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## The Effects of Enzyme Supplementation on Performance, Carcass Characteristics and Some Blood Parameters of Broilers Fed on Corn-Soybean Meal-Wheat Diets

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**Abstract:** In a completely randomized design the effects of a multi-enzyme (Endo-feed-W) supplementation on performance, carcass characteristics and some blood parameters of Cobb 500 broilers fed on corn-soybean meal-wheat diets were studied. The enzyme levels added to the diets were 0.00% (control) and 500mg kg<sup>-1</sup> DM. Enzyme supplementation significantly improved body weight gain, feed to gain ratio, relative growth, energy and protein efficiency from 11-28 d of age. Adding enzyme significantly increased body weight gain, decreased feed intake and improved feed to gain ratio, energy and protein efficiency from 29-44 d of age ( $p < 0.05$ ). Body weight gain, feed intake, relative growth, energy and protein efficiency was increased and feed to gain ratio was decreased by enzyme supplementation from 29-44 d ( $p < 0.05$ ). Enzyme addition significantly increased carcass and thigh percentages at 44 d of age. Adding enzyme significantly increased the concentration of blood triiodothyronine ( $T_3$ ) at 28 and 42 d and reduced the concentration of blood thyroxin ( $T_4$ ) at 42 d of age. Enzyme inclusion increased the concentration of blood total cholesterol at 10, 28 and 42 d of age, however HDL-cholesterol and triglyceride concentrations increased at d 10 and 42 ( $p < 0.05$ ). The concentration of blood uric acid was significantly decreased at d 28 and 42 in broilers fed enzyme supplemented diets.

**Key words:** Multi-enzyme, performance, carcass characteristics, blood parameters, broilers

### INTRODUCTION

Adding Non-Starch Polysaccharides (NSP) degrading enzymes in poultry diets has increased considerably in recent years. However, the effects of exogenous enzymes can be variable and are dependent on a large number of factors such as the age of the bird and the quality and type of diet (Bedford and Schulze 1998; Bedford 2000; Acamovic, 2001). The use of exogenous enzymes to improve the digestibility of corn-soybean meal diets for broilers is less well documented. Neither corn nor soybean meal is regarded as viscous feedstuffs even though they do contain appreciable amounts of NSP's. Corn contains approximately 0.9% soluble NSP and 6% insoluble NSP, whereas soybean meal contains approximately 6% soluble NSP and 18-21% insoluble NSP (Bach Knudsen, 1997). Noy and Sklan (1994) reported that ileal digestibility of corn starch rarely exceeds 85% in broilers between 4 and 21 d of age, indicating opportunities to further improve the digestibility of resistant starch in the jejunum and ileum through amylase supplementation. Furthermore, proteases could potentially degrade such soybean proteins as glycinin and  $\beta$ -conglycinin and some antinutritional factors (lectin and trypsin-inhibitor) in inadequately processed soybean meal (Thrope and Beal, 2001).

Legume NSP are much more complex in structure than those present in cereals and therefore, the use of "classical" NSP-degrading enzyme products tends to

provide limited and inconsistent responses (Broz and Ward, 2007). Wheat is an important source of energy in broiler diets because of its high Starch (ST) and CP content and is often the only cereal in grower and finisher diets (Gutierrez Del Alamo *et al.*, 2008). In a survey of 18 wheat cultivars, Kim *et al.* (2003) reported that ST content ranged between 58.5 and 73.7%, CP between 9.7 and 19.1%, NSP between 7.8% and 11% (on DM basis). The physical entrapment of wheat starch and protein by cell wall polysaccharides has been suggested as another important factor by which NSP exert their anti-nutritive properties (Theander *et al.*, 1989; Bedford and Autio, 1996; Wiseman *et al.*, 2000). When added to relevant poultry diets, NSP-degrading enzymes usually result in numerous beneficial effects, such as increased utilization of nutrients (e.g., fat and protein), improved AME values, increased growth rate, improved feed to gain ratio, decreased viscosity of intestinal digesta, reduced incidence of sticky excreta, improved litter conditions and reduced environmental pollution due to a decreased output of manure and gases such as ammonia (Broz and Ward, 2007; Fernando Guilherme Perazzo Costa *et al.*, 2008).

