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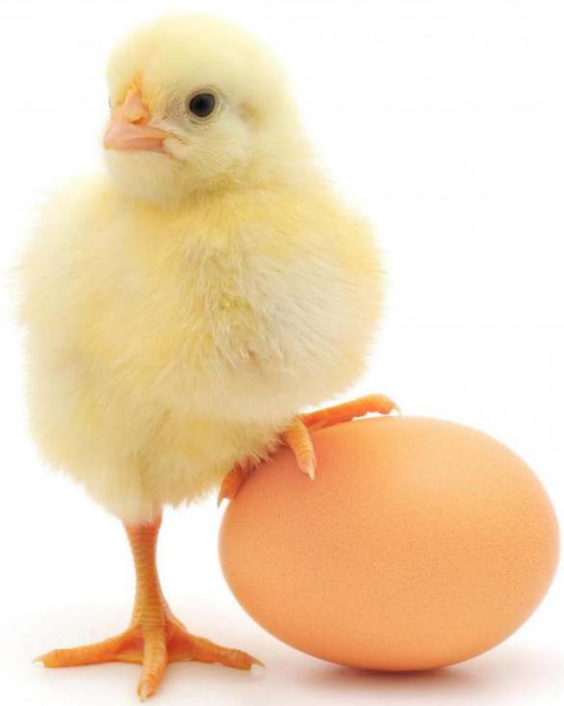

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The Influence of Supplemental Multi-enzyme Feed Additive on the Performance, Carcass Characteristics and Meat Quality Traits of Broiler Chickens

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Abstract: A study was conducted to investigate the effect of adding a commercial multienzyme feed additive (Tomoko, Biogenkoji Research Institute, Japan) on the performance of broilers. Four isoenergetic and isontirogenous diets consisting of control diet without enzyme (Con) and three test diets supplemented with Tomoko at 250 (T250), 500 (T500) and 750 (T750) g/tonne of feed were used for starter, grower and finisher phases. Each diet was offered to 10 replicates of 40 one-day-old straight-run Lohmann broiler chicks (n = 1600) in a randomized complete block design (10 blocks of 4 diets each). Data was analyzed using mixed procedure of SAS (repeated measures analysis) for a randomized complete block design, with level of significance set at $p = 0.05$. Enzyme used in the study was authenticated by the supplier to have minimum level of acidic protease (10,000 U/g), alpha-amylase (40 U/g), pectinase (30 U/g), phytase (10 U/g), glucoamylase (5 U/g) and cellulase (4 U/g). Enzyme supplementation had no significant effect on Feed Intake (FI) at 21 d, while at 42 d birds fed T250 and Con diets significantly consumed more feed than T500 and/or T750. No significant differences were observed for Feed Conversion Ratio (FCR). Body Weight (BW) and Body Weight Gain (BWG) were significantly higher ($p<0.05$) for birds fed Con diet at 42 d. Carcass characteristics showed no significant effects on whole carcass weight and/or dressing percent and weight and percent of breast, thighs and wings. Enzyme supplemented diets significantly ($p<0.05$) increased liver percent in contrast to Con diet, while no significant differences were reported for heart, gizzard and abdominal fat pad. The addition of enzyme did not significantly impact meat quality traits (pH, cooking loss, water holding capacity, shear force and colour attributes). Chemical analysis showed significantly ($p<0.05$) higher Dry Matter (DM) and ash percent for breast meat and significantly ($p<0.05$) higher DM, ash and Crude Protein (CP) percent for thigh meat, in birds fed Con diet. In conclusion, enzyme supplementation elicited few responses in birds when supplemented at three levels in contrast to a normal corn-soybean diet.

Key words*:* Multienzyme, feed additive, performance, meat quality, broilers

INTRODUCTION

Enzyme use is well documented across different types of poultry diets. The possibility of using exogenous enzymes in non-ruminant diets has provided nutritionists with a very important tool to improve feed digestibility, reduce environmental contamination and lower feed cost, thus, allowing for more flexibility in diet formulation. This is reflected in better flock performance, better litter quality and improved bird health, which in turn, has a positive influence on total production costs (Saleh *et al*., 2005; Cowieson and Ravindran, 2008a,b). Many commercial enzymes have been reported to be effective when added to poultry diets containing large amounts of Non-Starch Polysaccharides (NSP) such as wheat, barley sorghum, peas and lupins, due to well digestion of soluble and insoluble NSP (Hughes *et al*., 2000; Meng *et al*., 2005; Saleh *et al*., 2005; Wang *et al*., 2008; Selle *et al*., 2010).

