ISSN 1682-8356
ansinet.com/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

Science Alert
scialert.net

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS International Journal of Poultry Science
ISSN 1682-8356
DOI: 10.3923/ijps.2020.455.466

Research Article
Effect of β-Mannanase Enzymes Supplementation to Energy Deficient Diets on Productive Performance, Physiological and Carcass Traits of Broilers

[1]M.M. Hashim, [1,2]S.A. El-Safty, [3]K.G. El-Eraqi, [4]H.M.R. El-Sherif, [5]T. Azza, [6]K. Marwa, [7]H.M. Ibrahim and [2]A.Y.M. Abdelhady

[1]Applied Feed Research House (AFRH), Orabi Community, Qalyobia, Egypt
[2]Department of Poultry Production, Faculty of Agriculture, Ain Shams University, Hadayek Shoubra 11241, Cairo, Egypt
[3]Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[4]Department of Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt
[5]Department of Clinical Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[6]Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[7]Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt

Abstract

Objective: This experiment was conducted to assess the beneficial effect(s) of β-mannanase (Lycell® and Hemicell®) supplementation to low energy diet on productive performance, gross energy digestibility, hematological parameters and carcass traits of broiler chicks. **Materials and Methods:** A total of 540, day old Ross 308 broiler chicks, obtained from local hatchery with mean body weight of 33±0.05 g, were randomly assigned to three dietary treatments, 180 birds each. The treatments were T1 (low energy diet, 80 kcal kg^{-1}, as control), T2 (low energy diet +250 g t^{-1} β-mannanase from Lycell® product) and T3 (low energy diet +300 g t^{-1} β-mannanase from Hemicell® product). The parameters recorded were growth performance, carcass traits, haematological and blood biochemical parameters, dropping and litter microbiology, gut histomorphology and digestibility trial. **Results:** Results demonstrated that β-mannanase improved growth performance ($p<0.05$) in terms of body weight, weight gain, feed intake and feed conversion ratio from three to five weeks of age. Carcass traits criteria including dressing weight, dressing percentage, and abdominal fat were significantly ($p<0.05$) affected by β-mannanase while gizzard, heart and spleen weight were not affected by the dietary treatments. Moreover, the dietary treatments did not affect the dry matter digestibility (DMD) and organic matter digestibility (OMD) of broiler. **Conclusion:** β-mannanase enzymes, either Lycell® or Hemicell®, supplementation should be considered when low energy diets are formulated in broiler.

Key words: Broiler, β-mannanase, low energy diet, growth performance, carcass traits, digestibility

Citation: M.M. Hashim, S.A. El-Safty, K.G. El-Eraqi, H.M.R. El-Sherif, T. Azza, K. Marwa, H.M. Ibrahim and A.Y.M. Abdelhady, 2020. Effect of β-Mannanase enzymes supplementation to energy deficient diets on productive performance, physiological and carcass traits of broilers. Int. J. Poult. Sci., 19: 455-466.

Corresponding Author: K.G. El-Eraqi, Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

About 80% of the birds' diets are made up of ingredients from plant origin containing non-starch polysaccharides (NSPs) in the plants cell wall. Among NSP, β-mannans can be considered as the leading molecules and are the most prevalent in a wide variety of feed ingredients including soybean meal, which is the major protein source in feeds produced around the world[1].

The β-mannans have the ability to bind large quantity of water which subsequently results in an increase in the digesta viscosity, decrease in the diffusion of digestive enzymes and stimulates proliferation of bacteria inside the gastrointestinal tract[2]. Higher digesta viscosity also results in poor enzyme substrate interaction and thus reduces nutrient availability to the birds[3]. β-mannans is an anti-nutritional fiber and reduces metabolizable energy and nitrogen retention and increase fecal output of birds[4].

In practice, the poultry diets supplementation with exogenous enzymes is a universal strategy to improve nutrient utilization, growth performance and thus reduce feed cost[5]. Dietary β-mannanase supplementation is responsible for the hydrolysis of β-mannans, thus reduce intestinal viscosity, promote better nutrient digestibility and absorption in the gut and improve feed conversion[6,7]. Supplementation of β-mannanase to β-mannan-rich diets may boost the population of beneficial intestinal bacteria, increase the digestibility of mannans, enhance the immunity, suppresses the growth of harmful intestinal bacteria, enhance the digestion and absorption of nutrients in intestinal tracts and reduce the environmental pollution due to poultry excreta[8].

