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Effect of a ß-Mannanase on Nutrient Digestibility in Corn-Soybean Meal Diets for Broiler Chicks†

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Abstract: The possibility of improving digestibility of nonstarch polysaccharides present in broiler diets by the use of different carbohydrase enzymes appears as an opportunity to enhance feed utilization by the birds. In this study, the effect of a beta-mannanase product on nutrient digestibility in corn-soybean meal diets was investigated. One-day-old chicks received a nutritionally complete corn-soybean meal for 19 days. At that time birds were randomly allocated to four treatments, each of which had six replicates of five birds housed in battery brooders with wire floors. Aliquots of the basal diet were supplemented with four levels of CTCZYME (CTC Bio Inc., Seoul Korea): 0%, 0.025%, 0.05% (recommended level) and 0.10%. Chromic oxide was used as an indigestible marker. Feed was analyzed for gross energy, chromium and amino acid content. After eight days of acclimation to the test diets, birds were sacrificed and ileal contents collected. Analysis of the ileal contents indicated that digestibility of Lys, Met, Thr, Trp, Arg, Leu, Ile, Cys and Val were significantly ($p \leq 0.0001$) improved in a linear manner for each increment of enzyme. Ileal apparent metabolizable energy increased with each increment of CTCZYME level. These results show that the enzyme improves feed digestibility by making amino acids more available for the bird and increases energy utilization from the feed. These results suggest that lower levels of protein and energy could be used with the same results but further studies are required to estimate potential levels.

Key words: Broilers, beta mannanase, enzyme, digestibility

INTRODUCTION

Non-Starch Polysaccharides (NSP) have been classified as inhibitors of nutrient utilization due to their ability to increase the viscosity of the digesta, to modify the physiology of the gastrointestinal tract and to change the ecosystem of the gut (Choct, 2002). Viscosity is attributed to reduced digestive enzyme activities and macronutrient digestibility (Smits *et al*., 1997). The increased viscosity decreases rate of diffusion of substrates and digestive enzymes reducing the efficiency of nutrient absorption by interacting with the glycocalix of the intestinal brush border (Choct, 1997).

When beta-mannans, one type of NSP, are present in feed they depress growth and feed conversion and increase nitrogen and fecal output, decreasing metabolizable energy as well (Daskiran *et al*., 2004; Lee *et al*., 2003). The beta-mannans are present in soybean meal in a rate of 1.1-1.3% (Dierick, 1989). It has been reported that the use of a beta-Mannanase in corn-soybean meal diets has improved body weight gain and feed conversion in broilers and turkeys (Jackson *et al*., 2004; Zou *et al*., 2006; Odetallah *et al*., 2002). The present study was conducted to evaluate the effect of different inclusion levels of an exogenous beta-Mannanase on nutrient digestibility using corn-soybean meal based diets in broilers.

MATERIALS AND METHODS

In this experiment, 192, one-day-old Cobb 500 male chicks were obtained from a commercial hatchery and placed in wire floor starter batteries in a temperature-controlled room and fed a nutritionally complete diet to 19 days of age (Table 1). At that time, the birds were transferred and randomly assigned to unheated wire floored finisher batteries and weighed by pen. Eight birds were allocated per pen with six replicates per treatment for a total of 24 pens. The birds were given access to the experimental diets and tap water for *ad libitum* consumption. Lighting was continuous 24 hr during the experiment. Care and management of the animals followed recommended guidelines (FASS, 2010). All procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee. One large lot of feed was prepared with chromic oxide as a tracer (Table 1). It was also supplemented with Celite and titanium for a parallel study on comparison of

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Table 1: Composition (g/kg) and calculated nutrient content of basal diets