Different trials have given inconclusive results with several enzymes having significant effects (Ritz *et al*., 1995: Ghazi *et al*., 2003; Jiang *et al*., 2008; Liu *et al*., 2008; Wang *et al*., 2008), while others have shown a trend towards improvement (Bedford, 2000). However, there is a substantial body of evidence to the use of these enzymes when diets are formulated using corn and soybean meal, which make up the majority of the energy and protein components of poultry diets (Wyatt *et al*., 1997; Zanella *et al*., 1999; Café *et al*., 2002; Jiang *et al*., 2008; Cowieson and Ravindran, 2008a), although of the perception that corn is of a high and consistent nutritional value, therefore, may not benefit from the addition of enzymes to the same extent as diets based on viscous grains. The responsiveness of corn-based diets to exogenous enzymes has received considerable attention in recent years due to increasing pressure on feed formulators from rising ingredient prices

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(Cowieson and Ravindran, 2008a). Many different trials have shown that commercial enzymes have a positive effect on the growth of broilers fed on corn-soybean diets (Wyatt *et al*., 1997; Zanella *et al*., 1999; Olukosi *et al*., 2007; Jiang *et al*., 2008) however; these trials often examined one type of enzyme in isolation. Under certain economic conditions, nutritionists are tempted to incorporate in their diets more than one type of enzymes, assuming that the independent enzyme effects may be additive in their effect (Meng *et al*., 2005: Cowieson and Ravindran, 2008a,b).

Different kinds of interactions can occur between various supplemental enzymes. Carbohydrates, for example, may require the use of enzymes with diverse activities that are able to target different sugar components of feedstuffs used in a poultry diet. When an enzyme cocktail containing several activities is used in a broiler diet, it is more likely to have greater effect than when enzymes are added separately. It has been reported that supplementation of poultry diets with enzymes mixture including protease and amylase produced significant improvement in growth performance in broilers (Odetellah *et al*., 2003; Gracia *et al*., 2003).

Tomoko is a feed additive produced by fermentation using Koji-feed (*Aspergillus awamori*) produced from wheat bran and distillery by-product from rice, sweet potato, or barley and has growth promoting activity. It contains many valuable enzymes such as phytase, glucoamylase, alpha-glucosidase, alpha-amylase, cellulase and acidic protease (Yamamoto *et al*., 2007; Saleh *et al*., 2006). The practical application followed in this trial is called “over the top” which aims to improve performance more economically and consists of supplementing a standard diet with enzymes, without changing its nutritional levels (Costa *et al*., 2008).

Therefore, the objective of this study was to investigate the effect of an exogenous commercial enzyme cocktail composed of alpha amylase, pectinase, protease, glucoamylase and cellulase on the performance, nutrient utilization, carcass yield and meat quality of broiler chicks fed a regular corn/soybean-based diet.

MATERIALS AND METHODS

Experimental design: Four dietary treatments consisting of control (Con) and three test diets supplemented with Tomoko® enzyme at 250 (T250), 500 (T500) and 750 (T750) g/tonne of feed were fed to straight-run broilers during a 42 d trial . Each dietary treatment was randomly allotted to 10 replicates of one-day-old Lohmann[1] broiler chicks for a total of 40 replicate floor pens (40 chicks per pen). The experimental design was a randomized complete block design with 4 floor pens representing a block for a total of 10 blocks. Birds were weighed prior to commencement trial and randomly assigned to replicate pens according to body weight uniformity such that the average initial BW of birds was similar across pens.

Birds and housing: Lohmann chicks, obtained from a local hatchery were reared from one-day-old on experimental diets and were allowed *ad libitum* access to feed and water. All diets were fed in mash form throughout the 6-wk experimental period. Pens had a daily lighting regimen of 22 h of light and 2 h of dark. Room temperature was maintained at 35°C during the first week and reduced by 2°C per week thereafter, until maintained at 21°C. Birds were reared in an open-sided house on floor pens (2.5 x 1.85 m) and wood shavings were used as litter at a depth of 5 cm. All birds used in this trial were handled according to guidelines set forth by the Jordanian Society for the Protection of Animals.

