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Enzyme Supplementation of Corn-Soybean Meal Diets Improves Performance in Broiler Chicken

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Abstract: This study was performed to evaluate the effect of feeding Bergazyme P, a commercial enzyme supplement that contain β -pentosanase, α -amylase, gluconases and galactomannases, in standard corn-soy rations for broiler chicken from 1-49 days of age. A total of 800 one day old Cobb 500 chicks were obtained from a commercial hatchery and randomly distributed in a randomized complete block design among 12 floor pens with 4 replicate pens/treatment. Birds were divided into 3 groups: T1 = Control corn-soy diet; T2 = T1 + 250 g Bergazyme P/t or T3 = T1 + 500 g Bergazyme P/t. Significant treatment effects were determined using ANOVA. Enzyme supplementation significantly increased body weight at 42 and 49 d of age and significantly increased the breast yield at 49 d of age, suggesting that the improvement of body weight by Bergazyme is involved in the increase of the muscle weight. In both the T2 and T3 group, serum total protein concentration was significantly increased at 21 and 49 d of age. Ileal protein digestibility was significantly higher in the T2 compared to the control group. However, there is no significant difference in ileal protein digestibility between the T3 group and the control group. These findings suggest that the addition of Bergazyme P to the diets at the rate recommended by the manufacture (250g/t) uplifted productive performance mainly attributable to a higher degree of protein digestion.

Key words: Bergazyme P, broiler, digestibility, performance, serum metabolites

INTRODUCTION

A high protein content and well balanced amino acid profile make Soybean Meal (SBM) a valuable protein source in poultry diets. However, the nutritional value of SBM is decreased by the presence of Antinutritional Factors (ANFs) such as Nonstarch Polysaccharides (NSPs) and thus soybean meal is incompletely digested by poultry (Marsman *et al.*, 1997; Pack and Bedford, 1997). SBM is known for the variability of its nutritional content. This variability is found not only in the protein and amino-acid composition but also among presence of ANFs such as Non-starch Polysaccharides (NSPs) (Choct and Annison, 1992). Water soluble NSPs fed to young chicks are not only indigestible but also interfere with the digestion and absorption of other nutrients by increasing the viscosity of digesta in the gut (Ward and Marquardt, 1983).

Feed ingredient technologists have developed supplemental enzymes that 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). The latter effect allows nutrients, as well as the animal's own digestive enzymes, to move more freely, improving nutrient uptake and growth. The end result is better digestion and absorption of nutrients from wheat and other feed ingredients (Wyatt, 1992). Several

attempts have been made to increase the nutritional value of SBM by adding protease and carbohydrases either before or after processing (Bedford, 1993; Ghazi *et al.*, 1997; Rostango *et al.*, 2000).

Improvements in gain, feed efficiency, intestinal viscosity, digesta dry matter and digestibility are often associated with enzyme addition to poultry diets (Partridge and Wyatt, 1995; Reddy and Quadratullah, 1997; Zanella *et al.*, 1999; Scheideler *et al.*, 2005).

The objective of the present study is to evaluate the efficacy of Bergazyme P, a commercial enzyme supplement that contain β pentosanase, α amylase, β gluconases, gluconases and galactomannases, in a standard corn-soy rations for broiler chicken.

MATERIALS AND METHODS

Birds and management: A total of 800 one day old Cobb 500 broiler chicks were obtained from a commercial hatchery and randomly distributed among 12 floor pens with wood shavings with 50 chicks per pen. Pen allocation in experiment was such that all pens had similar average the starting body weight. The birds were reared in a conventional poultry house with raised side windows. The birds were maintained a 24 h light schedule.

Diets and experimental design: In order to test the effectiveness of Bergazyme P enzyme on the performance of broiler chicken, birds were divided into 3 groups: T1 =

Control diet; T2 = T1 + 250 g/t; T3 = T1 + 500 g/t Bergazyme P. Isocaloric and isonitrogenous corn-soybean meal diets were formulated (Table 1). The starter diet was fed from 1-10 days and contained 21% CP and 3000 kcal of ME/kg. The grower diet was fed from 11-22 days and contained 19% CP and 3100 kcal of ME/kg. In the finisher diet (23-49 days), CP and ME were 18% and 3180 kcal/kg, respectively. All diets were fed on an as fed basis. The experimental diets were formulated to meet National Research Council (1994) nutrient requirements for broiler, in particular the recommendations for Cobb 500 strain. Feed and water were provided *ad libitum*.

