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Original Research Article

Effects of a Multi-Enzyme on Humeral Immune Response against NDV and Performance of Broiler Chickens

Yasser Asaad Hameed Al Shareef[1*], Firas Hussain Kadim Abawi[1]
[1]Pathology and Poultry Disease Department, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq

***Corresponding Author:** Yasser Asaad Hameed Al Shareef
Pathology and Poultry Disease Department, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq

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Abstract: Non-starch polysaccharides (NSPs) are not fully digested by broiler's endogenous enzymes and consequently the soluble NSPs in feed results in high digesta viscosity and poor retention of nutrients. Supplementation of NSPs digesting enzymes may release the nutrients from feed and reduce the anti-nutritional effects of NSPs. This study was executed to investigate the effect of NSPs digesting enzymes (Endo-Power) multi enzyme supplementation levels (500g/ton of feed) with standard diet (SD) on immune response, blood biochemistry and growth performance of broilers chickens during 1–35 days of age. A total of 200 unsexed 1-day-old Ross308 broiler chicks were randomly distributed, to four treatments. There were fifty chicks per treatments. Results showed that diet with multi enzyme supplementation significantly increased the humoral immune response against NDV of broilers. Addition of multienzymes at levels (500g/ton of feed) significantly increased body weight gain BWG and improved Feed consumption ratio FCR, compared to the control treatments. Alanine aminotransferase (ALT) and aspirate aminotransferase (AST) were significantly higher for treatments without multi enzyme than the treatments with multi enzyme. Supplementation of multienzymes significantly decreased ALT and AST compared to the control group.

Keywords: Multi-enzyme, broiler chickens, humoral immune response, liver enzymes, poultry feed, NDV, FCR.

INTRODUCTION

Poultry diets that combine corn and soybean meal are considered beneficial due to their high nutritional value, but soybean meal contains oligosaccharides that have been shown to affect bird health and growth. (Iji and Tivey, 1998), and the cell wall of maize contains arabinoxylan. Glucan and cellulose are present. There is strong evidence that some nutrients in corn are not completely digested in the small intestine, and that significant amounts of starch and protein escape digestion and enter the midgut where they undergo fermentation with relatively low energy yields (Noy and Sklan, 1995). Potential factors that reduce nutrient bioavailability include non-starch polysaccharides (NSPs). Most studies have observed significant increases in NSP digestibility when enzymes or enzyme mixtures are added to corn and soybean feed, allowing enzymatic degradation of cell wall polysaccharides despite the complex nature of these polymers. It suggests that (Meng *et al*., 2005). Enzymes have been added to broiler feeds for over 30 years. Supplementing corn-soybean meal diets with enzymes or enzyme mixtures with a broad range of activities improves digestibility and therefore growth performance (Odetallah *et al*., 2002). Several studies have shown positive effects on apparent metabolizable energy (AME) and NSP digestibility of soybean diets, depending on the enzyme preparation used (Zhou *et al*., 2009; Kocher *et al*., 2002; Fuente *et al*., 1995). Hesselman and Aman (1986) suggested that Å-glucanases disrupt cell walls and release nutrients from cell contents during digestion. Recently, in vitro laboratory studies (Saleh *et al*., 2003a, b) have shown that excluding proteases from the enzyme mixture increases digestibility. Zhou *et al*., (2009) reported that supplementing a corn and soybean starter diet for broilers with an enzyme supplement containing a mixture of xylanase, protease, and amylase improved the AME value of the feed in the starter, grower, and finisher stages. did. (Endopower) is a multienzyme with β-glucanase, protease, and cellulose activities. Therefore, this study was conducted to investigate whether (Endopower) can affect his AME values and improve poultry performance.

MATERIALS AND METHODS

Two hundred one day old broiler chicks from Asaad hatchery/ Babel province the eggs were taken from breeder flocks (Ross308), and divided randomly to equally four groups and treated as follow:

Group A: without vaccine and without enzyme.
Group B: With enzyme with vaccine at 1,10,20 days.
Group C: Without enzyme with vaccine at 1,10,20 days.
Group D: With enzyme without vaccine.