Diets: The experimental diets were formulated in accordance with recommendations of the breeder’s manual and to meet National Research Council (1994) requirements of broilers. The diets were standard corn/soybean meal diets formulated for starter (0-21 d), grower (22-35 d) and finisher (36-42 d) periods and were isocaloric and iso-ntirogenous for each feeding phase as given in Table 1.

Tomoko® enzyme was supplemented to the control diets at three levels (250, 500 and 750 g/tonne of feed) to make up diets 2, 3 and 4. This enzyme is a natural multi-enzyme feed additive (Biogenkoji Research Institute[2]) produced by fermentation using *Aspergillus awamori* containing a minimum of 10,000 units of acidic protease/g, 40 units of alpha-amylase/g, 30 units of pectinase/g, 10 units of phytase/g, 5 units of glucoamylase/g and 4 units of cellulase/g (enzyme activity determined by manufacturer).

Statistical analysis: Data was analyzed using repeated measures analysis of SAS® (2000) (PROC MIXED) for a randomized complete block design. The data was tested for main effects of dietary treatments. The following GLM model was used:

$$Y_{ijk} = \mu + R_j + \alpha_i + \epsilon_{ijk}$$

Where:

Y_{ijk} = Measured response

μ = Overall Mean

Rj = Block

α_i = Dietary effect

ϵ_{ijk} = Residual error

Level of significance used was p = 0.05

Measurements

Production parameters: Production parameters measured on a weekly basis included Feed Intake (FI),

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Table 1: Diet composition

Ingredients	Starter (0-21 d)	Grower (22-35 d)	Finisher (36-42 d)
	---------------------- (%) ----------------------		
Corn	58.50	62.30	67.05
Soybean meal (48% CP)	35.65	31.00	26.00
Palm oil	1.69	2.62	3.00
Limestone (ground)	1.84	1.79	1.68
Dicalcium phosphate	1.00	0.96	1.02
NaCl	0.41	0.41	0.42
DL-methionine (98%)	0.20	0.20	0.20
L-Lysine-HCl (98.5%)	0.11	0.12	0.13
Coccidiostat	0.10	0.10	-
Vitamin premix[1]	0.10	0.10	0.10
Mineral premix[2]	0.10	0.10	0.10
Choline chloride (60%)	0.10	0.10	0.10
Antioxidant	0.10	0.10	0.10
Antifungal	0.10	0.10	0.10
Calculated nutrient composition:			
ME, kcal/kg feed	3,000	3,075	3,150
Protein (%)	22.00	20.00	18.00
TSAA (%)	0.90	0.86	0.81
Methionine (%)	0.54	0.51	0.50
Lysine (%)	1.31	1.20	1.07
Threonine (%)	0.84	0.76	0.68
Tryptophan (%)	0.29	0.27	0.23
Ca (%)	1.03	0.98	0.95
P, nonphytate (%)	0.45	0.42	0.40
Na (%)	0.18	0.18	0.18

[1]Vitamin premix provided per kilogram of diet: vitamin A, 120000 IU; vitamin D_3, 3500 IU; vitamin E, 40 mg; vitamin B_1, 2.5 mg; vitamin B_2, 8 mg; vitamin B_6, 5.0 mg; vitamin, riboflavin, 150 µg; B_{12}, 30 µg; biotin, 150 µg; folic acid, 1.5 mg; niacin, 45 mg; pantothenic acid, 13 mg

[2]Trace mineral premix provided per kilogram of diet: Fe, 30 mg; Cu, 15 mg; Mn, 60 mg; Zn, 550 mg; I, 1 mg; Se, 0.80 mg

Feed Conversion Ratio (FCR), Body Weight (BW) and Body Weight Gain (BWG). Mortality was observed and recorded daily and any bird that died was weighed and weight was adjusted to both FI and FCR. Feed: gain ratio was calculated by dividing total feed intake by weight gain of live plus dead birds.