Ingredient	g/kg
Yellow corn	638.35
Poultry oil	10.22
Soybean meal	287.26
Limestone	5.25
Defluorinated phosphate	18.39
Feed grade salt	3.03
Sodium bicarbonate	0.86
MHA 84[1]	2.48
L-Threonine	0.33
L-Lysine HCl	1.83
Vitamin premix[2]	5.00
Mintrex P_Se[3]	1.00
Celite	20.00
Chromic oxide	3.00
Titanium dioxide	3.00
Total	1000.00
ME kcal/kg	3020.00
Crude protein %	19.63
Calcium %	0.90
Total P %	0.68
Nonphytate P %	0.45
Methionine %	0.55
Cystine %	0.32
Lysine %	1.14
Tryptophan %	0.23
Threonine %	0.75
Isoleucine %	0.78
Valine %	0.88
Leucine %	1.64
Arginine %	1.25

[1]Methionine hydroxy analogue calcium salt. Novus International, Inc. St. Louis MO.

[2]Provides per kg of diet: vitamin A (from vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadione dimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg.

[3]Provides per kg of diet: Mn (as manganese methionine hydroxy analogue complex) 40 mg; Zn (as zinc methionine hydroxy analogue complex) 40 mg; Cu (as copper methionine hydroxy analogue complex) 20 mg; Se (as selenium yeast) 0.3 mg. Novus International, Inc., St. Louis MO

indigestible markers; this will be reported in a separate manuscript. The mixed diet was then divided into two aliquots. One had an added enzyme level of 0.10% CTCZYME and the other had no enzyme. Aliquots of these two diets were mixed in order to obtain four diets with 0% (no enzyme), 0.025% (half the recommended level), 0.05% (recommended level) and 0.10% (twice the recommended level). The four diets were presented to the birds as mash feed to avoid possible heat destruction during pelleting.

After eight days of acclimation to the experimental diets, the birds were killed by by CO_2 inhalation and the ileal contents collected. The distal section of the ileum (portion of the small intestine from Meckel's diverticulum to approximately 1 cm anterior to the ileo-cecal junction) was removed and the contents gently expelled and rinsed with distilled water. The contents were frozen and freeze dried.

Pooled samples of the freeze dried ileal contents were analyzed for crude protein, gross energy, amino acids, calcium, phosphorus and chromium levels by commercial laboratories specializing in these assays.

The Ileal Apparent Metabolizable Energy (IAME) and the digestibility coefficients of the amino acids were estimated by the following equations:

$$IAME = GE_{Diet} - \left[\frac{(GE_{Ileal} \times Cr_{Diet})}{Cr_{Ileal}}\right]$$

$$AAdigestion = AA_{Diet} - \left[\frac{(AA_{Ileal} \times Cr_{Diet})}{Cr_{Ileal}}\right]$$

RESULTS AND DISCUSSION

Results for crude protein, phosphorus, calcium, chromium and gross energy in ileal content are presented in Table 2. Levels of crude protein in ileal content decreased as the inclusion level of CTCZYME increased. This reduction suggests that CTCZYME increases protein digestibility and that the bird is absorbing more available protein. Gross energy of the ileal content decreased as the beta-mannanase inclusion level increased reflecting higher energy utilization from the feed and responsive to the higher usage levels. The amount of chromium increased as the inclusion levels of enzyme increased. Since the chromium is considered as undigestible material this increase supports the idea that the beta-mannanase increases digestibility of the dietary nutrients. Levels of phosphorus and calcium increased with the increment of the beta-mannanase level, possibly due to a concentration effect as a consequence of the higher nutrient utilization.

Results for amino acid ileal content are presented in Table 3. The levels for the nine amino acids analyzed decreased as the inclusion levels of the beta-mannanase increased in the diet. In support of the improved nutrient utilization, when the digestibility for these amino acids was calculated, the results (Table 4) showed that the digestibility for all the amino acids increased significantly ($p<0.0001$) when CTCZYME was included at levels of 0.025%, 0.05% and 0.1%. This is in agreement with the results of Kong *et al.* (2011).