Enzyme supplementation can change the nutritional status and improve growth performance of broiler chickens fed a wheat diet, but which are also closely related to the regulation of metabolism and functioning of the growth-related endocrine system. Nutritional status is an important factor in the regulation of plasma

hormones and intermediary metabolism in broiler chickens (Gao *et al.*, 2007; Buyse *et al.*, 2002; Swennen *et al.*, 2005). Evaluation of plasma biochemistry in birds allows for the identification of metabolic alterations due to realizing of factors such as age and husbandry conditions (Alonso-Alvarez and Ferrer, 2001). Therefore, the objective of this study was to examine the effects of multi-enzymes supplementation on performance, carcass characteristics and some blood parameters in broilers fed on corn-soybean meal-wheat diets.

## MATERIALS AND METHODS

A total of 150, one-day old mixed-sex broiler chicks (Cobb 500) were obtained from a local commercial hatchery on the hatching day. The experimental design was CRD with 2 treatments and 3 replicates with 25 chicks in each replicate. Treatments included 2 levels of a multi-enzyme (0.00 and 500 mg kg<sup>-1</sup> DM Endofeed-W produced from *Aspergillus niger*, GNC Bioferm Inc., Canada, with activity of 2250 U/g arabinoxylanase and 700 U/g Beta-glucanase). The enzyme contained *Aspergillus niger* fermentation product with barley malt sprouts dehydrated as carrier and standardizer. Furthermore, the calorimetric enzyme test kit method proposed by the manufacture was used to ensure the presence of the desired level of enzyme activity in each diet. According to the manufacture, this enzyme product also contained activities of other enzymes, including cellulase, hemicellulase, protease, alpha-amylase and alpha-galactosidase. The room was lit continuously during the whole experimental period and room temperature was controlled at 32°C from 1-3 d and then gradually reduced by 2-3°C per week to a final temperature of 20°C. All diets were formulated to meet the nutrient requirements according to Cobb 500 rearing guideline. The composition and nutrient levels of the basal diet are shown in Table 1. Feed and water were provided *ad libitum* during the whole trial. The experimental period lasted 44 d. Chicks were weighed at 1, 10, 28 and 42 d of age. The initial body weight mean of chicks (1 day of age) was about 40 g. There was not any mortality throughout the experiment. At 10 d of age, blood sample was collected from heart of 18 chicks (these chicks were culled from the experiment) and at 28 and 42 d of age blood sample was collected from a wing vein. To prevent coagulation, blood samples were mixed with EDTA and centrifuged at 3000x g for 10 min. Plasma stored at -20 C until hormone and metabolite analyses were carried out. Plasma glucose concentration was determined as mg/dL using commercial laboratory kits (Zistshimi and parsazmoon) with god-pap method at 546 nm wavelengths. Triglyceride, cholesterol, LDL-cholesterol and HDL-cholesterol measured by using commercial laboratory kits (Friedewald *et al.*, 1972; Gordon and Amer, 1977). The concentrations of plasma Thyroxin (T<sub>4</sub>) and Triiodothyronine (T<sub>3</sub>) were measured by

Table 1: Composition experimental diets in different periods of the experiment (g kg<sup>-1</sup> DM)

