EFFECT OF DIFFERENT LEVELS OF OPTIZYME AND PHYTASE ENZYMES AND THEIR INTERACTIONS ON THE PERFORMANCE OF BROILER CHICKENS FED CORN/SOYBEAN MEAL: 3. EUROPEAN PRODUCTION EFFICIENCY FACTOR, EUROPEAN BROILER INDEX AND SOME IMMUNE ORGANS

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SUMMARRY

A total of 180 unsexed 1-day old IR broiler chicks were randomly distributed into 6 treatments of 3 replicates each (10 birds each) in experiment for 5 weeks of age. A factorial design (3×2) was used in which there were three levels of multienzymes, optizyme (0, 250, 500 mg/kg diet) and two levels of phytase enzyme (0, 1500 FTU/kg diet). No significant effects due to treatments on both European production efficiency factor (EPEF) and European broiler index (EBI). Bursa percentage was significantly higher in birds fed 250 mg/kg optizyme plus 1500 FTU/kg phytase. Immune organs, such as spleen and bursa of Fabricius were significantly increased with both multienzyme, optizyme plus phytase supplementation. It could be concluded that multienzyme optizyme supplementation at the level of 250 or 500 mg/kg plus 1500 FTU/kg phytase improved significantly ($P<0.05$) immune status of broiler chicks.

Keywords: *Enzymes, European production, broiler index, immune organs and broilers.*

INTRODUCTION

Poultry industry is becoming increasingly receptive to the use of exogenous enzymes supplementation. Enzyme supplementation to the poultry rations has a positive effect on feeds digestibility and leads to better productivity and performance.

The cost of poultry feed ingredients represents about 60 –70% of the total production cost, and hence, feed formulation is a critical approach in poultry industry. Feed utilization can be met with inclusion of enzymes, antimicrobials, probiotics, or prebiotic or natural products (Al-Khalaifah, 2018).

Moreover, supplementation of commercial enzymes can increase the nutritive value of feed ingredients and diets as well as allow greater flexibility in diet formulation. It has also a potential effect on mitigation of the environmental pollution by reducing the excretion of some elements such as nitrogen and phosphorus in poultry manure. Broilers that received multienzymes through drinking water recorded the highest weekly weight gain when compared to those given enzymes through the feed, and both groups had higher growth than unsupplemented controls (Gupta *et al.*, 2014)

Liu *et al*. (2017) investigated the effect of multienzymes containing phytase, protease, and xylanase at 1,000, 2,000, and 2,000 U/kg of broiler feed, respectively. The authors showed that multienzymes significantly improved polymeric Ig receptor (pIgR), secretary IgA (sIgA), and ileal counts of Lactobacilli and Bifidobacteria and significantly reduced lesions in the intestine, serum a-toxin

antibodies, mucin 2 expression, and ileal count of Clostridium perfringens. However, the strength of the multienzyme effect depends on the protein content in the diet. The authors pointed that high non-conventional protein in the diet can lead to increased occurrence of subclinical necrotic enteritis, while multienzyme supplementation can reduce this effect in broiler chickens by enhancing the gut immunity.

Therefore, the objective of the present study was to investigate European production efficiency factor, European Broiler Index and some immune organs.

MATERIALS AND METHODS

The present study was carried out at the Poultry Research Farm, Poultry Production Dept., Faculty of Agriculture, South Valley University, Qena, Egypt.

Management and experimental design:

A total of 180 (IR) broiler chicks (one day old) were randomly divided into 6 treatments. Each treatment was divided into 3 replicates of 10 each. The birds were reared at 34°C temperature as standard brooding temperature and then, gradually reduced to reach 24°C at the end of the experiment. A light schedule used was 23 h of light during the entire period of the experiment, and the level of relative humidity ranged from 55 to 60%. The enzyme was supplemented in addition to the diet and was not included in the nutrient matrix. Birds were fed on starting commercial diet (Table 1) containing (23% crude protein, ME, 3000 Kcal. /Kg) from one day old of age and growing commercial diet containing (21% crude protein, ME, 3000 Kcal. /Kg) from 3 to 5 weeks of age(marketing), diets were formulated according to the Nutrient Recommendations for poultry (NRC, 1994).