Vaccination program

vaccine: 1day old
fluH9N2+ND (lasota) (cavac) vaccine was given at one day old by Subcutaneous injection method.
Innovax ND+IBD (hvp360) (MSD) vaccine was given at one day old by Subcutaneous injection method.
IB4-91, NDclon30+IBma5 (MSD) vaccine was given at one day old by intranasal and ocular drop method.

vaccine: 10 days old
NDclon (IZO) vaccine was given at ten days old by intranasal and ocular drop method.

vaccine: 20 days old
NDlasota (IZO) vaccine was given at twenty days old by intranasal and ocular drop method.

Amount of multienzyme feed (500 g per ton of animal feed, Endo-Power from EASY BIO, Inc., Korea). The room remained lit throughout the experimental period, and the room temperature was controlled at 32 °C for 1–3 days and then gradually lowered by 2–3 °C every week to a final temperature of 20 °C. Feed is formulated to meet nutritional requirements according to ROS308 feeding guidelines. The composition and nutritional components of the basic diet are shown in Table 1. Food and water were provided ad libitum during the experiment. This enzyme was obtained from Aspergillus niger and Aspergillus oryzae and manufactured in Korea. This includes at least 1,500 (units/g) of 1xylanase, 1,100 (units/g) of 1β-glucanase, 110 (units/g) of 2galactomannanase and 35 (units/g) of 3α-galactosidase. It was included. According to the manufacturer, this enzyme product also contained the activities of other enzymes such as phytase, protease, and amylase. The experimental period lasted 35 days. Chicks were weighed on days 1, 7, 14, 21, and 28 after birth to determine body weight, weight gain, and feed conversion ratio (F.C.R). Blood samples were collected from the hearts of chicks at 1, 7, 14, 21, 28, and 35 days of age to determine humoral immunity to Newcastle disease virus NDV using ELISA (Newcastle disease virus antibody test kit, IDEXX, USA). A diagnosis was made and a liver massage was performed. The amounts of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were quantified using a commercially available colorimetric kit (France Company for Biotechnology). The activities of (ALT, U/L) and (AST, U/L) were measured on a Unico UV-2000 spectrophotometer using the method of Reitman and Frankel (53). Spectra Lab Scientific Inc., USA) was calibrated at a wavelength of 545 nm after 21.35 days (Saleh *et al*., 2023). Blood samples were stored on ice and centrifuged, and plasma was stored at −20°C until assayed.

Table 1: The composition and chemical analysis of starter and grower diets

Ingredients %	**Starter diet**	**Grower diet**
Yellow corn	50.00	55.00
Soybean meal, 44%	32.00	27.00
wheat	5.00	4.00
Wheat bran	5.50	6.00
Vegetable oil	4.00	4.90
Limestone	2.00	1.6
Di-calcium phosphate	1.00	1.00
Nacl	0.25	0.25
Vit. & min. *	0.25	0.25
Total	100KG	100KG
Calculated analysis (NRC,1994):		
CP%	22.07	19.67
ME, k cal\kg	2996.00	3100.21
CF%	3.54	3.62
Ca%	1.03	0.95
Av. Ph%	0.69	0.65
Lys. %	1.10	0.95
Meth+Cys.	0.52	0.50
Chemical analysis:		
DM%	93.02	92.78
Ash%	7.20	6.76
CP%	22.00	19.80

Ingredients %	Starter diet	Grower diet
CF%	3.35	3.72
EE%	4.69	6.21
NFE%	62.76	63.51

According to NRC,1994 and Sasso farmer's guide* Each 3 kg contains contain: Vitamin A = 12,000,000 IU, D3 = 2,000,000 IU, E = 10,000 mg, K3= 2000mg, B1 = 1000 mg, B2 =5000 mg, B6 =1500 mg, B12= 10mg, Biotin= 50 mg, pantothenic acid= 10000 mg, Nicotinic acid = 30000 mg, Folic acid =1000 mg, Zinc = 50,000 mg, Manganese = 60,000 mg, Iron = 30,000 mg, Copper = 10,000 mg, Iodine =1,000 mg, Selenium = 100 mg, Cobalt = 100 mg, Cobalt = 1000 mg, and Calcium carbonate up to 3 Kg.