Table 1: Composition of experimental diets fed to broilers from 1-49 days

Ingredients (g/kg)	Starter	Grower	Finisher
Corn	607.5	654.1	668.8
Soybean meal (48% CP)	330.0	283.0	260.0
Fat	21.0	25.0	35.0
Salt	3.0	3.0	3.0
Limestone	6.0	4.0	5.0
Di Calcium phosphate	26.0	25.0	23.0
DL-methionine	1.5	1.4	1.2
L-Lysine	0	1.0	1.0
Vitamin-mineral premix ¹	3.0	3.0	3.0
Amprol	0.5	0.5	0
Choline chloride	1.5	0	0
Calculated analysis			
ME (Kcal/Kg)	3010	3000	3180
CP (%)	21.0	19.0	18.0
Methionine (%)	0.50	0.47	0.44
Methionine + Cystine (%)	0.85	0.78	0.73
Lysine	1.2	1.1	1.07
Linoleic acid	1.9	2.0	2.2
Calcium	1.0	0.9	0.9
Phosphorus	0.50	0.48	0.45

¹Vitamin-mineral mix is supplied in the following per kg of diet: Vitamin A, 17500 IU; Cholecalciferol, 5000 IU; Vitamin E, 25 IU; Vitamin B₁₂, 0.03 mg; Riboflavin, 15 mg; Niacin, 75 mg; D-panthothenic acid, 25 mg; Choline, 705.5 mg; Menadione, 5 mg; Folic acid, 1.5 mg; Pyridoxine, 6.25 mg; Thiamine, 3.03 mg; D-biotin, 0.127 mg. Manganese, 120 mg; Zinc, 100 mg; Copper, 10 mg; Iodine, 2.5 mg; Calcium, 135 mg, Iron, 75 mg; Selenium, 0.15 mg

Measurements: Body weight and feed consumption were recorded weekly by pen and feed conversion was computed.

At 21, 35 and 49 days of age, one bird from each pen was selected and kept without food for 3 h then was bled from cutaneous ulnar vein. Blood samples were centrifuged for 15 min at 2 500 x g and the serum was harvested and stored at -80°C. Protein, cholesterol, triglyceride, calcium and phosphorus concentrations were analyzed using enzymatic colorimetric kits (Biolabo Reagents)¹; all analyses were carried out in duplicate.

At 49 days of age, 3 males per pen were sampled for measurements of parts yield and the mean was computed. After euthanasia, feather, heads and shanks

were removed and the remaining carcasses were dissected to breast, thigh, wings, neck, gizzard, liver, abdominal fat and weighed. The percentage yield of each part was calculated on the basis of carcass weight (Izat *et al.*, 1990).

A digestion trial was performed at the end of the experiment. Twenty four birds (4 replications per each diet) were housed (2 birds/cage) in 12 cages with wire bottoms. Birds had free access to feed and water throughout the 4 days of adaptation period. All diets were supplemented with 3g/kg chromic oxide as an analytical marker for the digestibility trails. At 44 day of age, birds were fed chromium oxide mashed feed for 5 days and on the 5th day approximately 200 grams of clean excreta (free of feed and visible feather contaminants) was collected to determine the feces moisture. At 49 days of age, all birds were euthanized by cervical dislocation to determine ileal digestibility of nutrients (protein, ether extract and nitrogen free 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 digestibilities. Approximately 3 g of homogenized chyme sample was collected. Ileal digesta samples were dried and dry matter was determined by oven-drying at 105°C for 16 h. Diets and ileal digesta samples were ground pass a 1.0 mm screen. Crude protein (N x 6.25), ether extract and crude fiber were analyzed according to the procedures established by the Association of Official Analytical Chemists (AOAC, 1984). All analyses were carried out in duplicate. Chromic oxide was analyzed according to the procedure described in Williams *et al.* (1962). The following equations were used for calculation of percent digestibility (Scott *et al.*, 1976):

$$\% \text{ Nutrient Digestibility} = 100 - (\text{Diet Cr}_2\text{O}_3 / \text{Fecal Cr}_2\text{O}_3 \times \text{Fecal nutrient/diet nutrient}) \times 100.$$

Statistical analysis: Treatments were distributed following randomized complete block design, in which each experimental diet was fed to 4 replicate pens. The experimental unit was the pen mean. Data were analyzed by analysis of variance procedures appropriate for a randomized complete block design, using General Linear Model procedure of the Statistical Analysis System (SAS, 1996). When significant differences among treatments were found, means were separated using LSD test. Statistical significance was assessed at ($p < 0.05$).