Table (1): Feed ingredients and chemical analyses of basal diets.

Item	0 – 2 weeks (Starter)	3 – 5weeks (Grower)
Ingredient, %:		
Corn (grains)	54.00	59.20
Soybean Meal (44%)	32.85	28.00
Corn Gluten Meal (62%)	6.50	6.00
Soybean Oil	2.70	2.50
Di-Calcium Phosphate	1.46	1.52
Limestone	1.51	1.80
Premix	0.30	0.30
Salt (NaCl)	0.30	0.30
DL-Methionine	0.28	0.28
L-Lysine HCL	0.10	0.10
Total	100	100
Chemical analysis (Calculated):		
Crude Protein %	23.18	21.20
ME Kcal/ Kg diet	3009	3040
Calcium %	1.10	0.93
Available Phosphorus %	0.42	0.42
Lysine %	1.19	1.07
Methionine & Cysteine %	1.06	1.01

Each 3 Kg of premix contains: Vitamins: A: 12000000 IU; D3 2000000 IU; E: 10000 mg; K3: 2000 mg; B1:1000 mg; B2: 5000 mg; B6:1500 mg; B12: 10 mg; Biotin: 50 mg; Choline chloride: 250000 mg; Pantothenic acid: 10000 mg; Nicotinic acid: 30000 mg; Folic acid: 1000 mg; Minerals: Mn: 60000 mg; Zn: 50000 mg; Fe: 30000 mg; Cu: 10000 mg; I: 1000 mg; Se: 100 mg and Co: 100 mg.

Experimental design:

The chickens were fed three levels of Optizyme enzyme (0, 250 and 500 mg/kg diet and two levels of Phytase enzyme supplementation (0 and 1500 FTU/kg diet). One FTU of Phytase enzyme activity (FTU) is defined as the activity of 0.030 µg of Phytase. Optizyme is a commercial multienzyme consists of multienzymes product containing proteases, amyloglucosidase, xylanase, β-glucanase, cellulases and hemicellulases, (Product of Optivite International LTD). One unit (FTU) is equal to the enzyme activity that liberates 1 µmol or tho-phosphate from 5.1 mmol of sodium phytin per minute at 37º C and pH 5.5. (Marketed by BASF, Germany). The experimental treatments were as follows: T1 (0 mg/kg diet optizyme and 0 FTU/kg diet phytase); T2 (0 mg/kg diet optizyme and 1500 FTU/kg diet phytase); T3 (250 mg/kg diet optizyme and 0 FTU/kg diet phytase); T4 (250 mg/kg diet optizyme and 1500 FTU/kg diet phytase); T5 (500 mg/kg diet optizyme and 0 FTU/kg diet phytase); T6 (500 mg/kg diet optizyme and 1500 FTU/kg diet phytase).

Broilers in each replicate were weighed (g) as a group replicate and feed consumption was also weighed weekly till 5 wks of age. Body weight gain (BWG) (g/chick) and feed conversion (FCR, g feed/g gain) were calculated.

After calculation of viability percentage and FCR, the European Production Efficiency Factor (EPEF) and European Broiler Index (EBI) were used to evaluate the growing performance of broilers as suggested by Marcu et al. (2013**)** as follows:

EPEF= Viability (%) x BW (kg)*100/Age (d) x FCR (kg feed/kg gain)

EBI = Viability (%) x ADG (g/chick/day) *100 /FCR (kg feed/kg gain) x 10

Where: ADG (g/chick/d) = TWG (total weight gain)/ days of growth period.

At 35 days of age, a random sample of 3 growing birds from each replicate were slaughtered after 8 h fasting according to the Islamic method using a sharp knife and cutting into the jugular vein, carotid artery and windpipe, processed. Some organs (liver and immune organs as spleen, and bursa of fabricius) were removed and weighed.

