed consumption and efficiency: Growth performance, feed intake and feed conversion are shown in Table 2. At 10 day, no significant differences in body weight, feed intake and feed efficiency due to treatment; data were not included in the table. At 22 day of age, significant differences in body weight were observed due to density and enzyme ($p<0.001$ and 0.005, respectively); birds which had received normal density and enzyme gained more weight compared to those which had received low density and no enzyme. Birds which had received low density and enzyme consumed significantly more feed compared to the other two groups ($p<0.001$). As a result, birds which had received the normal and enzyme had a better feed efficiency compared to the other groups ($p<0.001$ and 0.05, respectively). At 42 day, birds which had received normal density and enzyme gained more weight compared to those which had received low density or no enzyme (129 and 126 g, respectively). A two way interaction was significant for feed intake ($p<0.005$). Feed intake increased by birds when normal density diets were supplemented with enzyme while it was decreased by birds when low density diets were supplemented. A two way interaction was significant for feed efficiency ($p<0.001$). Enzyme improve feed efficiency in birds which had received low or normal density; however, the magnitude of improvement was better for birds which had received the low density diets.

Carcass characteristics: The mean percentage of carcass parts is documented in Table 3. Both density and enzyme had significant effects on eviscerated percentage ($p<0.001$, 0.01,

Table 2: Live weight (BW), cumulative feed intake and feed conversion ratio (FC) of broiler chickens given experimental diets at 22 and 42 days

			Performance					
			22 day			42 day		
Treatment	Density	Enzyme (%)	BW (g)	Feed (g)	FC (g: g)	BW (g)	Feed (g)	FC (g: g)
1	Normal	0	888	1171	1.319	2345	4595	1.959
2	Normal	0.05	907	1182	1.303	2457	4638	1.888
3	Low	0	862	1193	1.385	2201	4740	2.153
4	Low	0.05	880	1202	1.366	2342	4660	1.989
SEM±			5.35	1.85	0.007	11.76	17.62	0.011
Density average								
Normal			898	1177	1.311	2400	4616	1.92
Low			871	1198	1.375	2271	4699	2.07
SEM±			3.78	1.31	0.005	8.31	12.46	0.007
Enzyme average								
Without			875	1182	1.352	2273	4668	2.056
With			894	1192	1.334	2399	4649	1.939
SEM±			3.78	1.31	0.005	8.31	12.46	0.007
Statistical probabilities								
Density			***	***	***	***	***	***
Enzyme			**	***	*	***	NS	***
Enzyme×Density			NS	NS	NS	NS	**	***

*p<0.05, **p< 0.01, ***p<0.001, NS: Not significant, SEM: Standard error of the mean

Table 3: Effect of different treatments on parts yield as percentages of broiler dressed weight at 42 days

Treatment	Density	Enzyme	Eviscerated[1]	Breast	Thigh	Drumstick (%)	TM[2]	Fat
1	Normal	0	72.3	20.1	13.5	10.5	36.2	2.47
2	Normal	0.05	73.4	21.3	13.4	10.7	36.9	2.3
3	Low	0	71.2	18.9	12.8	0.1	35.41	2.15
4	Low	0.05	71.9	19.7	13.1	10.5	35.8	2.15
SEM±			0.27	0.33	0.13	0.12	0.13	0.06
Density average								
Norma			72.7	20.7	13.5	10.6	36.5	2.4
Low			71.6	19.3	12.9	10.3	35.6	2.1
SEM±			0.19	0.23	0.09	0.08	0.09	0.04
Enzyme average								
Without			71.7	19.5	13.1	10.4	35.8	2.31
With			72.6	20.5	13.2	10.6	36.4	2.21
SEM±			0.19	0.23	0.09	0.08	0.09	0.04
Statistical probabilities								
Density			***	***	**	*	****	***
Enzyme			**	**	NS	NS	***	NS
Enzyme×Density			NS	NS	NS	NS	NS	NS

