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Influence of Dietary Enzymes Prepared at Ensiling (ZADO®) from Hatch to 42 Days of Age on Productivity, Slaughter Traits and Blood Constituents in Broiler Chickens

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Abstract: Four hundred Cobb-500 broiler chicks at one-day old were used to study the effects of exogenous xylanases, cellulases, protease and alpha-amylase enzyme preparations at ensiling (ZADO®) on the productive performance, slaughter traits and blood metabolites. Chicks were divided randomly into 4 treatments (broiler basal diets supplemented with 0, 2, 4 and 6‰ with ZADO®) and housed on deep litter at an open house system under commercial conditions. Each treatment replicated 4 times (30 chicks per replicate). Basal diet contained 23.1% CP and 3,103 kcal AME/kg for the starter diet (0-21 d) and 20.0% CP and 3,207 kcal AME/kg for the finisher diet (21-42 d). Results indicated that broiler productivity improved in response to dietary 4 or 6‰ ZADO®. Feed conversion ratio was 1.948, 1.903, 1.758 and 1.678 for birds fed diet supplemented with 0, 2, 4 and 6‰ ZADO® ($p = 0.0007$), respectively. In addition, birds fed diet supplemented with 6‰ ZADO® recorded higher dressing and immune organs relative weights at 42 days of age than other treatments ($p<0.05$). Moreover, enzyme supplementation increased significantly plasma total protein ($p = 0.0040$) and globulin ($p = 0.0131$) at 42 days of age. Also, dietary 6‰ ZADO® decreased plasma cholesterol ($p = 0.0466$) and increased HDL-:Total-cholesterol ratio ($p = 0.0040$). It could be concluded that ZADO® supplementation to broiler diets improved broiler productivity and might improve immunity. Moreover, dietary 6‰ ZADO® has a favor effects on lipid metabolism. It could be recommended from this study to supplement 4‰ or more of ZADO® to broiler diets up to 42 days of age.

Key words: ZADO®enzyme complex, broiler performance, slaughter traits, blood biochemical characteristics

INTRODUCTION

In the last decade, the addition of exogenous enzymes to broiler chicken feeds has gained increasing attention because of both economical and environmental aspects. Their prospect is to stimulate a better utilization of the diet, because less feed is needed to produce a certain amount of meat and fewer nutrients end up in the litter (Kalmendal and Tauson, 2012). Branded enzyme products can be categorized into single (mono-component) enzymes, blends of mono-component enzymes and fermentation products from wild type microorganism strains expressing a spectrum of enzyme activities (Freitas *et al*., 2011).

It is well known that exogenous enzymes have been used to enhance the feeding value of wheat and rye-based diets because these feed stuffs are high in soluble non-starch polysaccharides that induce viscosity (Mathlouthi *et al*., 2002; Lázaro *et al*., 2003). However, it has been reported also that enzyme cocktail (carbohydrase and protease) improve the productivity (Saleh *et al*., 2005) and the digestibility of corn and soybean meal (SBM), which induce less viscosity for broilers (Zanella *et al*., 1999; Gracia *et al*., 2003; Olukosi *et al*., 2007; Cowieson and Ravindran, 2008).

The efficacy of many commercial enzyme products has been well stated, but there is still some vagueness in their mode of action (Bedford, 2002). Moreover, several reports indicated that dietary enzymes improve the digestibility of nutrients in broilers (Gracia *et al*., 2003; Cowieson and Ravindran, 2008; Kalmendal and Tauson, 2012). In addition, Gao *et al*. (2007) suggested that enzyme supplementation accelerated the development of the immune organs. In this respect, the author hypothesized that the improvement of nutrient digestibility might be reflected in enhancing immunity and modifies blood metabolites profile especially, if these enzymes are prepared at ensiling from anaerobic bacterium (Safaa *et al*., 2010). Also, for this reason liver and kidney might be work better in response to dietary enzymes and their functions might be improved.