RESULTS

Feed consumption and feed efficiency: Growth performance, feed intake and feed conversion are shown in Table 2. No significant difference ($p > 0.05$) in feed intake was observed through day 42 or 49.

¹Biolabo Reagents. Biolabo SA, 02160, Maizy, France.

Table 2: Live Weight (BW), cumulative feed intake and Feed Conversion Ratio (FC) of broiler chickens given experimental diets at 42 and 49 days

	Treatments				
	T1	T2	T3	SEM	P*
Performance at 42 d					
BW (kg)	2.462 ^b	2.562 ^a	2.575 ^a	±0.012	**
Feed (kg)	4.770	4.687	4.785	±0.066	NS
FC (kg: kg)	1.939 ^a	1.829 ^b	1.858 ^{ab}	±0.022	*
Performance at 49 d					
BW(kg)	2.857 ^b	3.172 ^a	3.125 ^a	±0.028	***
Feed (kg)	5.825	6.045	6.020	±0.062	NS
FC (kg: kg)	2.038 ^a	1.905 ^b	1.926 ^b	±0.026	*

^{ab}Means in the row with different superscripts differ significantly (*p<0.05, **p< 0.01, ***p<0.001, NS: Not significant). SEM = Standard Error of the Mean

Significant differences in body weight were observed at 42 d [(2.462, 2.562 and 2.575 kg, (p<0.04)] and 49 d [2.857, 3.172 and 3.125 kg, (p<0.001)] for T1, T2 and T3, respectively. Corresponding improvements in feed conversion efficiency were shown when enzyme was added. Feed efficiency was lower for the treatments that contained the enzyme compared to the control while increasing the level of enzyme in the diet resulted in no further improvement in feed efficiency. At 42 d of age, T2 resulted in 11 points improvement in feed efficiency (p<0.04) compared to the control group (1.829 vs. 1.939, respectively). At 49 d of age, the effect of enzyme was more pronounced (p<0.03), more than 14 points improvement was reported due to T2 (1.905 vs. 2.038, respectively).

Blood serum metabolites: The data related to serum metabolites are shown in Table 3. Serum total protein concentration was significantly increased in the T2 group compared to the T1 group at 21 d of age (2.91 vs. 2.78 mg/dl). There were no significant differences between treatments in serum cholesterol, triglycerides, calcium and phosphorus (p>0.05). At 35 d of age, serum triglycerides concentration was significantly increased in the T2 and T3 groups compared to the T1 group (T1 = 149, T2 = 159.3, T3 = 156.3, p<0.05). Moreover, enzyme addition increased serum total protein, cholesterol, calcium and phosphorus numerically (p>0.05). At 49 d of age, enzyme addition improved serum total protein concentration by 3.9% (T1 vs. T2, p<0.01) while it improved the other metabolites numerically (p>0.05). Same trend was found in the three periods, such that increasing the level of enzyme from 250-500 g/t, did not result in further improvement in serum metabolites measured (p>0.05)

Feed ileal digestibility and excreta moisture: Table 4 shows excreta moisture and ileal digestibility data in this experiment. Enzyme supplementation (250 g/t) reduced excreta moisture content (from 68.1-66.1%), but this reduction was not significant (p = 0.07).

Table 3: Serum Total Protein (TP), cholesterol, Triacylglycerol (TG), Calcium (Ca) and Phosphorus (Phos) concentrations of broiler chickens at 21, 35 and 49 days

	Treatments				
	T1	T2	T3	SEM	P*
Age (21 d)					
TP (g/dl)	2.78 ^b	2.91 ^a	2.82 ^{ab}	±0.024	*
Cholesterol (mg/dl)	142.6	143.3	145.7	±2.19	NS
TG (mg/dl)	143.3	146.3	146.3	±1.61	NS
Ca (mg/dl)	9.53	10.0	9.83	±0.18	NS
Phos (mg/dl)	6.67	7.16	7.20	±0.13	NS
Age (35 days)					
TP (g/dl)	2.86	3.08	3.1	±0.08	NS
Cholesterol (mg/dl)	148.7	150.7	150.0	±1.46	NS
TG (mg/dl)	149.0 ^a	159.3 ^a	156.3 ^a	±1.85	*
Ca (mg/dl)	9.96	10.60	10.40	±0.22	NS
Phos (mg/dl)	7.20	7.40	7.30	±0.056	NS
Age (49 d)					
TP (g/dl)	3.01 ^b	3.13 ^a	3.21 ^a	±0.02	**
Cholesterol (mg/dl)	150.7	154.0	150.6	±0.81	NS
TG (mg/dl)	156.7	162.3	160.6	±0.15	NS
Ca (mg/dl)	11.3	11.4	11.6	±0.15	NS
Phos (mg/dl)	7.26	7.55	7.43	±0.13	NS