Ingredients (%)	Starter (1-10 d)	Grower (11-28 d)	Finisher (29-44 d)
Corn	436.9	374.2	308.6
Soybean meal (44% CP)	356.7	314.6	228.4
Wheat	150.0	250.0	400.0
Soybean oil	18.3	23.5	23.7
Dicalcium phosphate	13.0	11.9	11.8
Oyster shell	12.3	13.1	13.7
Salt	3.0	3.0	3.0
Mineral Premix <sup>1</sup>	2.5	2.5	2.5
Vitamin Premix <sup>2</sup>	2.5	2.5	2.5
DL-Met	3.2	3.0	3.2
L-Lysine HCl	1.7	1.8	2.6
Total	1000	1000	1000
<b>Calculated analysis</b>			
ME (MJ kg <sup>-1</sup> DM)	12.33	12.54	12.75
CP	220	210	180
Ca	8.6	8.6	8.6
Av. P	4.3	4.3	4.3
Na	1.4	1.4	1.4
Arg	14.7	13.6	10.9
Lys	13.3	12.3	10.8
Met	3.5	3.3	2.7
Met + Cys	10.0	9.4	8.8
Thr	8.6	8.0	6.6
Try	2.8	2.6	2.2

<sup>1</sup>Supplied per kilogram of diet: 6050 µg vitamin A (retinyl acetate + retinyl palmitate), 55 µg vitamin D<sub>3</sub>, 22.05 µg vitamin E (alpha-topheryl acetate), 2.0 mg vitamin K<sub>3</sub>, 5 mg vitamin B<sub>1</sub>, 6.0 mg vitamin B<sub>2</sub>, 60 mg vitamin B<sub>3</sub>, 4 mg vitamin B<sub>6</sub>, 0.02 mg vitamin B<sub>12</sub>, 10.0 mg pantothenic acid, 6.0 mg folic acid, 0.15 mg biotin, 0.625 mg ethoxyquin.

<sup>2</sup>Suppled per kilogram of diet: 500 mg CaCO<sub>3</sub>, 80 mg Fe, 80 mg Zn, 80 mg Mn, 10 mg Cu, 0.8 mg I, 0.3 mg Se

Radioimmunoassay (RIA) using standard commercial kits (Pishtazteb) according to the procedure of Kloss *et al.* (1994) (Gama manic1, Contron, Italy, with Automatic Gama Counter). At the end of the experiment (44 d) 2 birds from each pen with body weight close to the pen average body weight were selected for carcass analyses. After feed withdrawal for 9 h, the selected birds were transported to the university pilot for processing. The chickens were slaughtered by cervical dislocation to determine the carcass characteristics.

**Statistical analysis:** Data of this experiment were analyzed by analysis of variance using GLM procedures (SAS Institute, 2001). Differences among means were compared by Duncan's new multiple range test (1955). The model of this experiment was as follow:

$$X_{ij} = \mu + T_i + e_{ij}$$

Where  $\mu$ ,  $T_i$  and  $e_{ij}$  are overall mean, the treatment and experimental error effects respectively.

## RESULTS AND DISCUSSION

**Performance:** The effects of enzyme supplementation on broiler performance at 10, 28 and 44 d are shown in

Table 2: Effects of enzyme supplementation on body weight gain, feed intake and feed: gain of broiler chickens

Items	T1 (without enzyme)	T2 (with enzyme)	SEM	Significance
<b>1-10 d</b>				
Weight gain (g/bird)	198.2	192.3	2.31	NS
Feed intake (g/bird)	244.4	235.0	3.11	NS
Feed: gain (g/g)	1.23	1.21	0.01	NS
<b>11-28 d</b>				
Weight gain (g/bird)	1191.6	1243.3	5.65	**
Feed intake (g/bird)	1850.0	1860.0	12.92	NS
Feed to gain ratio (g/g)	1.55	1.49	0.009	*
<b>29-44 d</b>				
Weight gain (g/bird)	1460	1524.3	13.26	*
Feed intake (g/bird)	3020.5	2945.0	8.99	**
Feed: gain (g/g)	2.06	1.93	0.02	*
<b>1-44 d</b>				
Weight gain (g/bird)	2850.0	2960.0	7.36	**
Feed intake (g/bird)	5305.0	5350.0	7.38	*
Feed: gain (g/g)	1.86	1.80	0.006	**