An Egyptian patented product (ZADO®), which is a commercial exogenous enzyme mixture prepared from anaerobic bacterium, has been shown to improve ruminal fermentation, N balance and nutrient digestibility, as well as milk yield of cows fed diets containing Egyptian by-product feeds (Gado *et al*., 2007; Gado *et al*., 2009), as well as live body weight (BW) gain (BWG) and feed conversion ratio (FCR) of sheep and goats fed diets contained wheat straw (Gado, 1997; Gado and Salem, 2008; Gado *et al*., 2011). In addition, dietary serine protease derived from fermentation of *Bacillus licheniformis* in corn-SBM-based broiler diets resulted in improved BW (Peek *et al*., 2009), feed efficiency and digestibility of fat, protein (Freitas *et al*.,

2011) and amino acids (Angel *et al*., 2011). Therefore, the aim of this study was to evaluate the impacts of ZADO® supplementation to broiler diets on productive performance, slaughter traits and blood metabolites in chicken broilers.

MATERIALS AND METHODS

The trial was conducted at a private broiler farm in Shubramant, Giza, Egypt and the biochemical analysis was completed at the laboratory of biochemistry, Cairo University Research Park (CURP), Giza, Egypt. The study was conducted under the guidelines of the institutional animal care and use committee and approved by the Ethics of Animal Use in Research Committee (EAURC), Cairo University, Egypt.

Husbandry and experimental design: A total of 480 Cobb-500 broiler chicks (mixed sex) at one-day old with an initial BW of 44.3±2.3 g were obtained from a local commercial hatchery (Cairo Poultry Company, 10th of Ramadan City, Egypt) and used in this trial. Chicks were divided randomly into 4 treatments (broiler basal diets supplemented with 0, 2, 4 and 6‰ with ZADO®). ZADO® is a patented product manufactured by the Academy of Scientific Research and Technology, Egypt and contains a mix of anaerobic bacteria and their enzymes of xylanases (2.3 unit/g), cellulases (7.1 unit/g), alpha-amylase (61.5 unit/g) and protease (29.2 unit/g) in a powder form obtained through an anaerobic fermentation process (Gado *et al*., 2009; Gado *et al*., 2011).

At arrival to the farm, the birds were weighed individually and stratified by BW into four groups of 120 birds each. Sixteen uniform groups of 30 chicks each were formed and allocated in the experimental unit (a pen of 2×1.5 m). All chicks were housed on deep litter in open house system divided into 16 floor pens each one as a replicate (10 birds/m^2). Birds fed starter diet (23% crude protein and 3103 ME Kcal/kg) during the first 21 days then finisher diet (20% crude protein and 3207 ME Kcal/ kg) from 22 to 42 days of age (Table 1). Diets were formulated to have similar nutrient content and met or exceeded the nutritional recommendations of National Research Council (1994) for broilers.

Room temperature was maintained at 32±0.7°C during the first three days of life and then reduced gradually until reaching 24±0.9°C at 42 days of age. Birds were vaccinated against main diseases (Infectious bronchitis disease, Infectious bursal disease and Newcastle disease) according to commercial practices. Birds were exposed to 23 hours daily and the same managerial conditions. Feed and water were provided *ad-libitum*.

Analytical evaluation of feeds: Feeds were analyzed for dry matter by the oven-drying method (930.15), crude protein by Kjeldahl method (990.03), ether extract by Soxhlet fat analysis after 3 N Hcl acid hydrolysis (920.39) and crude fiber by sequential extraction with diluted acid and alkali (962.09) as described by Association of Official Analytical Chemists International (AOAC, 2000). The gross energy was determined by combustion using an adiabatic bomb calorimeter.

Table 1: Feed ingredients and nutrient composition of basal diets for broiler chickens

Item	Starter diet (1 to 21 d)	Finisher diet (22 to 42 d)
Ingredients (%)		
Yellow corn	52.03	61.38
Soya bean meal (44% CP)	29.60	22.50
Corn gluten meal (60% CP)	7.00	6.10
Vegetable oil	4.00	4.00
Wheat bran	2.80	2.60
Bone meal	3.30	2.15
Limestone	0.14	0.14
Permix[1]	0.30	0.30
NaCl (salt)	0.50	0.50
L-lysine-HCL	0.18	0.18
D.L. Methionine	0.15	0.15
Analyzed chemical composition[2]		
ME (Kcal./Kg)	3103	3207
Dry matter (%)	89.07	88.01
Crude protein (%)	23.07	20.03
Ether extract (%)	6.50	6.73
Crude fiber (%)	3.80	3.32
Lysine (%)[3]	1.22	1.05
Methionine (%)[3]	0.55	0.49
Methionine + Cystine (%)[3]	0.92	0.81
Calcium (%)[3]	0.92	0.90
Available P (%)[3]	0.48	0.46