Sample preparation: At the end of 42 d trial, 40 birds from each treatment were randomly selected, weighed, and fasted for 10 h prior to slaughter. Slaughtered birds were scalded, feathers mechanically plucked in a rotary drum picker and eviscerated. Feet, shanks, neck and head were removed and carcasses were immediately weighed to obtain post-slaughter hot carcass weight without giblets. Giblets are the total yield of liver, heart, and gizzard which were removed and weighed in addition to fat pad relative to body weight. Carcasses were refrigerated for 24 h at 2-3°C and thereafter, carcasses were weighed again to obtain cold carcass weight as a % of live-weight to determine dressing %. Carcasses were then dissected into different commercial parts (breast, thighs and wings) to determine part yield. Each part was weighed separately, put in sealed plastic and stored at -20°C for further chemical analysis. Cuts were related to the carcass weight and expressed in percentage. Meat samples were minced to a finely divided homogenous paste by passing them three times through a grinder fitted with a fine screen.

Proximate analysis was performed for feed and meat samples of breast and thighs. They were analyzed for dry matter by oven drying, ash by muffle furnace, crude protein by Kjeldahl method and ether extract by Soxhlet fat analysis. All analyses were conducted according to AOAC (2000).

pH and color measurements: The pH values were determined by using the iodoacetate method as described by Jeacocke (1977) and Sams and Janky (1986). One to 1.5 g of raw muscles were put into plastic test tube containing 10 ml of neutralized 5 mM iodoacetatereagent and 150 mM KCL and homogenized using homogenizer (Ultra-Turrax) T8, IKA Labortechnik, Janke & Kunkal GmbH & Co., Germany). Before recording the pH, values of the solutions on a pH meter (pH spear, model 35634-40, Eurotech Instruments, Malaysia). The pH was measured at three points on the cranial area of the pectoral superficial muscle (*Major Pectoralis*) at about 5 cm from the sternum line.

Color measurements were taken on the same area as pH for each sample using a colorimeter (12 MM Aperture U 59730-30, Cole-Parameter International Inc, Pittsford, NY, USA). Three measurements were taken at each point on the medial portion of the pectoralis muscle. Colors for each sample was expressed in terms of values for lightness (L*), redness (a*) and yellowness (b*) of the meat.

Water holding capacity: Water Holding Capacity (WHC) was measured using the method described by Grau and Hamm (1953) and modified by Safiudo *et al*. (1986), using a sample of an initial weight of 5 g of raw meat (1 sample per replication). Each sample was cut into smaller pieces and covered with two filter papers (qualitative, 185 mm circles, fine crystalline retention) and two thin plates of quartz material, then pressed with weight of 2500 gm for 5 min. The meat samples were then removed from filter paper and their weight was recorded (final weight). WHC was calculated as the difference between initial and final weight divided by initial sample weight and expressed as a percentage.

Cooking loss and shear force measurements: Meat samples of about 250 g were weighed and put in well sealed bags without air in a freezer at -20°C. Breasts were then thawed from freezer, taken out of plastic bags to determine loss in weight. The breasts were put individually in sealed plastic bags, cooked in thermostatically controlled water bath at 85°C for 25 min to achieve the maximum internal temperature of 80°C. Samples then were removed and put under running cold water to cool down for 45 min, then well dried and weighed to determine cooking loss. Cooking loss was reported as weight lost during cooking divided by fresh sample weight and expressed as percentage. The cooked pieces of meat were cut to obtain 6 cores (20 x 13 x 13 mm) on each breast sample (6-8 carrots) using cylindrical metal that measures 1.25 cm in diameter to determine shear force of meat according to Bratcher *et al.* (2005), (Warner-Bratzler Meat Shear Apparatus/ INSTRON, G-R manufacturing CO. 1317 Collins LN, Manhattan, Kansas, 66502, USA). This apparatus measures the maximum strength in Kg/cm^2.

RESULTS AND DISCUSSION

Bird performance: The effects of different concentrations of the exogenous enzyme (Tomoko) in diet on performance of broiler at different ages are presented in Table 2. Enzyme addition did not show any significant effect at 21 days of age, while there was a significant ($p<0.05$) effect at 42 days due to improvement in digestibility since the gastrointestinal tract of 21-d-old broilers is not fully developed to digest and ability of birds' to extract nutrients may be limited (Gracia *et al.*, 2003). As birds age, their digestive ability increases, as does the microbial population and the effect of exogenous enzymes is more evident and is mediated through the microfloral route (Bedford, 2000).

