

## **BROILER PERFORMANCE, ENZYMES ACTIVITY AND HISTOLOGICAL OBSERVATIONS AFFECTED BY MULTI ENZYMES COMPLEX (ZADO®)**

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### **SUMMARY**

The aim of this study was to evaluate multi enzymes complex (ZADO®) on broiler performance, enzymes activity, some blood parameters and histological observations. 225 one- day unsexed Hubbard chicks were distributed into 5 equal group (45 chicks). Each experimental group was divided into five replicates (9 chicks/each). The first group was served as control and fed basal diets. While, the other groups received the basal diet supplemented with 0.1, 0.3, 0.5 and 0.7kg ZADO® on ton diets, z1, z2, z3 and z4, respectively. This product 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). Our obtained results indicated that the use of this multi-enzyme at 0.5 Kg/ton affect positively in the BW of broilers. The enhancement in both stomach and intestine enzymes activities in all supplemented groups with ZADO® are good explanations for the improvements in the FCR. The histological observations for liver and intestine and blood pictures showed that non-significantly differences among groups with or without supplementations. These findings may indicate that this product seems to be safe on birds and accordingly birds are safe for human consumption. Good of Economic Efficiency for ZADO® 0.5 Kg/ton.

**Keywords:** ZADO®, broiler performance, enzyme activity, blood components.

### **INTRODUCTION**

It is well known that exogenous enzymes have been used to enhance the feeding values of feed stuffs are high in soluble non- starch polysaccharides that induce viscosity (Mathlouthiet *et al.*, 2002; Lazaro *et al.*, 2003). However, it has been reported also that enzyme cocktail (carbohydrase and protease) improve the productivity (saleh *et al.*, 2005) and digestibility of corn and soybean meal, which induce less viscosity for broilers (Zanella *et al.*, 1999; Graacia *et al.*, 2003; Olukosi *et al.*, 2007; Cowieson and Ravindran, 2008).

The addition of exogenous enzymes to broiler chicken feeds has gained increasing attention because of both environmental and economic 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).

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 the 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, 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

A total number of 225 one d-old Hubbard broiler chicks were used in this study. The broiler chicks were nearly equal in the live body weight and divided randomly into five treatment groups of 45 chicks each. Each experimental group was divided into five replicates (9 chicks/each). The first group was served as control and fed basal diets. While, the other groups received the basal diet supplemented with 0.1, 0.3, 0.5 and 0.7kg ZADO® on ton diets, z1, z2, z3 and z4 respectively. 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). This study was terminated when the birds were 35 days old. All chicks were vaccinated against Newcastle disease, at seven and twenty two days of age with Hitchener B1 and Lasota strain vaccine, respectively and Gumboro vaccine against Bursal disease at fourteen days of age.

The experimental period included two feeding phases (starter, from 1-21 days of age and finisher, from 21-35days of age). Experimental diets were formulated to nearly meet the nutrient requirements of the broiler chicks (NRC, 1994). The composition and chemical analysis of the control basal diets are presented in Table (1). The chemical composition of the experimental diets was analyzed according to A.O.A.C. (2000).

**Table (1): Composition, calculated and chemical analysis of the basal diets**

Ingredients (%)	Starter (0-3 Weeks)	Finisher (3-5 Weeks)
Yellow corn	57.77	71.00
Soybean meal (44%CP)	25.5	13.60
Corn gluten meal	10.00	10.00
Vegetable oil	3.00	2.00
Bone meal	2.60	2.00
Limestone	0.30	0.60
Vit& Min Premix*	0.30	0.30
NaCl	0.25	0.25
L-Lysine	0.18	0.20
DL-Methionine	0.10	0.05
Total	100	100
A-Chemical analysis:-		
Crude protein %	22.16	18.00
Crude fiber %	3.53	3.13
Ether extract %	2.86	3.12
Ash %	6.77	6.58
B-Calculated analysis:		
ME (Kcal/Kg diet)	3139	3221
Calcium %	1.02	0.90
Available phosphorous %	0.45	0.35
Lysine %	1.15	0.90
Methionine %	0.51	0.40
Cystine %	0.38	0.33
Meth. + Cys. %	0.89	0.73