Thus, the objective of this experiment was to determine the effect of two different commercial β-mannanase products on growth performance, nutrient digestibility and energy utilization of broilers fed energy deficient diet.

MATERIALS AND METHODS

The present trial was carried out in the Poultry Research Unit belonging to Applied Feed Research House (AFRH), Orabi Community, Egypt, during October and November 2019.

Experimental design: A total 540 one-day-old broiler chicks (Ross 308) with initial body weight of 44±1 g were raised in well ventilated pens with soft wood shaving bedding material. The broiler chicks were weighed and randomly allocated to three treatment groups each of 180 chicks, in six replicates, 30 chicks each. The first group (T1) was fed the negative Control diet (Basal diet -80 kcal). Lycell® consists of 1,4 β-mannanase 250000 Unit g^{-1} were obtained from *Bacillus Lentus* Fermentation with wheat bran carrier, whereas Hemicell® was a dried *Bacillus lentus* fermentation solubles with 158 000 unit g^{-1} minimum β-mannanase enzyme activity.

Experimental diets: Birds were fed on starter (0-14 day), grower (15-24 day) and finisher (25-35 day) diets which were formulated according to the nutritional recommendation of Ross 308 strain catalogue (Table 1). The birds were vaccinated against common viral diseases i.e. Newcastle Disease virus (NDV), Infectious Bursal Disease (IBD), Infectious bronchitis (IB) and Avian Influenza (AI). Fresh water and feed were offered *ad libitum* during the whole experimental period.

Data recorded and measurements

Productive performance traits: Live body weight (LBW), body weight gain, feed consumption and feed conversion ratio were recorded at weekly intervals throughout the experimental period. Feed consumption was calculated by measuring the difference between the amount of feed offered and residue left. Feed conversion ratio (FCR) was calculated as total feed consumed divided by total weight gain for a given period of growth.

The feeding economic efficiency was carried out according to the prices of feed ingredients, tested material during the experimental period.

- Performance index (PI) was calculated according to North[9] using the following equation:

$$PI = \frac{BW\ (kg)}{FCR} \times 100$$

- European Performance Efficiency Index (EPEI) was calculated using the following equation:

$$EPEI = \frac{LBW \times SU}{FCR \times age} \times 100$$

where, LBW is live body weight (kg), SU is survival rate (%) [100-Mortality rate], FCR is feed conversion ratio, and age is age of slaughter (days).

Carcass traits: At the end of the trial (35 day), four chickens from each treatment were randomly slaughtered for measurements of live body weight, dressing weight, dressing (%) abdominal fat, gizzard, heart and spleen weight.

Table 1: Feed ingredients and chemical composition of diets presented to birds during starter, grower and finisher phases (0-35 days of age)

	Dietary treatment		
Ingredient	Starter (80 kcal kg^{-1})	Grower (80 kcal kg^{-1})	Finisher (80 kcal kg^{-1})
Yellow corn	597.416	652.707	705.510
Soybean meal (46%)	311.344	243.277	171.353
Corn gluten meal (60%)	47.638	57.064	70.917
Soybean oil	5.000	10.000	15.000
Mono-calcium phosphate	10.433	9.635	9.288
Calcium carbonate	14.003	12.303	12.981
HCl-lysine	4.817	5.611	5.850
DL-methionine	1.823	1.405	1.256
L-threonine	0.279	0.711	0.521
Salt (NaCl)	3.247	3.287	3.324
Phytase enzyme	1.000	1.000	1.000
Premix	3.000	3.000	3.000
Total	1000.000	1000.000	1000.000
Calculated chemical composition			
Crude protein (%)	22.000	20.000	18.000
ME kcal kg^{-1} (calculated)	2920.000	3020.000	3120.000
Linoleic acid	1.500	1.835	2.156
Calcium (%)	1.000	0.900	0.900
Available phosphorus (%)	0.500	0.470	0.450
Lysine (%)	1.400	1.300	1.150
Methionine (%)	0.560	0.500	0.470
Methionine+cysteine (%)	0.983	0.900	0.850
Price/t ($) and (LE)	340$	335$	329$
	6300 LE	6207 LE	6095 LE

Vitamin and Mineral mixture provides diet; Vitamin A: 12000000 IU, D3: 4000000 IU, E:50000 mg, K3: 3500, B1: 3500 mg, B2: 60000 mg, B6: 4000 mg, B12: 180 mg, Niacin: 50000 mg, Pantothenic acid: 14000 mg, Folic acid: 2000 mg, Biotin: 150 mg, Minerals: Iron: 50000, Copper 9000, Zinc 66000 mg, Manganese: 66 mg, Selenium: 300 mg, Iodine: 1000 mg, Cobalt: 100

Haematobiochemical parameters: Blood samples were collected from the slaughtered birds (4 chicks/treatment) in two tubes, one with anticoagulant (EDTA) and the other without anticoagulant for harvesting serum by centrifugation at 4000 rpm for 15 min. The resulting serum was kept frozen at -20°C until the biochemical analyses were done.