Statistical analysis:

The data were statistically analyzed by factorial design (3 x 2), three levels of optizymes and two levels of phytase enzymes using ANOVA and General Linear Models (GLM) Procedure of SAS software, Version 9 2 (SAS (2009) procedure. Duncan'smultiple range tests (Duncan 1955) was used to determine differences among means when treatment effects were significant. Significant differences were considered to exist when ($P<0.05$). The mathematical model was as follows:

$Yijk= \mu+ Oi+ Pj+ (OV)ij+ Eijk$

Where:

Yijk: any observation; µ= the population mean.

Oi: Optizyme levels effect (i = 1, 2 and 3), Pj: Phytase levels effect (j = 1 and 2), (OP)ij: Interaction of Optizyme levels× Phytase levels.

Eijk: Experimental error.

RESULTS AND DISCUSSION

European production efficiency factor (EPEF) and European broiler index (EBI):

Date presented in (Table 2), showed that European Production efficiency Factor (EPEF) and European Broiler Index (EPI) did not significant ($P<0.05$) affected by either Optizyme or Phytase enzyme levels or by their interactions. The obtained results are disagreement with Attia *et al*. (2020) who showed that enzyme application increased EPI compared to the unsupplemented control. El Harthi *et al.* (2020) found that Phytase supplementation significantly improved production index.

It could be concluded that multienzyme supplementation at the level of 250 or 500 mg/kg optizyme plus 1500 FTU/kg phytase improved immune status in the form of immune organ relative to body weight of broiler chicks.

Table (2): Effect of treatments on European Production Efficiency Factor and European Broiler Index of broiler chickens.

Treatment	Production index	
	EPEF	EBI
Optizyme levels (mg/kg)		
0 (O)	297.07±15.53	289.60±15.36
250 (O1)	296.00±13.60	288.70±13.46
500 (O2)	299.68±9.49	292.29±9.39
Phytase levels (FTU/kg)		
0 (P)	299.17±8.98	291.76±8.90
1500 (P1)	295.99±11.76	288.63±11.62
Interactions		
O×P	279.73±14.88	272.46±14.87
O×P1	314.40±26.53	306.73±26.22
O1×P	307.67±19.09	300.20±18.94
O1×P1	284.32±20.21	277.19±19.97
O2×P	310.10±10.54	302.61±10.40
O2×P1	289.25±15.39	281.96±15.23

Means in the same columns with different superscript are significant different (P≤0.05).
EPEF= European Production efficiency Factor. EBI= European Broiler Index.

Liver and some immune organs (spleen and bursa of fabricius):

Data presented in (Table 3), showed that liver, some immune organs (spleen and bursa of fabricius relative to BW) had no significant (P<0.05) affected by either Optizyme, or Phytase enzyme alone. Concerning of liver weight and percentage, the obtained results are in agreed with Salem *et al.* (2008) who reported that dietary multienzyme avizyme supplementation had no significant effect on most of carcass traits studied liver relative weights. Ismail *et al*. (2006) found that avizyme had not significantly affected internal organs percentage (liver and heart) of broilers. Mohamed et al. (2005) found that adding avizyme to broiler diets did not affect liver, weight and relative to weights. Qota *et al*. (2002) found that phytase addition at 500 U/kg to broiler diets contained 10% linseed cake did not significantly affect liver percentage. Attia *et al*. (2001) found that phytase supplementation at 700 U/Kg diet did not affect body organs (liver eight and percentage) of broiler chicks fed low protein low-energy diet. El-Kelawy *et al*. (2012) found that the use enzymes supplementation (Avizyme) had no significant differences was shown in spleen and bursa absolute and relative weight.On the other hand, the obtained results are in disagreement with Viveros *et al*. (2002) who found that phytase reduce liver weight in broiler chickens.