\*Each 3kg of vit-mineral mixture contain vit A 10m IU, vit D3 1mIU, vit E 10g, vit B1 1g, vit B2 4.0g, vit B6 1.5g, Nicotinic acid 20g, Pantothenic acid 10g, vit B12 0.01g, Biotin 0.05g, Follic acid 30g, Choline chloride 50g, Iron 30g, Manganese 40g, Copper 3g, Iodine 0.45g, Zinc 45g and Selenium 0.1g.

Feed and water were supplied *ad-libitum* during the experimental periods and birds were exposed to 24 hours of constant light. All chicks were kept under the same managerial, hygienic and environmental conditions. Chicks were individually weight at the beginning of the experiment, then at weekly intervals until the end of experiment, live body weight (LBW), body weight gain (BWG), feed consumption (FC), feed conversion ratio (FCR, g feed/g gain) were recorded during these periods.

At the age of 35 d, ten birds (5 males and 5 females) from each experimental group were weighed and slaughtered by slitting the jugular vein, then scalded and defeathered. Carcasses were manually eviscerated and weighted. Liver, heart, gizzard, spleen, thymus (all lobes of both sides), bursa, small intestine and abdominal fat were removed and their relative percentages of live body weight were estimated. The intestinal length (cm) was also considered and the intestinal density (g weight/ length, cm) was calculated. The same located segments of their digestive tract (stomach and intestine) were emptied by gentle squeezing, contents of individual segments were taken and mixed and about 1g of the mixed content was immediately diluted with 10 ml of distilled water. All samples were centrifuged for 10 minutes. The supernatant fluid was taken and stored in sealed bottles at -20°C until analyzed. Enzymes activity in digestive content of stomach, and intestine of chicks were determined as follows: amylase (Osman, 1982), protease (Malik and Singh, 1982), cellulase (Halliwell, 1958) and carboxymethylcellulase (Mandels and Waber, 1969).

Blood samples were collected from the ten slaughtered birds in nonheparinized tubes. The blood samples were centrifuged at 3000 rpm for 15 min. and serum obtained was stored at -20C until analysis. Serum total protein, albumin, triglycerides, total cholesterol, creatinine, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and minerals (calcium and phosphorous) were determined calorimetrically by using available commercial kits purchased from Diamond Diagnostics Company. The globulin values were calculated by subtracting the values of albumin from the corresponding values of total protein. Serum concentration of triiodothyronine (T3) and thyroxine (T4) were determined using commercial enzyme immunoassay test kit purchased from Taytec Incorporation (7278 Aldercrest Dr., Mississauga, ON, L5N7N8, Canada).

The economic efficiency was calculated according to the price of local market at the time of carrying out the experiment as follows: Economic efficiency =  $(A-B/B) \times 100$ .

Where: A = Price of kg gain in Egyptian pounds B = Feed cost / kg gain in Egyptian pounds.

Performance index (PI) was calculated according to North (1981) as follows:

$$PI = [\text{live body weight (kg)} / \text{feed conversion ratio}] \times 100.$$

The production efficiency factor (PEF) was calculated according to Emmert (2000) as follows:

$$PEF = [\text{Livability} \times \text{Mass (Kg)} / \text{FCR} \times \text{Age in days}] \times 100$$

Where: Livability = 100 – Mortality rate (%)

Mass (Kg) = Final live body weight.

Data were statistically analyzed by using the General Linear models (GLM) procedures of SAS (SAS, 2004). The model was as follows:  $Y_{ij} = \mu + T_i + e_{ij}$

Where:  $Y_{ij}$  = The observation on the  $i$ th treatment  $\mu$  = Overall mean,  $T_i$  = Effect of the  $i$ th treatment,  $e_{ij}$  = Random error treatment. Significant differences among treatment means were determined by Duncan's multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

### *Performace traits:*

The effects of dietary treatments on BW, FCR, and mortality are shown in Table (2). The level of 0.5Kg ZADO® supplementation in the diets of broiler affect positively in the BW and the livability of the broilers at the growing period than this fed control diets.