Haemogram estimation: Erythrocytic and leukocytic counts were performed using an improved Neubauer hemocytometer and Natt and Herrick solution as a special diluent for chicken's blood according to Harrison and Harrison[10]. The packed cell volume was estimated by micro hematocrit centrifuge according to Coles[11]. Hemoglobin estimation was performed using the cyanomethemoglobin colorimetric method after centrifugation according to Zijlstra[12]. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were then calculated. Blood films were made, fixed by methyl alcohol, stained by Giemsa stain for differential leukocytic count and detection of abnormalities in RBCs morphology according to Weiss and Waldrop[13].

Serum biochemical analyses: Serum total proteins and albumin were calculated using the available commercial kits (DRI-CHEM NX500i, Japan) according to Weissman *et al.*[14] and Doumas *et al.*[15] respectively, while globulin was calculated by subtracting the value of albumin from the total protein whereas A/G ratio was calculated according to results of albumin and globulin. Serum Aspartate aminotransferase (AST) activity was determined using the method of Reitman and Frankel[16], creatinine and urea were also determined using method of Bartels *et al.*[17], serum total cholesterol and serum glucose was determined using the method of Allain *et al.*[18].

Microbiological analysis: Aerobic, anaerobic, and coliform bacteria populations were counted from each sample (litter or dropping). To process the samples, 1 g of litter sample or dropping was diluted 10-fold using sterile buffered peptone water. The diluted sample was serially diluted using sterile buffered peptone water until a final dilution of 1:10 was obtained. From each serial, 12 dilutions, 0.1 mL was then spread plated onto two different media: tryptic soya agar and MacConkey agar. The dilutions were plated in quadruplicate on the tryptic soya agar and in duplicate on the MacConkey

agar. Half of the tryptic soya agar plates and all of the MacConkey plates were incubated under aerobic conditions at 37°C for 24 h. To obtain anaerobic bacteria counts, the other tryptic soya plates were incubated anaerobically at 37°C for 24 h using Gaspack anaerobic jar[19].

Isolation and detection of *C. Perfringens*, were done according to Shaltout *et al.*[20]. The collected samples were inoculated into tubes of freshly prepared, boiled and cooled cooked meat medium (Oxoid) and incubated anaerobically for 24 h at 37°C. A loopful of inoculated fluid medium was streaked on to neomycin sulphate sheep blood agar plate[21] and incubated anaerobically at 37°C for 24 h using Gaspack anaerobic jar[22]. Suspected C. perfringens colonies were cultured onto 2 plates of sheep blood agar and egg yolk agar. One plate was incubated aerobically and the other plate was incubated anaerobically. The colonies that grew only in anaerobic condition and lecithinase producer and showed double zone of hemolysis on blood agar were picked up and purified for identification tests[21,23]. Isolated colonies with a typical appearance were then biochemically tested by using a commercial biochemical panel kit (API 20A, Bio Mérieux).

Histopathological examination: Tissue samples from duodenum, jejunum, liver and kidney were collected from the slaughtered chickens at the end of the experimental period. Samples were fixed in 10% neutral formalin buffer for latter processing by paraffin embedding technique. Tissue sections (4 micron thick) were then made by microtome (Leica 2135, Germany), deparaffinized and stained with hematoxylin and eosin[23]. The length of intestinal villi was measured from the tip of villus to its base. The mean length of 10 randomly selected villi/ intestinal cross section was measured in 3 cross sections for each chick[24,25]. Similarly, the width of villi and crypt depth were measured. The villus height/crypt depth and the absorption surface (AS) area were calculated using the formula reported by Oliveira *et al.*[26], as follow:

$$AS\ (um^2) = \text{villus height (um)} \times \text{width at half of the villus height (um)}$$