[1]Eviscerated %: Eviscerated carcass, without neck, abdominal fat and internal organs, as percentage of live weight. [2]Total meat: Sum of breast meat, thigh and drumstick deboned without skin, as percentage of live weight. * p<0.05, **p<0.01, ***p<0.001, NS: Not significant, SEM: Standard error of the mean

respectively). Birds which had received normal density and enzyme had a higher eviscerated percentage. Breast muscle yield followed the same trend; heavier breasts were obtained from birds which had received normal density diet supplemented with enzyme as compared to low density and without enzyme (20.7 and 20.5 vs. 19.3 and 19.5, respectively). Thigh and drumstick yield percentages were significantly affected by density ($p<0.01$ and 0.05, respectively) while enzyme had no effect ($p>0.05$). Both density and enzyme had significant effects on total meat percentage ($p<0.001$ and 0.001, respectively), with higher total meat percentages were obtained from birds on normal density and enzyme diets; enzyme improved total meat percentage by 0.6%. Abdominal fat was reduced significantly ($p<0.001$) in birds which had received low density diet. Enzyme had no effect on the abdominal fat percentage ($p>0.05$).

Feed ileal retention and blood serum metabolites: Table 4 shows excreta moisture and ileal retention data in this experiment. Neither density nor enzyme had a significant effect on in fecal moisture or ileal ether extract retention ($p<0.05$). A two way interaction was significant for ileal protein retention ($p<0.005$), a higher increase in protein retention was found in birds which had received the normal density diet supplemented with enzyme compared to birds on low density diet that was supplemented with enzyme.

The data related to serum metabolites are shown in Table 5. Serum total protein concentration was significantly affected by density and enzyme ($p<0.001$ and 0.001, respectively). Serum triglyceride was not affected by any treatment. Serum calcium and phosphorus were affected by enzyme ($p<0.05$ and 0.05, respectively) while diet density had no effect on concentrations.

Table 4: Excreta dry matter content and apparent ileal retention of nutrients in broiler chickens at 42 days

			Ileal retention coefficients		
Treatment	Density	Enzyme	Excreta moisture	Crudeprotein (%)	Ether extract
1	Normal	0	69.3	75.7	80.9
2	Normal	0.05	71	79.9	80.7
3	Low	0	69.5	73.8	79.6
4	Low	0.05	68.5	75	79.8
SEM±			1.19	0.39	0.74
Density average					
Normal			70.1	77.9	80.7
Low			8.9	74.46	79.7
SEM±			0.84	0.28	0.52
Enzyme average					
Without			69.4	74.8	80.2
With			69.7	77.5	80.2
SEM±			0.84	0.28	0.52
Statistical probabilities					
Density			NS	***	NS
Enzyme			NS	***	NS
Enzyme×Density			NS	**	NS

*p<0.05, **p<0.01, ***p<0.001, NS: Not significant, SEM: Standard error of the mean

Table 5: Serum total protein (TP), cholesterol, triacylglycerol (TG), calcium (Ca) and phosphorus (P) concentrations of broiler chickens at 42 days

			Serum metabolite			
Treatment	Density	Enzyme (%)	TP (g dL^{-1})	TG (mg dL^{-1})	Ca (mg dL^{-1})	P (mg dL^{-1})
1	Normal	0	3.04	161.3	11.55	7.62
2	Normal	0.05	3.16	161.6	11.85	7.79
3	Low	0	2.81	159.5	11.5	7.48
4	Low	0.05	2.93	159.3	11.75	7.73
SEM±			0.022	1.14	0.098	0.07
Density average						
Normal			3.1	161.4	11.7	7.72
Low			2.87	159.4	11.62	7.6
SEM±			0.01	0.81	0.07	0.05
Enzyme average						
Without			2.92	160.4	11.52	7.56
With			3.04	160.5	11.8	7.76
SEM±			0.02	0.81	0.06	0.05
Statistical probabilities						
Density			***	NS	NS	NS
Enzyme			***	NS	*	*
Enzyme×Density			NS	NS	NS	NS

*p<0.05, **p< 0.01, ***p<0.001, NS: Not significant, SEM: Standard error of the mean

DISCUSSION

The results revealed a significant interaction in feed efficiency at 42 day of age which could be explained by a difference in magnitude or response, birds on both density levels responded positively to enzyme supplementation however, those which had the low density diet had a higher response to the enzyme. On the other hand, enzyme increased feed intake for birds which had normal density diet while it decreased feed intake by those who received the low density diet and this explained the significant interaction in feed intake. The improvement in feed efficiency could be explained in part by the improvement in body weight that occurred as a result of the enzyme, final body weight for the low density group plus enzyme was restored and was comparable to that of the normal density without the enzyme, suggesting that the improvement in nutrient utilization brought about by enzyme supplementation completely compensated for the reduced energy and protein content. The improvements in body weight gain of broilers which had fed the enzyme supplemented diets could be ascribed to the increased nutrient retention especially protein as shown in this study. It has been reported that corn-soy diets supplemented with enzyme produced significant improvement in growth performance in broilers (Abudabos, 2010; Gracia *et al*., 2003; Ranade and Rajmane, 1992; Saleh *et al*., 2006; Wyatt, 1992; Zanella *et al*., 1999). On the other hand, Douglas *et al*. (2000) reported no corresponding feed efficiency improvements with enzyme.