The results showed significantly ( $P \leq 0.05$ ) higher body weight at 21 day of age for the group supplemented with Z3 than other groups and the same trend was recorded at 35 day of age. The best LBW ( $P \leq 0.01$ ) was recorded for Z3 group than control, Z2 and Z4 groups. Concerning daily weight gain, the groups supplemented with Z3 & Z4 and Z2 showed higher daily weight gain than control group (Table 2). The highest values were recorded for Z3 group followed by Z4 & Z2 groups which were equally similar at 7 – 21, 21 - 35 and at 7 - 35 day of age. The good FCR for chicken feed diet

supplemented with Z3 followed by Z4 and Z2 which were equally similar at 7 – 21, 21 - 35 and at 7 - 35 day of age. 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 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-SBM-based diets for broiler 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-SBM-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-nutrient 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 (cellulose, 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 broiler 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, improved 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.

**Table (2): Effect of feeding different levels of ZADO® on productive performance of growing chicks.**

Item	Control	Z1	Z2	Z3	Z4	Prob.
Live body weight (g) at:						
At 7 days	122.0±4.49	127.0±5.11	126.0±4.12	124.05±3.99	125.0±5.12	0.74
21days	658.37 <sup>c</sup> ± 10.13	690.35 <sup>b</sup> ±11.25	702.39 <sup>b</sup> ±13.22	765.58 <sup>a</sup> ±15.16	712.39 <sup>b</sup> ±12.92	0.032
35days	1614.96 <sup>c</sup> ± 15.3	1717.52 <sup>b</sup> ± 25.2	1823.44 <sup>ab</sup> ± 20.2	1925.72 <sup>a</sup> ± 30.3	1843.44 <sup>ab</sup> ± 22.2	0.006
Daily body weight gain (g) from:						
7-21 days	38.21 <sup>c</sup> ±1.09	40.21 <sup>b</sup> ±0.75	41.19 <sup>b</sup> ± 0.89	45.85 <sup>a</sup> ±0.95	41.95 <sup>b</sup> ± 0.99	0.04
21-35days	68.62 <sup>c</sup> ±1.99	73.75 <sup>b</sup> ± 2.02	79.87 <sup>b</sup> ± 2.99	82.81 <sup>a</sup> ±3.01	80.07 <sup>b</sup> ± 2.79	0.01
7-35 days	53.09 <sup>c</sup> ± 1.15	56.77 <sup>b</sup> ± 1.39	60.21 <sup>ab</sup> ± 1.29	64.27 <sup>a</sup> ± 2.58	61.08 <sup>ab</sup> ± 1.39	0.01
Daily feed consumption (g) from:						
7-21 days	58.92±2.53	59.83±3.46	59.93±2.78	61.93±2.98	59.93±2.68	0.58
21-35days	125.79±4.58	126.5±4.82	130.79±3.93	133.07±4.15	130.79±3.83	0.63
7-35 days	92.36±6.23	93.21±5.16	95.36±6.16	97.5±7.12	95.36±5.96	0.63
Feed conversion ratio (Feed/Gain) from:						
7-21 days	1.54 <sup>a</sup> ± 0.01	1.49 <sup>b</sup> ± 0.03	1.45 <sup>b</sup> ±0.04	1.35 <sup>c</sup> ± 0.03	1.42 <sup>b</sup> ±0.04	0.002
21-35days	1.84 <sup>a</sup> ± 0.05	1.72 <sup>b</sup> ± 0.06	1.64 <sup>b</sup> ± 0.02	1.61 <sup>c</sup> ± 0.01	1.63 <sup>b</sup> ± 0.01	0.020
7-35 days	1.74 <sup>a</sup> ±0.02	1.64 <sup>b</sup> ±0.05	1.58 <sup>b</sup> ±0.05	1.52 <sup>c</sup> ± 0.02	1.56 <sup>b</sup> ±0.01	0.008
Mortality %	5	4	3	2	3	-

<sup>a, b</sup> Means within the same row with different superscripts are significantly different.