There was significant (P<0.05) interaction effect between Optizyme and Phytase enzyme levels on spleen weight, bursa weight and bursa percentage relative to BW. Birds in the groups (O1×P1) and (O2×P) had significant (P<0.05) the highest spleen weight (P≤0.05) than other groups. However, birds in the group (O1×P1) had significant (P<0.05) the highest bursa weight and bursa percentage than other groups. The obtained results are consistent with Saleh et al., (2019) who found that dietary xylanase (Xyl) and arabinofuranosidase (Abf) supplementation had beneficial impacts on immune response in broiler chickens fed low-energy diets. Saleh *et al*. (2018) mentioned that the exogenous multienzyme complexes may be included in the low-energy diet to enhance the performance of broiler chickens (Avi- zyme® >Hemicell® >Megazyme®), improving the immunity of broiler chickens.(Seidavi *et al*. (2017) reported that broilers treated with Probio enzyme (mixture of probiotic cultures and enzymes) showed a satisfactory immune response compared with control. Improved immunity against New Castle virus (Ghosh *et al*., 2016)

Sadeghi *et al*. (2015) reported that the dietary inclusion of a probiotic-enzyme mixture had no significant effect on the immune parameters of chickens but improving the immune response in birds when challenged with a pathogen. Exogenous enzymes can enhance broiler productivity and likely immunity too, that may represent an alternative to the use of sub-therapeutic doses of antibiotics (Talebi *et al*., 2008).

No significant effects due to interactions between optizyme and phytase on liver weight and liver and spleen percentages.

Table (3): Effect of treatments on liver, spleen and bursa of fabricius weight and percentages of broiler chickens.

Treatments	Liver		Spleen		Bursa	
	Wt.	%	Wt.	%	Wt.	%
Optizyme levels (mg/kg)						
0 (O)	44.3±1	2.15±0.08	3.23±0.49	0.15±0.02	4.20±0.45	0.20±0.02
250 (O1)	46.4±2	2.20±0.07	3.58±0.30	0.17±0.01	4.95±0.51	0.23±0.02
500 (O2)	48.4±1	2.26±0.07	3.46±0.37	0.16±0.01	4.36±0.42	0.20±0.01
Phytase levels (FTU/kg)						
0 (P)	45.1±1	2.17±0.05	3.25±0.32	0.15±0.01	4.64±0.34	0.22±0.01
1500 (P1)	45.1±1	2.17±0.05	3.25±0.32	0.15±0.01	4.64±0.34	0.22±0.01
Interactions						
O×P	42.0±2	2.09±0.06	2.56±0.54^{b}	0.12±0.02	4.63±0.87^{ab}	0.23±0.04^{ab}
O×P1	46.5±2	2.20±0.16	3.90±0.70^{ab}	0.18±0.03	3.76±0.28^{ab}	0.17±0.01^{ab}
O1×P	45.5±3	2.20±0.10	3.00±0.28^{ab}	0.14±0.01	4.20±0.51^{ab}	0.20±0.01^{ab}
O1×P1	47.3±3	2.21±0.13	4.16±0.18^{a}	0.19±0.00	5.70±0.72^{a}	0.26±0.02^{a}
O2×P	47.7±1	2.22±0.11	4.20±0.37^{a}	0.19±0.02	5.10±0.40^{ab}	0.26±0.01^{ab}
O2×P1	49.1±2	2.30±0.12	3.73±0.12^{b}	0.12±0.00	3.63±0.43^{b}	0.17±0.01^{b}

$^{a-c}$*Means in the same columns with different superscript are significant different (P≤0.05).*

This work was executed to study the effect of supplementing multienzymes, optizyme and phytase levels on broiler production index and some immune organs of broilers chickens during 1–35 days of age .

The enhanced in BWG as a result of multienzyme supplementation as mentioned in articles 1&2 may be due to the increased nutrient availability and absorption as a result of increased digestibility of the ingested diets, as suggested by Choct (2006), Attia *et al*. (2012), and El-Kelawy.(2012)

Adding exogenous enzymes that hydrolyze the NSP of vegetable ingredients in the diets for monogastric enhances the energy availability and use of nutrients, and thus enhances feed conversion ratio (Shirmohammad and Mehr, 2011). The increase in the release of nutrient due to enzyme supplementation as phytase resulted in higher nutrient available for absorption, as demonstrated by the increase in intestinal villi length, and thus for biochemical reaction in favor of anabolic reaction and muscle buildup (Attia *et al*., 2012) and for immune function as well (Yang *et al*., 2010 and Attia *et al*., 2017).