[1]Supplied per Kg diet: Vit.A, 12000 IU; Vit.E, 10 mg; Vit.K, 2 mg; Vit.B_1, 1 mg; Vit.B_2, 5 mg; Vit.B_6, 1.5 mg; Vit.B_{12}, 10 mg; Nicotinic acid 30 mg; Folic acid 1 mg; Pantothenic acid 10 mg; Biotin 50 mg; Choline chloride 500 mg; Copper 10 mg; Iron 30 mg; Manganese 60 mg; Zinc 50 mg; Iodine 1 mg; Selenium 0.1 mg; cobalt 0.1 mg

[2]Triplicate samples

[3]Calculated according to National Research Council (1994)

Productive performance: The BW and feed consumption were recorded by replicate weekly and mortality was recorded daily. Feed wastage was observed to be negligible and was not measured. From these data BWG, Average Daily Feed Intake (ADFI), FCR corrected for mortality and mortality rate were calculated cumulatively.

Slaughter traits and blood metabolites: At the end of the trial (42 days of age), five chicks at random per each replicate were chosen, weighted, slaughtered and eviscerated. Carcass, heart, liver, gizzard, spleen and bursa were weighed and relative weights to live BW (g/100 g of BW) were calculated (El-Sayed *et al*., 2011). At the same day of slaughter, 3 mL blood samples were

collected from slaughtered chicks (chosen at random per each replicate) and obtained in heparinized tubes. Blood samples were centrifuged at 3000 rpm/minute for 10 minutes. Clear plasma samples were separated into Ependorph tubes and kept in the deep freezer at -20°C until chemical analyses. Total protein (mg/dL) was analyzed in plasma according to Gornall *et al.* (1949) and plasma albumin (g/dL) according to Doumas *et al.* (1971). Plasma globulin (g/dL) was calculated by the difference between total protein and albumin (Zhang *et al.*, 2009). Total cholesterol was determined in plasma according to Allain *et al.* (1974). Heparin and sodium citrate were used selectively to precipitate all lipoproteins except the Low Density Lipoprotein (LDL) fractions in plasma which were present in the supernatant (Steinberg, 1981). Also, phosphotungstic acid and magnesium ions were used selectively for precipitating all lipoproteins except the High Density Lipoprotein (HDL) fractions in plasma which were present in the supernatant (Lopes-Virella *et al.*, 1977). Then, the LDL- and HDL-cholesterol were determined in the supernatant using the same method described for the total cholesterol. Moreover, form these data ratios of HDL-:Total-, HDL-:LDL- and LDL-:HDL-cholesterol were calculated. Plasma aspartate transaminase (AST) and Alanine transaminase (ALT) (U/L) were determined according to the method of Reitman and Frankel (1957).

Statistical analysis: The experimental design was completely randomized with four treatments (each with 4 replicates). The experimental unit (replicate) consisted of a group of 30 chicks per pen for productive traits. For slaughter and blood metabolites traits, the experimental unit consisted of five birds chosen at random from each replicate. Data were statistically analyzed by analysis of Variance (ANOVA) one-way according to Snedecor and Cochran (1967) using General Linear Model (GLM) procedure of SAS software (SAS Institute, 2004). When the model was significant, Tukey's test was used to separate treatment means. Differences between treatment means were considered significant at $p<0.05$ and $p<0.10$ were considered as a trend (Steel and Torrie, 1980). Orthogonal polynomial contrasts were performed to study the linear and quadratic effects of dietary ZADO® levels on all traits. Results in tables are presented as means.