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; 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 enzyme. Moreover, Onilude and Oso (1999a) reported that the supplementation of three enzyme mixture (amylase from *macrophominaphaseolina*, cellulase from fermentation of cassava root fiber by a *Trichoderma* sp. and pectinase from banana peel fermentation by *Fusariumtricitum* 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. 2237.7g), 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.* (2013) 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. However, in the second experiment they reported a reduction in feed intake in response to dietary Avizyme vs. un-supplemented group. 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.

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, hemicellulose 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, Barekainet *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.

#### **Digestive enzymatic activity:**

Digestive enzymes activities (amylase, protease, cellulase and xylanase) in different segments of gastrointestinal tract are presented in Table (3). As a result of stomach pH, among groups, low amylase activity but significant differences in samples taken from stomach. Although, high amylase activity was recorded in the small intestine contents. The activity of amylase enzyme was increased more than 5 folds in Z3 and Z4 compared with either Z2 & Z1 or control group. On the other hand, protease activity was significantly different among groups when estimated in stomach and in small intestine. In stomach, protease activity was more than 4 folds in Z3 and 2 folds comparing with Z1 and control groups. Whereas, the same trend was found for its activity in small intestine. These data may explain our results for the superiority of supplemented broilers with ZADO® in live body weights and blood profile. These findings may reflect a good feed utilization, absorption and metabolism for birds when diets supplemented with ZADO®.

#### **Blood parameters:**

Data presented in Table (4) clarify the effect of feeding broilers on diets with different levels of ZADO® on plasma protein profile. Significant increases have been recorded in total protein and globulin in Z3 & Z4 groups followed by Z2 and Z1. No significant differences in albumin were observed compared with control group. These findings in turn have influenced the A/G ratio as it declined from 0.523 in the control to the range of 0.220 to 0.294 in the other treatments. The reduction in A/G ratio may reflect an enhancement of broilers immunity.

**Table (3): Effect of different levels of ZADO® on enzymes activity of broiler**

Item	Control	Z 1	Z 2	Z3	Z4	Prob.
In stomach content:-						
Amylase	0.35 <sup>c</sup> ±0.199	0.75 <sup>c</sup> ±0.184	1.13 <sup>c</sup> ±0.172	9.54 <sup>a</sup> ±0.189	5.98 <sup>b</sup> ±0.153	0.017
Protease	10.22 <sup>c</sup> ±3.25	13.57 <sup>c</sup> ±5.75	26.33 <sup>b</sup> ±5.65	65.95 <sup>a</sup> ±4.89	27.50 <sup>b</sup> ±5.63	0.010
Cellulase	3.45 <sup>d</sup> ±3.11	9.23 <sup>c</sup> ±1.05	16.33 <sup>b</sup> ±1.99	27.93 <sup>a</sup> ±2.11	18.42 <sup>b</sup> ±2.45	0.008
Xylanase	6.73 <sup>d</sup> ±2.56	13.59 <sup>c</sup> ±3.29	24.76 <sup>b</sup> ±1.85	35.19 <sup>a</sup> ±3.15	26.59 <sup>b</sup> ±2.56	0.020
In small intestine content:-						
Amylase	35.84 <sup>d</sup> ±5.17	43.18 <sup>c</sup> ±8.25	112.92 <sup>b</sup> ±15.02	162.86 <sup>a</sup> ±6.12	114.80 <sup>b</sup> ±4.16	0.012
Protease	7.89 <sup>c</sup> ±2.45	10.27 <sup>b</sup> ±3.15	16.25 <sup>b</sup> ±4.23	28.75 <sup>a</sup> ±2.01	18.25 <sup>b</sup> ±3.76	0.009
Cellulase	24.27 <sup>f</sup> ±3.99	32.51 <sup>d</sup> ±3.31	119.27 <sup>c</sup> ±9.75	168.02 <sup>b</sup> ±11.36	227.11 <sup>a</sup> ±13.25	0.011
Xylanase	24.04 <sup>d</sup> ±7.35	56.43 <sup>c</sup> ±6.29	105.26 <sup>b</sup> ±6.10	165.49 <sup>a</sup> ±5.17	116.85 <sup>b</sup> ±5.12	0.019