Growth rate decreased significantly ($p<0.05$) with the addition of different levels of enzymes. While feed intake was the highest with the lowest enzyme concentration (T250) and as the concentration of enzyme increased to (T750), feed intake decreased significantly ($p<0.05$) compared to the control and T250 group. This is congruent with previous findings (Richter *et al.*, 1995; Manickam *et al.*, 1994; Ranade and Rajmane, 1992; Samarasinghe *et al.*, 2000; Douglas *et al.*, 2000; Kocher *et al.*, 2002) and in disagreement with others (Ritz *et al.*, 1995; Kocher *et al.*, 2003; Gracia *et al.*, 2003; Olukosi *et al.*, 2007; Cowieson and Ravindran, 2008a,b). The inconsistency between results might be due to differences in microbial species, or strains of microorganism used, or methods in preparing the supplement (El-Husseiny *et al.*, 2008). Feed intake decrement by enzyme addition might be attributed to birds fulfilling their nutrient requirement by taking less amount of feed due to changes in the digestibility of energy and amino acids rather than improved digestible nutrient intake. The lack of response might also be attributed to the possibility that the diets fed were extremely of good quality and allowed the birds to perform close to their genetic potential (Acamovic, 2001). As a result there was no significant effect on Feed Conversion Ratio (FCR) which meant that the enzyme cocktail was not capable of modifying the gastrointestinal environment to improve efficiency of feed utilization. Performance was not affected by enzyme supplementation, which is in agreement with Douglas *et al.* (2000), who reported no corresponding production improvements with Avizyme 1502. Production improvements depend on the specific enzyme preparation and mixture (Pack *et al.*, 1998; Vieira *et al.*, 2006). Some sort of toxicity can occur due to the influence of substances released after enzymes action (Vieira *et al.*, 2006). No apparent effects were detected and no detrimental responses have been noted, consistent with Gilbert *et al.* (2000).

Carcass characteristics: The meat yield characteristics of different diets with and without enzymes are presented in Table 3 and 4. Similar yields for the carcass and its different parts such as deboned breast, thighs and wings were noted between the control and the different enzyme concentration groups (Table 3) and are in agreement with (Mohamad and Hamza, 1991; Vranjes and Wenk, 1995; Saleh *et al.*, 2004, 2005; Café *et al.*, 2002) who reported no apparent effects on carcass yields when enzymes were added. The liver percent to the whole carcass decreased significantly ($p<0.05$) in the control diet compared to the other two enzyme concentrations (T250 and T500), but not with the higher concentration of the enzyme (T750) (Table 4). No significant effect was shown on the percent of heart,

Table 2: Production parameters-feed intake, body weight, body weight gain and feed conversion ratio

Production parameter	Feed intake (g)		Body weight (g)		Body weight gain (g/bird)		Feed conversion ratio (g feed: g weight gain)	
Days	21	42	21	42	21	42	21	42
Diets[1]								
Con	848.99	3615.43^{ab}	542.13	1755.65^{a}	498.43	1715.95^{a}	1.71	2.14
T250	867.16	3650.81^{a}	538.00	1686.30^{b}	496.55	1652.85^{b}	1.75	2.21
T500	846.21	3586.55^{bc}	532.88	1706.20^{b}	493.25	1667.20^{b}	1.71	2.24
T750	846.74	3533.49^{c}	539.00	1683.88^{b}	497.75	1645.13^{b}	1.71	2.22
SEM	9.13	19.81	25.01	19.55	6.31	19.49	0.029	0.046
Diet effect	NS	0.003	NS	0.03	NS	0.04	NS	NS
Contrasts								
Con vs. Enz	NS	NS	NS	0.006	NS	0.007	NS	NS

$^{a-c}$Means with varying superscripts differ significantly ($p<0.05$).