RESULTS AND DISSCUSION

Performance:

The effect of Endo-Power multienzyme supplementation on broiler performance on days 1, 7, 14, 21, and 28 of age is shown in Table 2a, b, c. Feed conversion ratio (FCR), body weight, and weight gain were significantly improved by enzyme supplementation from 7 to 28 days of age. The need for exogenous enzyme supplements in corn and soy flour diets is generally ignored. However, several studies have reported significant positive growth performance responses in corn-based diets enriched with enzymes using multiple enzymes including xylanase, protease, amylase, or a single protease enzyme (Zanella *et al*., 1999; Ghazi *et al*., 2002; Yu *et al*., 2007). The response to enzyme supplementation depends on the age of the bird, and this appears to be related to both the type of gut microbiota present and the bird's physiology. The increased fermentative capacity of the gut microbiota allows older birds to better cope with the effects of high viscosity (Choct *et al*., 1996; Vranjes and Wenk, 1995). Enzyme supplementation can improve nutrient digestibility and improve broiler performance. This mechanism may be caused, at least in part, by a decrease in viscosity (Lazaro *et al*., (2003). The results of this study are consistent with previous results (Gutierrez del Alamo *et al*., 2008; Gao *et al*., 2007; Yu *et al*., 2007).

Table-2a: Live body weight

Age Day Treatments	A	B	C	D
1	44±0.4 A e	44±0.2 A e	43.8±0.2 A e	43.9±0.1 A e
7	206.3±1.9 B d	206.7±0.1 A d	206.2±0.2 B d	206.4±0.8 A d
14	511.1±3.1 B c	515.4±0.8 A c	511.9±0.7 B d	515.2±0.2 A d
21	937±1.6 B b	950.8±0.6 A b	938±1.2 B b	949.6±2.4 A b
28	1520.5±3.3 B a	1632.6±0.8 A a	1521.6±3.2 B a	1631.8±0.8 A a
* Means ± Std Error *Means with the same Capital letters in the same column are not significantly different *Means with the same Small letters in the same Row are not significantly different				

Table-2b: Total Weight Gain

Age Day Treatments	A	B	C	D
7	162.3±1.5 B d	162.7±0.3 A d	162.4±0 B d	162.5±0.7 A d
14	304.8±1.2 B c	308.7±0.7 A c	305.7±0.5 B c	308.8±1 A c
21	425.9±1.5 B b	435.4±0.2 A b	426.1±1.9 B b	434.4±2.2 A b
28	583.5±1.7 B a	681.8±0.2 A a	583.6±4.4 B a	682.2±1.6 A a
* Means ± Std Error *Means with the same Capital letters in the same column are not significantly different *Means with the same Small letters in the same Row are not significantly different				

Table-2c: Food Conversion Rate (FCR)

Age Day Treatments	A	B	C	D
7	1.05±0.01 A d	1.045±0.005 B d	1.05±0 A d	1.045±0.005 B d
14	1.77±0.01 A c	1.75±0 B c	1.765±0.005 A c	1.745±0.005 B c
21	2.82±0.01 A b	2.755±0.005 B b	2.815±0.015 A b	2.765±0.015 B b
28	3.77±0.01 A a	3.23±0 B a	3.77±0.03 A a	3.225±0.005 B a

* Means ± Std Error
*Means with the same Capital letters in the same column are not significantly different
*Means with the same Small letters in the same Row are not significantly different

Measurement the antibody of NDV

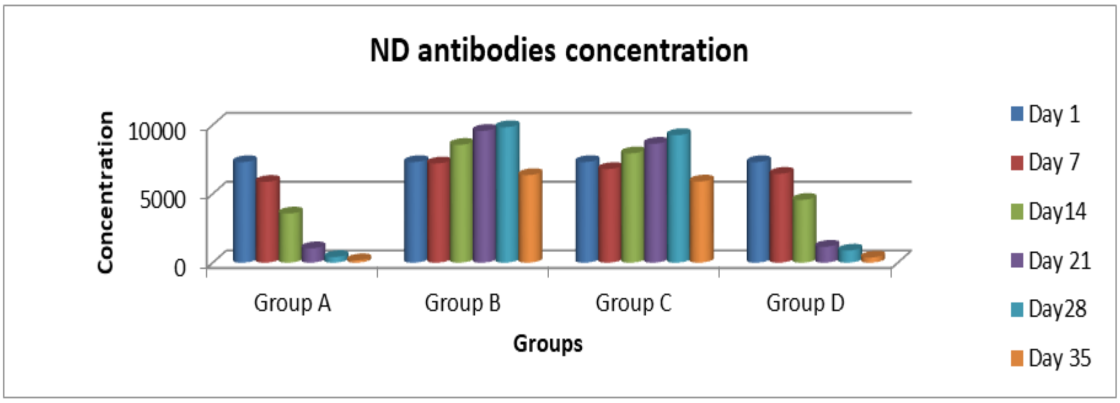
The effects of Endo-Power Multi-enzyme supplementation on broiler immune response against NDV of broiler chickens are show in Table 3 The vaccine antibody titers of GB and GC have increased with age and have shown a significant difference at days 14,21,28 and 35 days as compared to GA, GD; also, there was a significant difference between the treated groups (GD) in comparable to GA at 14,21,28 and 35 days.