<sup>a, b, c</sup> Means within the same row with different letters are significantly differ.

**Table (4): Effect of different levels of ZADO® on blood parameters of broiler.**

Item	Control	Z 1	Z2	Z3	Z4	Prob.
Total Protein g/dl	3.686 <sup>c</sup> ±0.15	4.824 <sup>b</sup> ±0.11	5.029 <sup>ab</sup> ±0.07	5.572 <sup>a</sup> ±0.12	5.386 <sup>a</sup> ±0.09	0.04
Albumin g/dl	1.266±0.08	1.097±0.10	1.057±0.19	1.005±0.08	1.021±0.15	0.07
Globulin g/dl	2.420 <sup>c</sup> ±0.18	3.727 <sup>b</sup> ±0.20	3.972 <sup>b</sup> ±0.16	4.567 <sup>a</sup> ±0.21	4.365 <sup>a</sup> ±0.19	0.05
A/G ratio	0.523	0.294	0.266	0.220	0.234	
Total lipids mg/dl	454.27±22.5	407.02±24.2	507.02±29.4	455.41±21.3	384.06±26.2	0.08
cholesterol mg/dl	117.085±23	93.60±20	92.43±22	100.70±21	112.43±24	0.09
AST IU/L	30.20±3.12	30.20±1.10	25.00±2.29	23.40±1.19	24.20±2.74	0.75
ALT U/L	44.20±2.98	43.83±3.11	43.34±2.22	42.86±1.67	43.18±2.67	0.68
Ca (mg/dl)	9.86±0.16	10.21±0.76	10.19±0.27	10.15±0.33	10.09±0.18	0.68
P (mg/dl)	5.86±0.51	6.23±0.17	6.19±0.15	6.05±0.15	6.17±0.24	0.73
creatinine (mg/dl)	0.793±0.05	0.789±0.03	0.783±0.04	0.723±0.05	0.746±0.02	0.59
T3 ng/ml	1.70 <sup>d</sup> ±0.16	2.18 <sup>c</sup> ±0.07	2.46 <sup>b</sup> ±0.09	2.94 <sup>a</sup> ±0.04	2.53 <sup>b</sup> ±0.08	0.04
T4 ng/ml	13.73±0.28	13.37±0.48	13.11±0.46	13.43±0.21	14.12±0.36	0.07

<sup>a, b, c</sup> Means within the same row with different letters are significantly differ.

The values of plasma constituents in broilers at 35 days of age (Table 4) were within the normal ranges for plasma total cholesterol, total protein and albumin.

Regarding to liver function expressed as plasma AST and ALT enzymes, data recorded in Table (4) clearly indicate non-significant variations between control and other treatments. The histological sections on liver confirmed these findings. The same trend was recorded for creatinine levels, as an indicator for kidney function, where no effects of ZADO® were recorded. Plasma liver 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.

Concerning calcium and phosphorus levels, it was noticed that there were no significant differences among all groups. On the other hand, concerning thyroid hormones, our results in Table (4) indicate that plasma thyroid hormones concentrations were seems to be significantly differed among treatments. The highest values were recorded for chicken given ZADO® 0.5 Kg/ton. No effect of treatment on T4. T3 levels raised in treated groups and free T4 levels declined compared with control group. These findings may reflect a good feed utilization, absorption and metabolism for birds when diets supplemented with ZADO®.