RESULTS

Productive performance: Productive performance traits of broilers from hatch to 42 d of age in response to dietary different levels of ZADO® are presented in Table 2. Results indicated that BW and average daily BWG were improved in response to dietary 6‰ ZADO® comparing to the other treatments ($p = 0.0112$ and 0.0097, respectively). In addition, ADFI was decreased when birds fed diets supplemented with 4‰ ZADO® or more from hatch to 42 d of age. Moreover, FCR was improved and mortality rate was decreased in response to dietary 4 or 6‰ of ZADO® comparing to 0 and 2‰. The FCR was 1.948, 1.903, 1.758 and 1.678 for birds fed diet supplemented with 0, 2, 4 and 6‰ ZADO® ($p = 0.0007$), respectively. Linear effects were noted for all productive traits. However, no quadratic effects were observed for these traits.

Slaughter traits and blood metabolites: Results in Table 3 shown the slaughter traits of broiler chickens at 42 days of age dietary different levels of ZADO®. Birds fed diets supplemented with 6‰ ZADO® recorded higher carcass ($p = 0.0198$) and immune organs ($p = 0.0028$ and 0.0003 for spleen and bursa, respectively) relative weights than other treatments. Linear effects were noted also for these traits. However, no significant effects were observed for relative weights of other internal organs (heart, liver and spleen) in response to dietary ZADO® levels.

The values of plasma constituents in broilers at 42 days of age which fed different levels of ZADO® during 42 d are presented in Table 4. Enzyme supplementation increased significantly plasma total protein ($p = 0.0040$) and globulin ($p = 0.0131$). Regarding lipid metabolites, total plasma cholesterol was reduced in response to dietary 6‰ ZADO® when compared to chicks fed 0 or 2‰ ZADO® with chicks fed 4‰ ZADO® intermediate. The same trend was observed for the HDL-:Total-cholesterol ratio and HDL-cholesterol content tended to be significant ($p = 0.0953$). In addition, linear effects were noted for total plasma cholesterol and HDL-:Total-cholesterol ratio. However, no significant effects were observed for the other plasma metabolites levels, including albumin and kidney enzymes activity, in response to dietary ZADO® levels. Moreover, no quadratic effects of dietary ZADO® levels were observed for all plasma metabolites traits.

DISCUSSION

Productive performance: Results of this trial proved that ZADO® enzyme cocktail, which prepared at ensiling and contains xylanases, cellulases, alpha-amylase and protease, has beneficial effects on broiler productivity when birds fed a corn-SBM based diet, which logically reflected on economic benefits for producers. These results are in agreement with several reports regarding enzyme addition in broiler corn-SBM-based diets. Kocher *et al.* (2003) reported that using an enzyme cocktail containing pectinase, amylase and protease in corn-SBM-based diets for chicks resulted in improved performance. Also, Cowieson *et al.* (2006) indicated that exogenous xylanase, amylase, protease and phytase (Avizyme) can be used successfully in a strategically

Table 2: Effect of dietary ZADO® on productive performance traits in broilers from 1 to 42 days of age

	ZADO® ‰					Effects		
Trait	0	2	4	6	SEM[1]	P-value	Linear	Quadratic
Body weight at 42 d (g)	1966[b]	1974[b]	2012[b]	2071[a]	17.5	0.0112	0.0050	0.5709
Body weight gain (g/bird/d)	46.8[b]	47.0[b]	47.9[b]	49.3[a]	0.47	0.0097	0.0043	0.6142
Feed intake (g/bird/d)	91.2[a]	89.4[a]	84.2[b]	82.5[b]	1.34	0.0017	0.0034	0.2884
Feed conversion ratio (g:g)	1.948[a]	1.903[a]	1.758[b]	1.678[b]	0.0365	0.0007	0.0009	0.4810
Mortality rate (%)	5.50[a]	5.25[a]	4.00[b]	3.50[b]	0.473	0.0309	0.0226	0.5298

®Exogenous xylanases, cellulases, protease and alpha-amylase enzyme preparations at ensiling

[1]Standard error of the mean (n = 4 replicates with 30 chicks per each)

[a,b]Means within rows with different superscripts are significantly different (p<0.05)

Table 3: Effect of dietary ZADO® on relative weights of carcass and internal organs (g/100 g of BW) in broilers at 42 days of age