There was no significant difference (P>0.05) in the maternal antibody titre between all groups. The maternal antibodies have reduced in all groups during the time before vaccination; this finding is in agreement with other studies (Allan & Gough, 1976; ERGANİŞ & Uçan, 2003).

After vaccination, the vaccine antibody titers of GB and GC have increased with age and have shown a significant difference at days 14,21,28 and 35 days as compared to GA, GD; also, there was a significant difference between the treated groups with vaccine and enzyme GB in comparable to groups with only vaccine GC at days 14,21,28 and 35 days. Therefore, it can be suggested that Endo-Power Multi-enzyme supplementation which included β -glucans and other enzyme showed high immunomodulatory property at a 500g/ton in feed concentration. In studies (Nochta *et al*., 2009; Sakai, 1999); have been found out that highly purified β- 1,3-1,6- glucans in diets stimulate cellular and humoral immune responses and increase disease resistance. feed supplementation of Endo-Power Multi-enzyme which included β -glucans and other enzyme increased the production of antibodies against NDV and IgA concentrations in the intestinal and tracheal mucosa (Elmusharaf *et al*., 2007); also β -glucans increase the concentration of serum IgG, IgM, and A (Yun *et al*., 2003); these results, taken together, provide new and clear evidence that β -glucan has an immunoregulatory effect at the local and systemic level (Gómez-Verduzco *et al*., 2009).

Table 3: Effect of supplementation Endo-Power enzyme (500g/ton of feed) on antibody titer for NDV for each group

Age / Groups	Day1	Day7	Day14	Day21	Day28	Day35
G A	7303±11.15	5866±15.56	3569 ±5.7	1046± 2.29	408±3.17	173 ±2.15
G B	7306±10.78	7204±17.31	8556±12.25	9571 ± 11.78	9839±13.12	6361±9.55
G C	7309±11.18	6805±18.11	7940 ±9.52	8636 ± 10.25	9261±13.22	5893±10.56
G D	7301±11.09	6451±19.35	4560 ±8.38	1158± 12.34	879±4.52	375 ±9.88
P value	0.08	0.05	0.04	0.05	0.03	0.04



Measurement Liver Enzymes AST and ALT

The ALT and AST were significantly higher in GA, GC than in GB, GD Table 4 Supplementation of multienzymes at 500g/ton significantly decreased the ALT and AST compared to the control group (Table 4).

As demonstrated in Table 4, throughout the 35-day study, hepatic function enzymes (ALT and AST), exhibited a significant ($P \leq 0.05$) reduction in GB, GD in contrast to GA, GC mainly in 21 days of age.

It appears that the Endo-Power Multi-enzyme supplementation effectively ameliorates the adverse effects of mycotoxins on hepatic tissues, possibly through the presence of glucose oxidase and mycotoxin detoxification enzymes, as discussed by Qu and Liu (2021), as their research suggested that the glucose oxidase supply reduces the inflammatory reaction induced by mycotoxin exposure by decreasing liver function enzyme levels. Additionally, Alharthi *et al*., (2022) reported that the addition of bentonite has a similar ameliorative effect on aflatoxin-exposed livers. These results align with those of Amer *et al*., (2018), who reported that bentonite supplementation overcomes the negative effects of aflatoxins, improving growth performance and decreasing the relative weights of hepatic tissues, which are typically elevated by aflatoxin exposure.

Table 4: AST and ALT parameters of the blood of broiler chickens

Enzymes / Groups	AST		ALT	
	21day	**35day**	**21day**	**35day**
G A	261.6±14.05	374.08 ±3.17	198.7 ±6.4	200.02 ±8.4
G B	238.6±3.5	298.6±11.9	187.6±3.6	183.4±3.4
G C	250.2±4.5	343.5 ±10.2	191.6 ±6.7	196.5 ±5.4
G D	249.7±7.3	307.7 ±5.4	186.5 ±5.4	194.3 ±8.1
P value	0.05	0.03	0.04	0.05

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