When ileal apparent metabolizable energy was calculated, the results showed that the IAME increased with each increment in the CTCZYME inclusion level (Table 5) These results are consistent with Schulze *et al.* (2006) who found similar results using different levels of beta-mannanase and with Daskiran *et al.* (2004) and Kong *et al.* (2011).

Table 2: Effect of CTCZYME on the protein, phosphorus, calcium, chromium and gross energy in ileal contents of broilers

Enzyme level (%)	Crude protein (%)	Phosphorus (ppm)	Calcium (ppm)	Chromium (ppm)	Gross energy (kcal/kg)
0	11.75	12,382	19,226	4,170	3,774
0.025	10.93	14,655	23,667	4,477	3,646
0.050	11.00	12,798	19,895	5,077	3,649
0.100	10.93	14,848	25,756	5,740	3,504

Table 3: Effect of different levels of CTZYME on amino acid levels (%) in ileal content

		% CTCZYME in diet			
Measurement[1]	Basal diet	0.00	0.025	0.050	0.100
Lysine %	1.144	0.411	0.380	0.389	0.400
Methionine %	0.550	0.095	0.085	0.088	0.092
Threonine %	0.741	0.531	0.479	0.498	0.495
Tryptophan %	0.241	0.158	0.148	0.151	0.143
Arginine %	1.244	0.418	0.392	0.384	0.373
Leucine %	1.522	0.816	0.757	0.778	0.761
Isoleucine %	0.756	0.378	0.348	0.358	0.353
Cysteine %	0.277	0.239	0.211	0.220	0.211
Valine %	0.874	0.486	0.449	0.461	0.453

[1]Amino acid values are an average of triplicate assays

Table 4: Ileal amino acid digestibility coefficients of chicks fed diets with different levels of CTCZYME

	Digestion coefficient								
Enzyme level (%)	Lys	Met	Thr	Trp	Arg	Leu	Ile	Cys	Val
0	0.790^{d}	0.899^{d}	0.589^{d}	0.601^{d}	0.806^{d}	0.711^{d}	0.719^{d}	0.565^{d}	0.679^{d}
0.025	0.819^{c}	0.916^{c}	0.654^{c}	0.651^{c}	0.830^{c}	0.750^{c}	0.759^{c}	0.644^{c}	0.724^{c}
0.050	0.837^{b}	0.923^{b}	0.683^{b}	0.687^{b}	0.853^{b}	0.774^{b}	0.781^{b}	0.671^{b}	0.750^{b}
0.100	0.852^{a}	0.929^{a}	0.722^{a}	0.738^{a}	0.874^{a}	0.804^{a}	0.809^{a}	0.722^{a}	0.782^{a}
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SEM	0.0009	0.0004	0.0024	0.0050	0.0006	0.0018	0.0008	0.0016	0.0007

[a-d]Means within a row with different superscripts differ significantly ($p<0.05$)

Table 5: Effect of dietary CTCZYME on Ileal apparent Metabolizable energy

Enzyme level (%)	GE diet (kcal/kg)	GE ileal (kcal/kg)	Cr diet (ppm)	Cr ileal (ppm)	Ileal AME (kcal/kg)
0	3898	3774	2417	4170	1711
0.025	3898	3646	2417	4477	1930
0.050	3898	3649	2417	5077	2161
0.100	3898	3504	2417	5740	2423

As the basal diet had approximately 0.37% inclusion of beta-mannans it is improbable that the improvement in nutrient utilization is due to the fact that the enzyme would have released nutrients previously unavailable for the bird. It is more likely that the enzyme activity has modified the digesta viscosity improving nutrient digestibility and absorption, as reported by Almirall *et al.* (1995) and Lee *et al.* (2003).

The results in the current study suggest that the inclusion of beta-mannanase at different levels increases nutrient digestibility and energy utilization. Although further studies are required, this could mean that CTCZYME could be used to reduce feed costs by reducing energy and protein levels in the diet. At the same time it opens the possibility to include in the formulation other feedstuff rich in beta-mannans that could lower the costs of the feed without reducing broiler's performance.

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