	ZADO® ‰					Effects		
Trait	0	2	4	6	SEM[1]	P-value	Linear	Quadratic
Carcass	67.8[b]	68.4[b]	68.9[b]	71.3[a]	0.77	0.0198	0.0126	0.3485
Internal organs								
Heart	0.867	0.880	0.889	0.892	0.0469	0.9815	0.8666	0.9564
Liver	3.24	3.26	3.30	3.32	0.125	0.9664	0.7226	0.9340
Gizzard	2.71	2.75	2.76	2.77	0.120	0.9844	0.9120	0.9790
Immune organs								
Spleen	0.149[b]	0.150[b]	0.158[b]	0.184[a]	0.0067	0.0028	0.0012	0.2708
Bursa	0.080[b]	0.081[b]	0.085[b]	0.130[a]	0.0084	0.0003	0.0002	0.0572

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[1]Standard error of the mean (n = 4 replicates with 30 chicks per each)

[a,b]Means within rows with different superscripts are significantly different (p<0.05)

formulated low nutrient density diet to maintain performance to that of birds fed on a nutritionally adequate diet. In addition, Cowieson and Ravindran (2008) stated that supplementing corn-SBM-based broiler diets with an enzyme product containing xylanase, amylase and protease improved BWG and feed efficiency compared with the un-supplemented diets, but feed intake did not affected. They also, reported that the energy and amino acid values of corn-based diets for broilers can be enhanced by supplementation with an enzyme cocktail of xylanase, amylase and protease, offering potential economic benefits to producers. The mode of action of enzymes in corn-based diets has been linked to improved starch digestibility associated with augmentation of endogenous alpha-amylase or improved digestion of resistant starches, improved access to cell contents via a reduction in cell wall integrity, modification of the intestinal microbial communities, improved protein solubility and digestibility and a reduction in the inimical effects of maize and/or soy-derived anti-nutritive factors. In the same context, Saleh *et al*. (2005) reported that the commercial enzymes, which are mostly comprised of carbohydrases and contain small amount of protease activity (Energex) improved significantly the productivity (BWG and FCR) of broilers fed corn-SBM based diets in compare to pure carbohydrases (cellulase, hemicellulose and pectinase) supplementation, which tended to affect in compare to control group (without enzyme supplementation). However, they noted that feed intakes were not affected by dietary enzymes. Similar results have been found earlier by Zanella *et al*. (1999) when they supplemented a corn-SBM diet with Avizyme, a commercial enzyme; BWG and FCR were significantly improved by Avizyme. They demonstrated that the energy and amino acid digestibility of a corn-SBM-based diet for broilers could be improved by around 3% when supplemented with xylanase, amylase and protease allowing performance to be maintained on a diet with a lower nutritional plane. In addition, Kalmendal and Tauson (2012) observed that the combination of xylanase and serine protease improved FCR, compared with the control diet but, BW and FI were not affected by enzyme addition sole or mixed. Moreover, Gracia *et al*. (2003) demonstrated that amylase was a critical enzyme to improve the nutritional value of corn-based broiler diets, improving BWG and FCR by 4 to 9% compared with an un-supplemented control diet. Remus *et al*. (2005) summarized the effect of a combination of xylanase, amylase and protease on ileal digestibility of amino acids for 5 broiler trials and found a mean response of around 2%. However, though highly significant, these effects were amino acid dependent, for example, for threonine (>2%) vs. methionine (<0.5%) and the reasons for the differential responses are not clear.

Frigard *et al*. (1994) reported also, a significant improvement in live BW and feed efficiency in broilers at 14 and 20 d of age fed rye-corn-SBM based diet supplemented with commercial enzyme (2 g/kg diet;

Table 4: Effect of dietary ZADO® on blood metabolites in plasma of broilers at 42 days of age