^{ab}Means in the row with different superscripts differ significantly (*p<0.05, **p<0.01, ***p<0.001, NS: Not significant). SEM = Standard Error of the Mean

Ileal protein digestibility was influenced by treatment (p<0.03), with protein being more completely digested by chicken on T2 compared to those on the control diet (79.3 vs. 75.3%, respectively). Enzyme supplementation was shown to increase fat ileal digestibility (80.8 vs. 79.5% for the control diet), but this was not a significant difference (p = 0.07). Moreover, enzyme supplementation improved numerically (p = 0.06) NFE ileal digestibility (82.2 vs. 80.2% for control diet). However, increasing the enzyme level did not show further improvement in digestibility.

Carcass characteristics: The mean percentage of carcass parts in different treatments is documented in Table 5. Except for breast yield, no significant effects of treatments on carcass parts could be found. A higher percentage of breast yields (7.9%) were obtained from chicken that were fed the enzyme supplemented diets [23.2 vs. 21.5%, respectively, (p<0.04)]. A similar result was found for carcass yield, which was numerically higher in enzyme supplemented group.

Table 4: Excreta dry matter content and apparent ileal digestibility of nutrients in duodenum of broiler chickens given experimental diets

	Treatments (%)				P*
	T1	T2	T3	SEM	
Excreta moisture	68.12	66.07	66.8	±0.51	NS
Ileal digestibility coefficients					
Protein	75.32 ^b	79.32 ^a	76.67 ^{ab}	±0.76	*
Ether extract	79.85	80.77	80.45	±0.07	NS
NFE	80.22	82.15	81.48	±0.45	NS

^{a,b}Means in the row with different superscripts differ significantly (*p<0.05, **p<0.01, ***p<0.001, NS: Not significant). SEM = Standard Error of the Mean

Table 5: Effect of different treatments on parts yield as percentages of broiler carcass weight at 49 days

	Treatments (%)				P*
	T1	T2	T3	SEM	
Dressing	70.6	71.3	71.2	±0.16	NS
Leg quarter	33.9	34.2	34.2	±0.20	NS
Breast	21.5 ^a	23.2 ^a	23.3 ^a	±0.03	*
Wing	12.2	12.3	12.6	±0.10	NS
Abdominal fat	2.43	2.77	2.83	±0.01	NS
Gizzard	1.95	2.27	2.2	±0.12	NS
Liver	3.2	3.1	3.1	±0.01	NS

^aMeans in the row with different superscripts differ significantly (*p<0.05, **p<0.01, ***p<0.001, NS: Not significant). SEM = Standard Error of the Mean

DISCUSSION

The results revealed a significant improvement in feed efficiency at 42 and 49 d of age for birds received the enzyme. This could be explained by the improvement in body weight for the birds received the enzyme supplemented diet. The results indicating that the efficiency of dietary utilization increased in chicks fed enzyme compared to those fed the control diet, since it was observed that broilers fed diets supplemented with enzyme consumed similar amount of feed compared to those fed the control diet. This result disagree with the findings of Ranade and Rajmane (1992) who reported a lower feed intake of broilers fed supplemented diets with enzyme preparation containing cellulase, protease, xylanase, β-glucanase and α-amylase activities. On the other hand, the improvements in body weight gain of broilers fed the enzyme supplemented diets could be ascribed to the increased nutrient digestibility especially protein as shown in this study. The result of this experiment is also supported by the findings of Zanella *et al.* (1999) who reported a significant increase in body weight gain in the broilers fed corn-soy diets supplemented with enzyme.

The results of feed efficiency are in agreement with the work of Ranade and Rajmane (1992) who reported a significant improvement in feed conversion efficiency of broilers fed with diets supplemented with enzyme preparation containing cellulase, protease, xylanase, beta-glucanase and alpha-amylase activities. Also, Cowan (1990) reported a 2-3% increase in efficiency of

feed utilization by birds fed diets containing enzymes compared to those fed control diet. An improvement in feed efficiency of broilers was observed with enzyme supplementation of corn-soy based diets (Zanella *et al.*, 1999).