The current experiment showed a favour effect of enzyme addition to broiler diets up to 35 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 fibre 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.

**Carcass traits:**

The main results of carcass traits are set out in Table (5). Carcass, breast and thigh weights (as a percentage of carcass) were significantly greater ( $P \leq 0.01$ ) in birds fed supplement of ZADO® than the control group. No effect of treatment on % gizzard, liver, heart, spleen, bursa, abdominal fat and drum.

**Table (5): Effect of different levels of ZADO® on carcass traits.**

Item	control	Z 1	Z 2	Z3	Z4	Prob.
carcass%	61.13 <sup>b</sup> ±1.81	65.92 <sup>ab</sup> ±1.71	68.66 <sup>a</sup> ±3.21	69.84 <sup>a</sup> ±4.32	69.46 <sup>a</sup> ±1.45	0.009
gizzard%	2.02±0.33	2.49±0.51	2.24±0.26	2.49±0.42	1.91±0.35	0.076
liver%	2.68±0.44	3.22±0.25	2.75±0.34	2.69±0.31	2.68±0.28	0.081
heart%	0.42±0.04	0.48±0.05	0.51±0.04	0.54±0.05	0.42±0.03	0.059
spleen%	0.13±0.03	0.19±0.05	0.51±0.02	0.54±0.03	0.42±0.04	0.067
bursa%	0.12±0.06	0.15±0.05	0.19±0.06	0.13±0.06	0.12±0.03	0.064
abdominal fat%	1.49±0.59	0.80±0.57	0.74±0.85	0.71±0.77	1.15±0.63	0.052
breast%	22.11 <sup>c</sup> ±0.19	25.90 <sup>b</sup> ±0.36	25.19 <sup>b</sup> ±0.41	27.86 <sup>a</sup> ±0.19	25.17 <sup>b</sup> ±0.39	0.038
thigh%	4.43 <sup>d</sup> ±0.16	4.90 <sup>c</sup> ±0.14	5.41 <sup>b</sup> ±0.19	5.93 <sup>a</sup> ±0.16	5.41 <sup>b</sup> ±0.11	0.045
drum%	4.916±0.92	5.201±0.92	5.16±1.00	5.84±0.94	5.19±0.85	0.076
Intestine %	8.02±0.95	7.86±0.89	7.94±0.92	7.12±0.99	6.85±0.75	0.064

<sup>a, b, c</sup> Means within the same row with different letters are significantly differ.

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, Barekain *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 0.5Kg ZADO® supplementation to corn-based diets slightly increased (but not significantly) 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.

### ***Economic Efficiency***

The profitability of using peanut hay in rabbit feeds depends mainly on both total feed cost and growth performance as presented in Table 7. The highest values of Economic Efficiency, performance index (PI) and Production Efficiency Factor (PEF) were recorded for chicken given ZADO® 0.5 Kg/ton. Chickens fed ZADO® 0.5 Kg/ton have better livability percentage.

**Table (7): Effect of different levels of ZADO® on Economic Efficiency.**

Parameters	Control	Z1	Z2	Z3	Z4
Price Kg feed(LE)	2.29	2.31	2.35	2.39	2.43
Total feed cost(LE)	7.38	7.52	7.84	8.14	8.10
Average Weight Gain(Kg/Chick)	1.575	1.685	1.785	1.895	1.805
Total Return (LE)	18.9	20.22	21.42	22.74	21.66
Net Return (LE)	11.52	12.70	13.58	14.60	13.56
Economic Efficiency (%)	155.97	168.97	173.37	179.49	167.31
PI	80.25	90.75	99.17	109.23	101.37
PEF	217.82	248.91	274.85	305.86	280.93
livability %	95	96	97	98	97

### ***Histological results:***

Histological examinations of the intestinal sections are illustrated in Figure (1). It is clear from the transverse sections (T.S) that the villi height increased in treated groups compared with the control one.