	ZADO® ‰					Effects		
Trait	0	2	4	6	SEM[1]	P-value	Linear	Quadratic
Protein metabolites in plasma								
Total protein (mg/dL)	3.53[b]	3.96[a]	4.17[a]	4.27[a]	0.133	0.0040	0.1179	0.7386
Albumin (mg/dL)	1.91	1.93	2.04	2.07	0.059	0.1957	0.1176	0.6039
Globulin (mg/dL)	1.62[b]	2.03[a]	2.14[a]	2.20[a]	0.122	0.0131	0.3338	0.9101
Lipid metabolites in plasma								
Total cholesterol (mg/dL)	133.5[a]	132.2[a]	129.7[ab]	126.8[b]	1.65	0.0466	0.0333	0.9351
LDL-cholesterol (mg/dL)[2]	31.77	31.35	31.13	30.85	0.499	0.6190	0.4886	0.9582
HDL-cholesterol (mg/dL)[2]	67.58	68.47	69.08	69.35	0.504	0.0953	0.2296	0.7777
HDL-:Total-cholesterol ratio[2]	0.51[c]	0.52[bc]	0.54[ab]	0.55[a]	0.008	0.0040	0.0105	0.7208
HDL-:LDL-cholesterol ratio[2]	2.13	2.19	2.23	2.25	0.041	0.1825	0.2536	0.9089
LDL-:HDL-cholesterol ratio[2]	0.47	0.46	0.45	0.45	0.008	0.2061	0.2475	0.8652
Kidney enzyme activity in serum								
GOT=AST (U/L)[2]	312.3	309.4	294.6	291.7	8.80	0.2761	0.1704	0.5902
GPT=ALT (U/L)[2]	2.25	2.18	2.14	2.11	0.042	0.1001	0.2263	0.8616

®Exogenous xylanases, cellulases, protease and alpha-amylase enzyme preparations at ensiling

[1]Standard error of the mean (n = 4 replicates with 30 chicks/each)

[2]LDL = Low density lipoprotein HDL = High density lipoprotein GOT = Glutamate oxaloacetate transaminase
AST = Aspartate aminotransferase GPT = Glutamate pyruvate transaminase ALT = Alanine aminotransferase

[a,b,c]Means within rows with different superscripts are significantly different ($p<0.05$)

GP-5000, based on beta-glucanases and xylanases) than those of birds fed the corresponding un-supplemented diet. However, they noted an increase ($p = 0.001$) in the cumulative feed intake, at the same ages, in response to dietary enzymes. They attributed that to the elimination of the depression of feed intake, caused by dietary rye-based diets in chickens, by enzyme supplementation, which might explain, at least in part, the differences between these findings and the results obtained for ADFI in the current trial. Moreover, Onilude and Oso (1999a) reported that the supplementation of three enzyme mixture (amylase from *Macrophomina phaseolina*, cellulase from fermentation of cassava root fiber by a *Trichoderma* sp. and pectinase from banana peel fermentation by *Fusarium tricitum*) to broiler fiber-containing diets (containing 20.24% rice bran and 37.00% wheat bran) from 1 to 42 d of age improved live BW, BWG and FCR at 42 d of age. In addition, Sarica *et al*. (2005) reported that xylanase supplementation in broiler diets based on wheat-corn-SBM up to 42 d did not affect BWG (2244.2 vs. 2237.7 g), feed intake (4005.8 vs. 3951.2 g) or FCR (1.79 vs. 1.77 g:g) for control and treated birds, respectively. In the same trend, Cowieson *et al*. (2006) have done 2 experiments with the same diets but, lower stocking density and different batches of maize and SBM were used in experiment 2. For birds fed adequate nutrients diets, in the first experiment, they noted an increase in feed intake (3767.4 g/42 d) in response to Avizyme (exogenous xylanase, amylase, protease and phytase) supplementation comparing to un-supplemented group (3621.3 g/42 d). However, in the second experiment they reported a reduction in feed intake (4291.3 g/42 d) in response to dietary Avizyme vs. un-supplemented group (4307.6 g/42 d). They attributed that to the lower stocking density in experiment 2 and to the increase of the ambient temperature during the first 2 weeks of experiment 1. This might explain, at least in part, the reduction in ADFI obtained in the current trial.