NS: Not Significant, \*p<0.05, \*\*p<0.01

Table 3: Effects of enzyme supplementation on relative growth, energy and protein efficiency in broiler chicks

Items	T1 (without enzyme)	T2 (with enzyme)	SEM	Significance
<b>1-10 d</b>				
Relative growth	4.95	4.80	0.05	NS
Energy efficiency <sup>1</sup>	0.274	0.276	0.003	NS
Protein efficiency <sup>2</sup>	3.68	3.71	0.05	NS
<b>11-28 d</b>				
Relative growth	4.99	5.34	0.04	**
Energy efficiency	0.214	0.222	0.001	**
Protein efficiency	3.06	3.18	0.01	*
<b>29-44 d</b>				
Relative growth	1.02	1.03	0.013	NS
Energy efficiency	0.158	0.169	0.001	*
Protein efficiency	2.68	2.87	0.02	*
<b>1-44 d</b>				
Relative growth	71.24	74	0.18	**
Energy efficiency	0.183	0.193	0.001	**
Protein efficiency	2.89	3.04	0.01	**

<sup>1</sup>Calculated as body weight gain divided by ME intake, <sup>2</sup>Calculated as body weight gain divided by protein intake.

NS: Not Significant, \*p<0.05, \*\*p<0.01

Table 2 and 3. Body weight gain, feed to gain ratio, relative growth, energy and protein efficiency was significantly improved (p<0.05) by enzyme supplementation from 11-28 d of age. Enzyme inclusion increased body weight gain, energy and protein efficiency and decreased feed intake and feed to gain ratio from 29-42 d of age (p<0.05). The need for exogenous enzyme supplements in corn-soybean meal diets is generally ignored. However, some studies reported a positive growth performance response in corn-based diets supplemented with enzymes, either multiple enzymes which contained xylanase, protease and amylase or a single protease enzyme (Zanella *et al.*, 1999; Ghazi *et al.*, 2002; Yu *et al.*, 2007). Responses to enzyme supplementation depend on the bird's age, which is apparently related to both the type of gut microflora present and the physiology of the bird. In old birds, due to enhanced fermentation capacity of the microflora in their intestines, have a greater capacity to deal with the effects of high viscosity (Choct *et al.*, 1996; Vukic-Vranjes and Wenk, 1995). Body weight gain, feed intake, relative growth, energy and protein efficiency was

increased and feed to gain ratio was decreased by enzyme supplementation from 29-44 d of age (p<0.05). Enzyme supplementation might improve broiler performance by at least two mechanisms: Increasing feed intake and improving nutrient digestibility. Both mechanisms might be induced, at least partially, by a reduction of the viscosity at reduced viscosity cause to decreased retention time of digesta in the gut, allowing more consumption and therefore improving growth and feed conversion ratio (Lazaro *et al.*, 2003). The results of this study are in agreements with findings of previous studies (Gutierrez Del Alamo *et al.*, 2008; Gao *et al.*, 2007; Yu *et al.*, 2007). Further experiments should be done with using Endofeed-W enzyme to identify the factors which mainly promote broiler chick's performance.

**Carcass characteristics:** Effects of enzyme supplementation on carcass characteristics at 44 d are shown in Table 4. Enzyme supplementation increased carcass, and thigh percentage significantly (p<0.05). Enzyme addition had no significant effect on breast,

Table 4: Effects of enzyme supplementation on carcass characteristics of broiler chickens at 44 d of age