[1]Diets: Con, control, T250: Tomoko enzyme at a rate of 250 g/tonne, T500: Tomoko enzyme at 500g/tonne, T750: Tomoko enzyme at 750 g/tonne

Table 3: Carcass characteristics-whole carcass, breast, thighs and wings

	Carcass		Breast		Thighs		Wings		Abdominal fat	
Characteristic	(g)	DP (%)[1]	(g)	(%)[1]	(g)	(%)[1]	(g)	(%)[1]	(g)	(%)[1]
Diets[2]										
Con	1153.13	69.83	297.50	25.77	336.25	29.27	126.87	11.02	24.06	2.14
T250	1085.00	69.37	315.63	29.16	338.13	31.25	130.00	12.09	22.19	2.10
T500	1045.63	69.58	298.25	28.76	332.50	32.07	125.62	12.15	26.56	2.56
T750	1098.13	70.54	311.25	28.28	315.63	28.72	124.36	11.33	24.38	2.15
SEM	33.960	0.593	14.056	1.175	10.534	1.043	3.267	0.343	2.394	0.214
Diet effect	NS	NS	NS	NS	NS	0.08	NS	0.08	NS	NS
Contrasts										
Con vs. Enz	0.04	NS	NS	0.04	NS	NS	NS	0.05	NS	NS

[a-b]Means with varying superscripts differ significantly (p<0.05).
[1]DP: dressing %. [2]Diets: Con, control, T250: Tomoko enzyme at a rate of 250 g/tonne, T500: Tomoko enzyme at 500 g/tonne, T750: Tomoko enzyme at 750 g/tonne

Table 4: Organ weight and percent-liver, heart and gizzard

	Liver		Heart		Gizzard	
Organ	(g)	(%)[1]	(g)	(%)[1]	(g)	(%)[1]
Diets[2]						
Con	40.63	3.92[b]	15.31	1.37	54.38	4.85
T250	43.75	4.59[a]	15.31	1.47	55.94	5.36
T500	46.63	4.52[a]	18.13	1.75	57.19	5.52
T750	41.25	4.10[b]	15.94	1.42	55.63	4.92
SEM	2.540	0.178	2.394	0.214	2.213	0.205
Diet effect	NS	0.005	NS	NS	NS	0.06
Contrasts						
Con vs. Enz	NS	0.006	NS	NS	NS	0.08

[a-b]Means with varying superscripts differ significantly (p<0.05).
[1]Organ% = weight of organ (g)/carcass weight (g).
[2]Diets: Con, control, T250: Tomoko enzyme at a rate of 250 g/tonne, T500: Tomoko enzyme at 500 g/tonne, T750: Tomoko enzyme at 750 g/tonne

gizzard and abdominal fat pad, which suggests that there was no response to increase calorie-protein ratio when enzyme was supplemented. These results are consistent with Biswas *et al*. (1999) and Kidd *et al*. (2001) who found that carcass yields and internal organs were not affected due to enzyme addition but in contrast to Café *et al*. (2002) who reported that birds fed the diet supplemented with enzyme had significantly higher proportion of abdominal fat. Yamamoto *et al*. (2007) stated that higher levels of the Tomoko Koji-feed had no effect on carcass yields and breast muscle weights because of the high amount of enzymes (especially protease) and unknown factor which might have negative effect on the bird's performance.

Meat quality parameters: Dietary enzyme had no effect on the different meat quality traits such as pH, cooking loss, water holding capacity, shear force and the different color parameters (Table 5). Enzymes addition did not affect the different meat quality parameters which are related to each other, such as the pH value and color which are manly affected by the haem concentration and cooking loss (Werner *et al*., 2009). Chemical analysis of the breast and thighs which shows the percent of Dry Matter (DM), ash, Crude Protein (CP) and Ether Extract (EE) indicated significant effects (p<0.05) on some analyses as shown in Table 6. Dry matter of breast was significantly higher (p<0.05) in the control diet compared to the enzyme supplemented groups and there was a significant difference (p<0.05) between the lower (T250) and higher enzyme concentration (T500 and T750) consequently results for the other different analysis based on DM were affected. Ash percent was significant (p<0.05) between the control group and the (T250 and T750) indicating more minerals content in the carcass which is directly related to the use of minerals in the diet (Olukosi *et al*., 2008). In regard to CP and EE% for the breast portion, no significant difference was noted between the control and the enzyme added groups. The thigh part showed significant difference (p<0.05) in DM, ash and CP percent between the control group and the enzyme added groups. Crude protein was decreased significantly (p<0.05) with the higher enzyme concentration by an average of 0.43 point comparing the control group with the higher concentration of enzyme supplemented groups, this applies also to the ash% which decreased significantly with higher enzyme concentration. A significant reduction in protein and ash concentration in the carcass of broilers receiving the higher concentration of enzyme supplementation is