Enzyme supplementation decreased feed intake in the low density by 43 g while it increased it by 80 g in the normal density group. This is congruent with previous findings of Gracia *et al*. (2003) and Cowieson and Ravindran (2008) and in disagreement with Douglas *et al*. (2000), Ranade and Rajmane (1992) and Saleh *et al*. (2006).

In the current study, enzyme increased serum total protein, calcium and phosphorus significantly suggesting that the efficiency of dietary utilization of protein, calcium and phosphorus increased in chicken which had fed the enzyme and consequently nutrient uptake was improved.

This could be due to the action of the exogenous protease and phytase in the enzyme preparation directly or indirectly by providing better environment for the endogenous digestive enzymes to work on substrates. Smits *et al.* (1997) demonstrated a significant reduction in apparent nitrogen retention after feeding higher NSP to the birds. Angkanaporn *et al.* (1994) demonstrated an increase in endogenous nitrogen losses in broiler birds as a result of water-soluble pentosans in the diet. These findings suggest that dietary NSP inhibits the ileal protein retention. Thus, the improvement of ileal protein retention could be due to the digestion of NSP in diet by the enzyme. Ileal protein retention improved by 4.4% due to enzyme supplementation in the normal density diet compared to 1.2 % in the low density diet which caused a significant interaction between diet density and enzyme. Similar results were shown in previous work; for example, a 3% improvement in crude protein retention of corn-SBM based diets of broiler supplemented with enzyme preparation containing protease, cellulase and amylase (Rostango *et al.*, 2000). Zanella *et al.* (1999) observed 2.9% improvement in the crude protein retention with addition of enzyme that contained protease. Sakomura *et al.* (1998) found that the addition enzyme preparation which contains xylanase, protease and amylase to a corn-SBM diet improved protein ileal retention. Wyatt (1992) postulated that exogenous enzymes cause cell wall disruption and the release of cell-bound nutrient and thus make more nutrients available for digestion by the animal.

A significant increase in the eviscerated percentage of broilers fed diets containing the enzyme was shown in this experiment (0.9%) this agrees with previous findings (Saleh *et al.*, 2006). A higher percentage of breast parts and total meat were associated with enzyme supplementation, this could be due to higher deposition of protein in this part. These results are in disagreement with others who reported that enzyme had no effect on carcass yields (Cafe *et al.*, 2002; Saleh *et al.*, 2004; Yamamoto *et al.*, 2007). Abdominal fat percentage was significantly higher in the control group compared to the low energy and protein group. Enzyme had no effect on abdominal fat percentage, this result disagree with Cafe *et al.* (2002) who reported that broilers fed enzyme supplemented diet had significantly higher proportion of abdominal fat. Deposits of fat in the abdominal region of the broiler are considered a waste by the poultry industry and it also added expense for the processing effluent treatment.

Enzyme supplementation at the rate recommended by the manufacture and used in this experiment had a positive effect on broiler performance, feed efficiency, breast yield, total meat, eviscerated percentage and ileal protein retention. The beneficial effect of enzyme in corn-SBM diet was the best when the enzyme was added at the top of the control diet. No difference in performance of birds was seen between the control without enzyme and the low density group plus enzyme, showing that the improvement of nutrient utilization brought about by enzyme supplementation completely compensated for the reduced protein and energy content of the diet. This result offers potential to reduce diet cost commensurate with no losses in production. It is important to mention that broilers in this experiment were fed a mashed diet that was not subjected to any heat; further study is required to examine the effect of pelleting on the enzyme activity. One constraint to the use of enzyme in broiler diets is that enzymes are generally heat-labile and the activity of the enzyme is lost or reduced when feed is pelleted and this could explain partly the wide discrepancy of the effect of exogenous enzyme on broilers.

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