Items	T1 (without enzyme)	T2 (with enzyme)	SEM	Significance
Carcass (% LW <sup>1</sup> )	78.10	80.11	0.330	**
Breast (% CW <sup>2</sup> )	32.50	33.13	0.270	NS
Thighs + Drumsticks (% CW)	24.35	25.46	0.320	**
Wings (% CW)	7.34	7.36	0.150	NS
Heart (% CW)	0.59	0.61	0.006	NS
Liver (% CW)	2.35	2.36	0.010	NS
Proventriculus (% CW)	0.341	0.338	0.005	NS
Gizzard (% CW)	1.30	1.29	0.010	NS
Splint (% CW)	0.096	0.095	0.004	NS
Abdominal fat pad (% CW)	2.98	3.02	0.030	NS
Feet (% LW)	3.46	3.47	0.060	NS

<sup>1</sup>Live weight, <sup>2</sup>Carcass weight, NS: Not Significant, \*p<0.05, \*\*p<0.01

wings, Heart, liver, proventriculus, gizzard, splint, abdominal fat pad and feet percentage. In general, enzyme supplementation decreased the relative size of the digestive organs and increased carcass yield. This results are in agreements with findings of Wang *et al.* (2005), Alam *et al.* (2003), Jamroz *et al.* (1996), Pisarski and Wojcik (1995); Leeson *et al.* (1996). They reported increased carcass yield by addition of enzymes in diet attributable to higher fat deposition in carcass and also for increased breast meat yield. On the other hand, this result contradict with the report of Biswas *et al.* (1999) who found that carcass yield was similar in enzyme treated and non-treated diets.

**Blood parameters:** The effects of multi-enzyme supplementation on blood parameters at 10, 28 and 42 d are shown in Table 5. Similar to mammals, the hormonal control of growth in birds is complex and involves a variety of hormones, including thyroid hormones, Growth Hormone (GH), somatomedins and other circulating growth factors, androgens, insulin, prolactin and glucocorticoids (Scanes and Lauterio, 1984). A close relationship between the somatotrophic and thyrotropic axis in regulation of growth and development of broiler chickens has been found, and they play an important role in poultry growth (Cogburn *et al.*, 1995). Some studies suggest that nutrition is an important factor in the regulation of plasma hormones and of their receptors gene expression in many tissues of chickens. For example, protein deficiency changes the ratio of circulating concentrations of T3 and T4, reduces circulating concentrations of IGF-I and increases that of G (Scanes and Griminger, 1990). Another possibility is that there are physiologically active peptides and oligosaccharides or some other growth-promoting substances in digesta of gut produced by exoenzymes and endoenzymes. These active substances might have affected the cell receptors, therefore, producing physiological effects (Han, 1997). The results of this study showed that enzyme addition increased the concentration of blood T3 (p<0.05) at 28 and 42 d and reduced the concentration of blood T4 at 42 d of age (p<0.05). This suggests that enzyme addition directly or

indirectly promoted an enhanced activity of deiodinase in liver and kidney tissues, promoting the transformation of T<sub>4</sub> into T<sub>3</sub> (Collin *et al.*, 2003).

Nutritional factors (diet quantity and composition) also affect intermediary metabolism, resulting in the changes of plasma metabolite levels in poultry (Buyse *et al.*, 2002; Swennen *et al.*, 2005). In this study the concentration of blood glucose was significantly elevated by enzyme supplementation at 42 d of age. Exogenous enzymes used in the diets of young chicks would be very beneficial in improving nutrient digestibility in at least 2 ways: 1) by supplying enzymes that the chick can not produce in sufficient quantity by itself, or 2) even though the chick can produce enough of an enzyme by itself, exogenous enzyme may reduce the requirement for the enzyme, thus making more nutrients and energy available for growth of the chick at that critical stage (Olukosi *et al.*, 2007).