The nutritional value of SBM is decreased by the presence of anti-nutritional factors, it contains 204 g/kg NSP and thus soybean meal is incompletely digested by poultry and there is a possibility to improve its nutritional value (Marsman *et al.*, 1997; Pack and Bedford, 1997). The mechanism by which soluble NSP reduce nutrient digestion and absorption is not fully understood but the increased viscosity associated with soluble dietary fibers has been shown to increase the thickness of the intestinal boundary layer and consequently impede nutrient uptake (Johnson and Gee, 1981; Flourie *et al.*, 1984). The physiological importance of soluble dietary fibers lies in their ability to reduce the diffusion and absorption of nutrients (most notably dietary fat) in the small intestine (Furda, 1990). This finding could be partly explained by the capacity of NSPs to bind water (Langhout and Schutte, 1996). The increase in digesta water is thus the primary mechanism by which water-soluble nonstarch polysaccharides exert antinutritive properties (Marquardt, 1997). In the current experiment, water content of excreta was numerically lower with enzyme supplementation.

In the current study, dietary Bergazyme P increased serum total protein and triglycerides significantly while it improved all other metabolites measured numerically. The results indicating that the efficiency of dietary utilization especially protein and fat increased in chicken fed enzyme-supplemented diet-compared to those fed the control diet and consequently nutrient uptake was improved. This could be due to the action of the exogenous enzyme I Bergazyme P directly or indirectly by providing better environment for the endogenous digestive enzymes to move more freely. Cell disruption has been advanced as one mechanism by which enzymes make more protein and starch available for digestion (Morgan *et al.*, 1995). Cone *et al.* (1994) suggested that xylanase treatment increased nitrogen solubility in SBM by causing cell-wall degradation leading to release of proteins, as well as by some proteolytic activity in the crude enzyme preparation. Wyatt (1992) postulated that cell wall disruption and the release of cell-bound nutrients might be the mechanism of improvement in digestibility values associated with addition of feed enzymes.

Various authors have suggested that the low lipid digestibility in broiler chickens fed diets with a high content of NSPs may be due to bacterial overgrowth in the small intestine and subsequent excessive deconjugation of bile acids, which reduces their efficacy in solubilizing lipids (Huhtanen and Pensack, 1965; Salih *et al.*, 1991). On the other hand, Smits *et al.* (1997) demonstrated a significant reduction in apparent nitrogen digestibility after feeding higher NSP to the birds. Angkanaporn *et al.* (1994) demonstrated that

water-soluble pentosans in the diet significantly raised the endogenous losses in broiler birds. This agrees with the results obtained here for protein digestibility. These findings suggest that dietary NSP inhibits the ileal protein digestibility. Thus, the improvement of ileal protein digestibility in the T2 and T3 groups is due to the digestion of NSP in diet by Bergazyme P. Similar results were shown in previous work. For example, a previous work showed a 3% improvement in the real digestibility of crude protein in corn-soybean meal based diets of broiler supplemented with enzymes containing protease, cellulase and amylase (Rostagno *et al.*, 2000). Zanella *et al.* (1999) observed 2.9% improvement in the crude protein digestibility with addition of enzymes. Marsman *et al.* (1997) found that enzyme supplementation improved apparent ileal digestibility of NSPs when compared with untreated soybean meal.

A slight increase in the dressing percent of broilers fed diets containing enzyme was reported in this experiment. An improvement in dressing percent of broilers fed diets supplemented with enzymes has been reported (Osei and Oduro, 2000). This difference in dressed yield of broilers could be due to different diet and kind and level of enzyme used. A higher percentage of breast parts were associated with enzyme supplementation. This could be due to lower deposition of fat or higher protein in this part. Higher protein is associated with higher water, which altogether increased the breast weight. Deposits of fat in the abdominal region of the broiler are considered a waste by the poultry industry. Abdominal fat is not only a loss, but also it represents an added expense for the processing effluent treatment. In this study, no differences in abdominal fat percentage due to treatment were reported.

In summary, Bergazyme P supplementation at the rate recommended by the manufacture (250 g/t) had a positive effect on protein bioavailability and broiler performance while increasing the level of enzyme from 250-500 g/t, did not result in further improvement in performance. The beneficial effect of Bergazyme P in corn-soy diet is based on the hydrolysis of the viscous NSPs. Feed efficiency improved by increasing body weight of chicken and the overall response is an increase in nutrient availability from the SMB and other dietary components. Increasing the nutritive value of feedstuffs such as SBM by the use of Bergazyme P offers potential to reduce diet cost commensurate with enhanced production. Increasing the digestibility of dietary protein has the potential to reduce total dietary nitrogen concentration which, coupled with the improved digestibility, will reduce the amount of nitrogen entering the environment.

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