The numerically improved EPPI due to enzymes supplementation was concurred with greater villi length for the group on 250 or 500 mg/kg optizymes multienzyme plus 1500 FTU/; g phytase supplementation. However, the effect of multienzyme depends on diet composition and the type of enzyme (Zanella *et al*., 1999). The addition of exogenous enzymes that hydrolyze the NSP of vegetable ingredients in the feeds for monogastric enhances the availability of energy and use of nutrients and thus enhances FCR (Shirmohammad and Mehr, 2011).

The use of multienzymes optizymes and phytase enhanced immune organs. This enhancement in immunity could be attributed to redistribution of nutrients toward immunity, the availability of nutrients increased for physiological response, immunity, and antioxidant utilization for eliminating free radical resulting from both non-enzymatic and enzymatic reactions due to decreasing nutrient demands for growth. In addition, phytase enzyme, which can improve nutrient availability by chickens, such as protein, energy, and minerals (Choct *et al*., 2006). The absolute and relative weight of the spleen was significantly different compared to the control group. The weights of the bursa of Fabricius were not different (Seidavi et al., 2017).

In addition, Khaksar *et al*. (2012) found that the relative weight of immune organs (thymus, spleen, and the bursa of Fabricius) was not influenced by enzyme supplementation.

These results suggest that it is possible to dilute nutrient profiles of broiler diets during the growing and finishing phases without negative effect on EPEI and FCR while improving immune response as in immune organs relative to body weight. These results agree with those reported by Abudabos (2012). The present results indicate that enzymes supplementations improved the immunity of broiler chicks as measured by organ changes. This was concurred with an increasing diameter of large follicle of the bursa of Fabricius for the group supplemented with 250 or 500 mg/kg multienzymes. The increase in the follicular diameter of the bursa of Fabricius indicates an increase in the number of B-lymphoblasts that leads to the formulation of B-lymphocytes that internally form antibodies.

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تأثير مستويات مختلفة من إنزيمات الأوبتزيم والفيتيز والتداخل بينهما على معدل آداء دجاج اللحم المغذى على عليقة الذرة/كسب فول الصويا :3 . معامل كفاءة الإنتاج الأوروبي ، مؤشر دجاج اللحم الأوروبي وبعض الأعضاء المناعية

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تم إستخدام 180 كتكوت تسمين IR عمر يوم وتم توزيعها بشكل عشوائي في 6 معاملات كل منها 3 مكررات (10 طيور فى كل منها) في تجربة مدتها 5 أسابيع. تم إستخدام تصميم عاملي (3 × 2) حيث كان هناك ثلاثة مستويات من الإنزيمات المتعددة، الأوبتزيم (صفر و250 و 500 ملليجرام/كجم) ومستويان من إنزيم الفيتيز (صفر و 1500 وحدة دولية/ كجم) .اوضحت النتائج انه لا يوجد تأثير معنوى للمعاملات على كل من معامل كفاءة الإنتاج الأوروبى (EPEF) ، ومؤشر دجاج اللحم الأوروبى (EBI) . تأثرت نسبة غدة فبريشى أعلى بشكل ملحوظ في الطيور التي تمت تغذيتها على 250 ملليجرام/كجم من الأوبتزيم بالإضافة إلى 1500 وحدة دولية من الفيتيز. الاعضاء المناعية كالطحال وغدة البرسا ازدات معنوبا فى مجموعات الطيور المغذاه على كل من مخلوط الانزيمات والفيتيز.

استنتج من التجربة ان مخلوط الانزيمات الاوبتزايم عند مستوى 250 أو 500 ملليجرام/كجم بالإضافة إلى انزيم الفيتيز بمستوى 1500 وحدة دولية/ كجم أدى الى تحسن معنوى على الحالة المناعية لكتاكيت التسمين.