Table 5: Meat quality traits-pH, cooking Loss, water holding capacity, shear force and color attributes (L, a and b)

Quality trait	pH	CL[1] (%)	WHC[1] (%)	SF[1] (kg/cm^2)	L[2]	a[2]	b[2]
Diets[3]							
Con	6.11	29.70	23.59	3.15	52.25	1.78	18.26
T250	6.16	28.57	23.78	3.04	50.03	1.92	16.23
T500	6.20	29.53	28.25	2.99	51.74	2.56	19.54
T750	6.18	29.13	25.69	2.72	48.47	2.55	19.26
SEM	0.047	1.776	2.055	0.456	1.583	0.656	2.055
Diet effect	NS	NS	0.08	NS	NS	NS	NS
Contrasts							
Con vs. Enz	NS	NS	NS	NS	NS	NS	NS

[1]CL: Cooking Loss, WHC: Water Holding Capacity, SF: Shear Force

[2]L: Lightness, a: Redness, b: Yellowness.

[3]Diets: Con, control, T250: Tomoko enzyme at a rate of 250 g/tonne, T500: Tomoko enzyme at 500 g/tonne, T750: Tomoko enzyme at 750 g/tonne

Table 6: Chemical analysis of meat (breast and thigh)-dry Matter, ash, crude protein and ether extract

Chemical analysis	Breast DM[1] (%)	Breast Ash (%)	Breast CP[1] (%)	Breast EE[1] (%)	Thigh DM[1] (%)	Thigh Ash (%)	Thigh CP[1] (%)	Thigh EE[1] (%)
Diets[2]								
Con	24.06[a]	1.14[a]	21.68	0.98	24.18[a]	1.12[a]	17.90[a]	5.07
T250	23.92[b]	1.10[bc]	21.81	0.91	23.71[b]	1.07[b]	17.96[a]	4.87
T500	23.80[c]	1.11[ab]	21.68	0.95	23.64[b]	1.05[b]	17.47[b]	4.88
T750	23.80[c]	1.06[c]	21.72	0.98	23.75[b]	1.06[b]	17.47[b]	4.73
SEM	0.084	0.020	0.063	0.022	0.106	0.014	0.053	0.108
Diet effect	0.001	0.01	NS	NS	0.002	0.001	0.001	NS
Contrasts								
Con vs. Enz	0.001	0.005	NS	NS	0.001	0.001	0.001	0.05

[1]DM: Dry Matter, CP: Crude Protein, EE: Ether Extract.

[2]Diets: Con, control, T250: Tomoko enzyme at a rate of 250 g/tonne, T500: Tomoko enzyme at 500 g/tonne, T750: Tomoko enzyme at 750 g/tonne

consistent with work reported by Olukosi *et al.* (2008). Comparing the CP% of breast and thighs, it is higher in the breast part over the thigh with an average of 3.78 point for the control groups and 4.10 points for the supplemented groups. This is mainly related to growth rate and fat % which is inversely correlated with the protein % which did not give significant effect. These results are consistent with previous data (Kirk and Sawyer, 1991) and the presented data and its variation for broilers are “within the normal range” (Werner *et al.*, 2009).

Conclusion: The current study failed to demonstrate improvements of the mixture of enzymes which were oriented to corn-soybean meal feeds and was not effective in improving the performance of birds. It is possible that many of the adverse or poor responses are due to a lack of understanding of enzyme effect leading to the creation of nutrient imbalances and overlap of reactions than to a true failure of the enzyme. Responses to enzymes vary widely and are difficult to predict since enzyme action may be affected by many factors, including environment, amount of enzyme in the reaction, and interactions between enzyme and other substances, which are still not fully understood. Yet, despite the results that were obtained, there are still challenges for the use of exogenous enzymes to improve nutrient utilization even on diets based on corn and soybean by the poultry industry. This may continue well into the future since enzymatic supplementation can reduce environmental problems and improve welfare of birds.

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