On the other hand, Marsman *et al*. (1997) showed no improvement in FCR or BWG when mixed-enzyme preparation (carbohydrases and proteases) was added to the broiler diets from 7 to 25 d of age. Moreover, Kocher *et al*. (2002) reported that the addition of the enzymes complex containing glucanase, hemicellulase and pectinase from 4 to 38 d of age had no effect on BWG or FCR of male Cobb broilers fed on a corn-SBM diet. Also, Meng *et al*. (2006) stated that 0.05% enzyme (contained cellulase, pectinase, mannanase, xylanase and glucanase as main activities) supplementation to broiler diets based on corn-SBM and containing 15% canola seed (20% CP) improved FCR (from 1.412 to 1.370) from 5 to 18 d of age. However, no effects were observed by enzyme supplementation for feed intakes of 702.3 vs. 692.8 g/bird and BWG of 497.0 vs. 505.9 g/bird for control vs. supplemented enzyme group, respectively. In addition, Walk *et al*. (2011) used mono-component xylanase and protease products derived from other microorganisms, for 18 days post hatch, but found no positive effects on production performance in broiler chickens fed a corn-SBM-based diet. Also, Barekatain *et al*. (2013) observed that an admixture of xylanase and protease to broiler corn-SBM based diets up to 21 d of ages did not result in further improvement in productive performance represented by BWG, feed intake and FCR. If the enzymes were additive in their effect, it would be expected that the sum of the effect attributed to each enzyme individually should not be different from the effect attributed to the use of the enzymes in combination (Olukosi *et al*., 2007). From this point of view, the author

suggesting that the accumulation of the additive effect of the enzymes and the effect of continuous enzyme supplementation from hatch to marketing (42 d of age) in the current trial might explain the differences between the above mentioned findings and the results of broiler productivity in the current trial.

Slaughter traits and blood metabolites: Dressing of broilers in the current trial represented by carcass relative weight was increased in response to dietary 6‰ ZADO®. Onilude and Oso (1999a) reported that the supplementation of three enzyme mixture (amylase, cellulase and pectinase) to broiler fiber-containing diets from 1 to 42 d of age increased carcass weight with a favor increase in its crude protein and ash content. Moreover, Café *et al*. (2002) noted a significant increase in dressing percentage at 42 d of age in broilers given a corn-SBM diet supplemented with commercial enzymes. These also, are in agreement with Saleh *et al*. (2005), who reported that carcass relative weight was higher (70.3 g/100 BW) for broilers fed pure carbohydrases (cellulase, hemicellulose and pectinase) than control group (68.6 g/100 BW) with broilers fed a commercial enzymes (Energex) intermediate (70.0 g/100 BW). They attributed the improvement of carcass yield to the effects on crude protein metabolize ability. An increase in carcass is a typical response to increased protein:ME ratio (Donaldson *et al*., 1958; Mabray and Waldroup, 1981; Donaldson, 1985). This also, might be explained and supported by the improvement in BWG in this trial. In the current experiment, internal organs were not affected by the enzyme addition. These results are in agreement with Saleh *et al*. (2005), who stated no differences in liver relative weight in response to dietary mixed enzymes. Also, Gao *et al*. (2007) observed that xylanase supplementation in broiler diets based on wheat-corn-SBM did not affect gizzard relative weight. In addition, Sarica *et al*. (2005) reported that xylanase supplementation in broiler diets based on wheat-corn-SBM did not affect heart, liver or gizzard relative weights and Gracia *et al*. (2003) reported that alpha-amylase supplementation in broiler diets based on corn did not affect liver or gizzard relative weights. However, Iji *et al*. (2001) indicated that the maximum weights of these organs are reached before 9 d of life. Also, Barekatain *et al*. (2013) observed a negative interaction between xylanase and protease resulting in a reduction in relative weight of gizzard at 21 d of age but, these differences were not noted at 7 d of age. They attributed that to the high insoluble fiber content, which played a stimulating role in gizzard development.

The present study showed that 6‰ ZADO® supplementation to corn-based diets significantly increased the relative weight of the spleen and bursa, suggesting that enzyme supplement accelerated the development of the immune organ. To my knowledge, few studies have been studied the effects of enzyme supplementation to corn-based diets on the immunity of poultry. However, Gao *et al*. (2007) observed that xylanase supplementation to wheat-based diets for cockerels from 7 to 21 d of age significantly increased the relative weight of the spleen. They attributed that to the improvement of feed digestion, the enhancement of nutrients absorption and the regulation of metabolic hormones in response to the addition of the enzyme, which in turn could have an effect on body immunity.