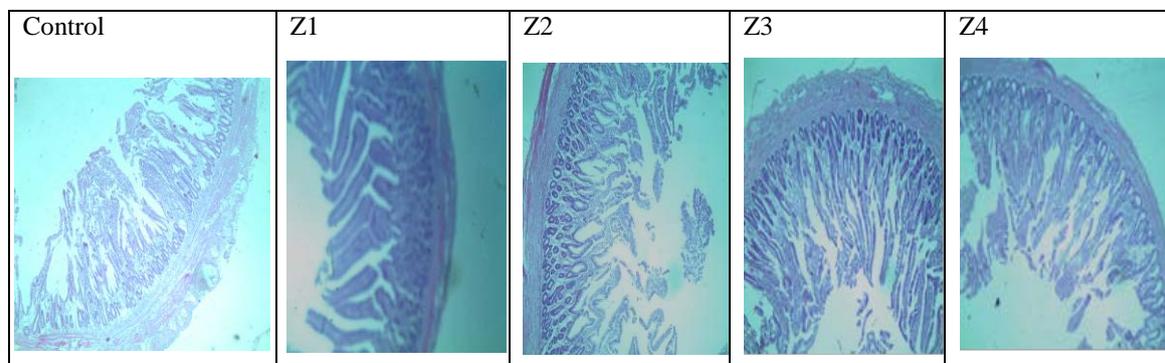
The observations from the histological sections for small intestine in broiler group fed with ZADO® 0.5% showed developmental increase in intestinal villi length and number more than other groups fed with ZADO® 0.3 and 0.7 Kg when compared with sections taken from ZADO® 0.1 group and control group fed with basal diet.

The observations from the liver histological sections in broiler group fed with ZADO® concentrations showed normal distribution for cells and ducts, absence of any lesions when compared with control group fed with basal diet without ZADO® supplementation.

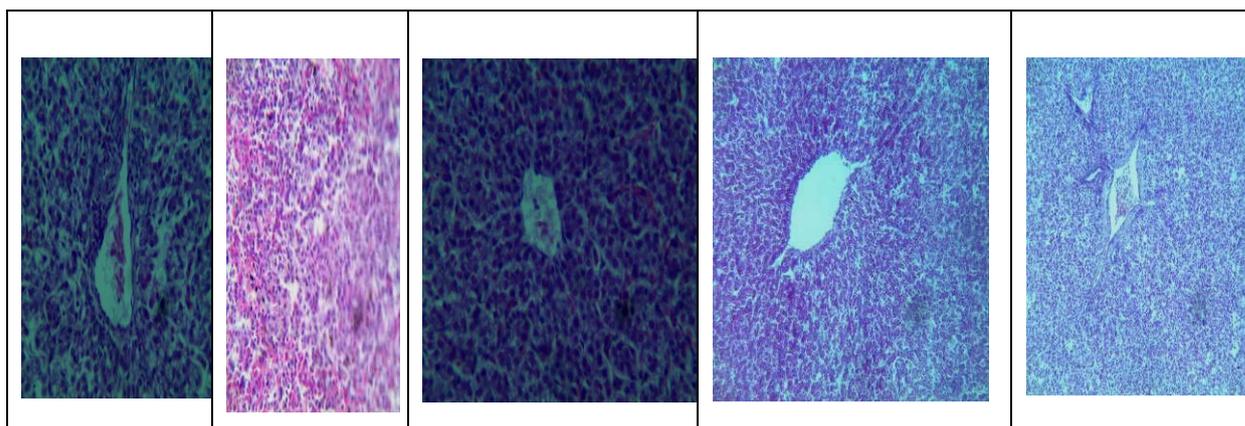
However, this increase was more obvious in groups that were fed basal diets supplemented with ZADO® groups. Furthermore, there were great variations in the size and number of Crypts of Lieberkuhn associated with the supplemental additives. These Crypts are known to secrete fluids containing different vital substances essential for the internal micro-environment of the small intestine segments (Hodges, 1974). While the sections from the liver parenchyma of the control treatment Fig. (2) has normal hepatocytic structure with dilated central vein engorged with blood. Also, there were dark stained eosinophilic cells surrounding or near the central veins. There is moderate hypertrophy of liver cells

especially in (Z4 group) which may reveal hyperactivity of the liver cells or a compensatory effect due to more degenerative (necrotic) areas in these sections. The above mentioned changes in liver sections may be related to the higher metabolic activity associated with the higher growth rates of broilers which depends on their genetic background.