Present study showed that adding Endofeed-W multi-enzyme to broilers diet significantly increased the concentration of blood total cholesterol at d 10, 28 and 42 d (p<0.05). The concentration of blood HDL-cholesterol and triglyceride was increased by enzyme addition at d 10 and d 42 (p<0.05). Studies with animal models have shown that high level of dietary cholesterol, saturated fatty acids and an increased small intestinal uptake of these components due to, for example, a low dietary fiber concentration or enzyme supplementation of the diet may increase plasma cholesterol levels (Mancini and Parillo 1991; Pettersson and Aman 1992; Sutton *et al.*, 1985). LDL and HDL-cholesterol is formed when cholesterol and fats get together in circulatory system. With changing the physico-chemical properties of intestinal chyme due to the presence of soluble NSPs in wheat and the known interaction effects of them with saturated fatty acids (Kussaibati *et al.*, 1982) and the effect of NSP-degrading enzymes might explain some of these results. Adding enzyme may alleviate the limitations present for the function of bile salts and the emulsifying properties of them in intestinal chyme and therefore it might be a reason for increasing total fat in blood. It is reported that the digestion of big molecules of carbohydrates with pentosanase (arabinoxylanase)

Table 5: Effects of enzyme supplementation on plasma T<sub>3</sub>, T<sub>4</sub>, glucose, cholesterol, HDL, LDL, triglyceride, uric acid of broiler chicks

Items	T1 (without enzyme)	T2 (with enzyme)	SEM	Significance
<b>10 d</b>				
T <sub>3</sub> (nmol/L)	2.2	2.3	0.08	NS
T <sub>4</sub> (nmol/L)	30.7	30.4	1.2	NS
Glucose (mg/dL)	240.1	255.9	5.25	NS
Cholesterol (mg/dL)	137.9	139.5	0.37	**
HDL <sup>1</sup> (%)	58.5	59.4	0.32	**
LDL <sup>1</sup> (%)	36.5	35.3	0.44	NS
Triglyceride (mg/dL)	33.4	36.1	0.40	*
Uric acid (mg/L)	148.3	145.1	0.89	NS
<b>28 d</b>				
T <sub>3</sub> (nmol/L)	3.2	3.6	0.09	*
T <sub>4</sub> (nmol/L)	46.3	41.7	1.04	NS
Glucose (mg/dL)	235.9	237.7	0.66	NS
Cholesterol (mg/dL)	137	140.3	0.69	**
HDL <sup>1</sup> (%)	55.6	55.1	0.51	NS
LDL <sup>1</sup> (%)	39.4	39.7	0.48	NS
Triglyceride (mg/dL)	34.1	34.9	0.37	NS
Uric acid (mg/L)	147.3	137.1	1.07	**
<b>42 d</b>				
T <sub>3</sub> (nmol/L)	3.5	4	0.11	*
T <sub>4</sub> (nmol/L)	32.8	26.9	1.34	*
Glucose (mg/dL)	232.9	235.3	0.57	*
Cholesterol (mg/dL)	140	146.4	1.02	**
HDL <sup>1</sup> (%)	56.8	57.4	0.31	**
LDL <sup>1</sup> (%)	37.7	36.7	0.52	NS
Triglyceride (mg/dL)	37.2	42.2	0.7	**
Uric acid (mg/L)	168.1	145.8	1.12	**

<sup>1</sup>Percentages of total lipoprotein, NS: Not Significant, \*p<0.05, \*\*p<0.01

can change the viscous nature of intestinal chyme and therefore improves fat digestibility (Bedford *et al.*, 1991; Van Der Klis *et al.*, 1995).

In birds, purine bases are degraded to uric acid. In addition, purines are formed from excess amino-N, which are subsequently degraded to uric acid and excreted in urine (Buyse *et al.*, 2002). Correlation analysis showed a negative relationship between plasma uric acid levels and efficiency of protein retention (Swennen *et al.*, 2005). In this study, enzyme supplementation reduced the concentration of blood uric acid at 28 and 42 d (p<0.05). This suggests that the enzyme preparation increased nutrient metabolism, particularly protein anabolism of birds, therefore, promoting the growth of chickens. In conclusion, multi-enzyme supplementation in corn-soybean meal-wheat based diet can improve broilers performance and carcass yield and it can change the concentrations of thyroid hormones and some metabolites in blood.

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