The values of plasma constituents in broilers at 42 days of age (Table 4) were within the normal ranges for plasma total cholesterol, total protein and albumin (Meluzzi *et al*., 1992; Del Bianchi *et al*., 2005). Moreover, the values of plasma lipid metabolites (total-, LDL- and HDL-cholesterol and their ratios) are within the normal ranges (Khalaji *et al*., 2011). Also, plasma kidney enzymes activities values (AST and ALT) at 42 d of age of broilers (Cobb strain) in the current trial are within the normal range (Viveros *et al*., 2002). Enzyme supplementation of chicken diets is employed in order to increase the availability of starch, protein and other macronutrients that are entrapped by intact cell wall structures or viscous polymers that are resistant to digestion by endogenous host enzymes (Frigard *et al*., 1994). The current trial indicated also, that broiler diets supplemented with ZADO® increased significantly the protein and globulin levels in plasma, which might supported by the enhancement of immune organs (spleen and bursa). It is well stated that gama-globulin is the main component of anti-body production, which presents the humoral immune response. So, findings of globulin levels in plasma in the current study are supported by Gao *et al*. (2007), who suggested that xylanase supplementation, to wheat-based diets for cockerels from 7 to 21 d of age enhanced the humoral immune response.

The current experiment showed a favor effect of enzyme addition to broiler diets up to 42 d of age on reducing the cholesterol level in plasma, suggesting that enzyme supplementation might play a role in broiler lipid metabolism. Unfortunately, little information has been published on the effects of enzyme supplementation in broiler diets on blood lipid metabolites. However, Onilude and Oso (1999b) reported that the supplementation of enzyme mixture including amylase, cellulase and pectinase to broiler fiber-containing diets from hatch to 42 d of age reduced blood lipid metabolites including plasma cholesterol level from 246 to 136 mg/dL at 42 d of age. Also, Cowieson *et al*. (2013) reported that phytase addition to broiler diets reduced total cholesterol concentration in the blood of chickens fed the positive control diet (adequate in P and Ca) but, increased cholesterol concentrations in the blood of chickens fed the negative control diet (with P and Ca levels reduced by 0.12 and 0.14%, respectively)

however, no effects of phytase on total- and HDL-cholesterol were noted. They hypothesized that enzyme addition with adequate minerals levels (Ca and P) might reduce the cholesterol content in the plasma, which might explain, at least in part, the reduction of cholesterol level in plasma in response to dietary ZADO® by providing improvement of feed digestion and enhancement of mineral absorption. In contrast, Sarica *et al*. (2005) reported that xylanase supplementation in broiler diets based on wheat-corn-SBM did not affect cholesterol content in plasma (169.4 vs. 180.6 mg/dL for treated and control groups, respectively). Frigard *et al*. (1994) noted a higher serum cholesterol level in broilers at 21 d of age fed rye-corn-SBM based diet supplemented with commercial enzyme (2 g/kg diet; GP-5000, based on beta-glucanases and xylanases) than those of birds fed the corresponding un-supplemented diet and attributed that to the elimination of the dietary fiber effect on reducing cholesterol content in the serum by the enzyme supplementation. In conclusion, the response of broiler blood metabolites to enzyme supplementation is based not only on ingredient 'quality' but also on bird age, environmental conditions, managerial conditions, enzymes preparations, duration of enzyme supplementations and the dose of supplementation.

Conclusions: It could be concluded that broiler diets supplement with 4‰ or more of ZADO® improved broiler productivity from hatch to 42 days of age. In addition, ZADO® supplementation might improve broiler immunity by accelerating the improvement of immune organs and increasing the total protein and globulin levels in plasma. Also, dietary 6‰ ZADO® has a favor effect on lipid metabolism by reducing cholesterol content in plasma and enhance HDL-:Total-cholesterol ratio. In this respect, more studied are required to explain the mode of action of the effect of enzyme supplementation on immunity and blood constituents in broilers. Therefore, it could be recommended from this study to supplement 4‰ or more of ZADO® to broiler diets from hatch to 42 days of age.

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