The histological observations in the present study may explain our results concerning the enhanced live body weight and immune responses of treated broiler groups.



**Fig. (1): The histological structure (at 40X) of the small intestine from broilers fed different biological additives and a control group.**



**Fig. (2): The histological structure (at 40X) of the liver from broilers fed different biological additives and a control group.**

## CONCLUSIONS

It could be concluded that broiler diets supplement with 0.5Kg of ZADO® improved broiler productivity from hatch to 35 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 0.5Kg ZADO® has a favor effect on enzymes activity by the increase in stomach and intestine enzymes in ZADO® groups are good explanations for the improvements in the FCR. The histological and blood pictures showed that ZADO® products are very safe on birds and accordingly birds are safe for human consumption. 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. Good of Economic Efficiency for ZADO® 0.5 Kg/ton. Therefore, it could be recommended from this study to supplement 0.5Kg of ZADO® to broiler diets from hatch to 35 days of age.

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## الاداء الانتاجي ، النشاط الانزيمي و القطاعات الهستولوجية لدجاج التسمين المغذي علي معقد الانزيمات المختلفة (زادو)

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تهدف هذه الدراسة الي تقييم الاداء الانتاجي و النشاط الانزيمي و بعض مكونات الدم و القطاعات الهستولوجية لدجاج التسمين المغذي علي معقد الانزيمات (زادو). تم تقسيم عدد ٢٢٥ كتكوت عمر يوم هابرذ الي ٥ مجموعات متساوية العدد من ٤٥ كتكوت و كل مجموعة بها ٥ مكررات من ٩ كتاكيت. المجموعة الاولى كانت كتنترول بدون اضافة المعقد الانزيمي ، بينما كانت المجموعات الاخرى تمت تغذيتها علي نفس العليقة مضاف اليها المعقد الانزيمي (الزادو) 0.1 و 0.3 و 0.5 و 0.7 كجم لكل طن من العليقة. هذا المنتج يحتوي علي خليط من البكتريا الهوائية بانزيماتها من الزايلانيز (٢,٣ وحدة بالجـم) و سيلبوليز (٧,١ وحدة بالجـم) و الفا أميليز (٦١,٥ وحدة بالجـم) و بروتينيز (٢٩,٢ وحدة بالجـم). وضحت نتائجنا بهذا البحث ان استخدام هذا المعقد الانزيمي بتركيز ٠,٥ كجم/طن أثر ايجابيا علي وزن الجسم للدجاج في نهاية التجربة. كان التحسن واضح للنشاط الانزيمي بالمعدة و الامعاء للطيور المغذاه علي المعقد الانزيمي بالعليقة ايا كان تركيزه مما فسر نتائجنا علي تحسن التحويل الغذائي للطيور. بينما كانت نتائجنا علي مكونات الدم المختلفة و كذلك مشاهداتنا علي القطاعات الهستولوجية للكبد و الامعاء لم تظهر اختلافات معنوية بين المجاميع المختلفة و بين المجموعه الكتنترول. هذه النتائج يمكن ان تبين أن هذا المنتج يبدو آمنا لتغذية الطيور و بالتالي آمنا لاستهلاك الانسان لهذه الطيور. و في النهايه كان استخدام تركيز ٠,٥ كجم/طن عليقة لهذا المنتج هو الافضل اقتصاديا لتغذية دجاج التسمين بهذه الدراسة.