Digestion trial: A digestibility trial was conducted at the end of experiment. Four birds from each treatment were housed in digestion cages, (47×47×47 cm^3 dimensions) with nibble drinkers and separate feeders for 7 days. Feed and water were offered *ad libitum* during the preliminarily (4 days) followed by a collection period of 3 days. The feed consumption was recorded and excreta amounts were collected every 24 h. The collected samples were dried at 60°C until constant weight then, excreta were weighed, ground, mixed well and stored for analysis according to AOAC[27]. The parameters recorded were; Feed intake (g $bird^{-1}$ day^{-1}), Excreta Weight (EW) (g $bird^{-1}$ day^{-1}), Excreta weight % (EWP), dry matter digestibility (DMD), organic matter digestibility (OMD); either as air dried or dried basis; and Excreta moisture content (%).

Statistical analyses: Data were analyzed with the help of the general linear model procedure using SAS[28] and the corresponding means were compared using Duncan multiple test[29]. For all statistical analyses, differences were considered significant at $p<0.05$.

RESULTS

Table 2 shows non-significant differences in LBW of chicks during the first two weeks of age. At the 3rd week, chicks from treatment groups T2 and T3 had significantly heavier LBW than the control (T1) group. This trend was also observed at the 4th and 5th weeks of age, with chicks from T3 treatment having the best values. Moreover, BWG was significantly better for both T2 and T3 group than that of the control group. On the other hand, feed consumption ratio was significantly increased in response to feed additives (T2 and T3) compared to the control chicks at the 3rd week of age, whereas at the 4th week, chicks from all treatments consumed nearly similar amounts of feed. At the 5th week of age and for the whole experimental period, chicks from T2 and T3 treatments consumed significantly less amount of feed compared to the control chicks. This reduction in FC was 5.68 and 6.02% for chicks in T2 and T3, respectively. It is well-known that FCR is a function of BWG and FC, since our results revealed significant improvement in FCR of chicks from T2 and T3 for the whole period. This improvement was 11.11 and 12.78% for T2 and T3, respectively.

Supplementation of β-mannanase (Lycell® or Hemicell®) enzyme in the diets of broilers significantly ($p<0.05$) improved PI and EPEI. Minimum and significantly ($p<0.05$) lower EPEI (286) was observed in chicks fed negative control (T1) diet, however it was improved significantly ($p<0.05$) in T2 and T3 group (356, 371) respectively.

Carcass traits: Table 3 shows that dressing weight and dressing percentage were significantly increased in chicks from T2 and T3 compared to the control group. However, abdominal fat was decreased significantly.

Table 2: Effect of different treatments on productive performance of broiler chicks at different growth periods

Age	T1	T2	T3	SEM	Sig.
Live body weight (LBW, kg)					
1st week	0.152	0.171	0.162	0.0095	0.09860
2nd week	0.480	0.481	0.525	0.0257	0.04610
3rd week	0.784^{b}	0.980^{a}	0.967^{a}	0.0521	0.00060
4th week	1.288^{b}	1.532^{a}	1.551^{a}	0.0537	0.00300
5th week	1.982^{b}	2.096^{ab}	2.125^{a}	0.0756	0.00340
Body weight gain (BWG, kg)					
1st week	0.109	0.128	0.120	0.0095	0.11570
2nd week	0.329	0.311	0.363	0.0264	0.07090
3rd week	0.304^{b}	0.499^{a}	0.442^{a}	0.0478	0.04150
4th week	0.504	0.552	0.584	0.0403	0.06540
5th week	0.694^{a}	0.564^{b}	0.574^{b}	0.0153	0.12470
Overall	1.939^{b}	2.054^{a}	2.083^{a}	0.0427	0.01280
Feed consumption (FC, kg)					
1st week	0.131	0.156	0.153	0.0137	0.09094
2nd week	0.445	0.383	0.461	0.0412	0.15192
3rd week	0.403^{b}	0.740^{a}	0.636^{a}	0.0715	0.01023
4th week	0.879^{b}	0.948^{a}	0.947^{a}	0.0396	0.03440
5th week	1.627^{a}	1.061^{b}	1.078^{b}	0.0913	0.00673
Overall	3.486^{a}	3.288^{b}	3.276^{b}	0.1179	0.02676
Feed conversion ratio (FCR, kg)					
1st week	1.20	1.220	1.28	0.0346	0.81416
2nd week	1.35^{b}	1.230^{c}	1.27^{a}	0.0200	0.01097
3rd week	1.33^{b}	1.480^{a}	1.44^{a}	0.0265	0.04062
4th week	1.75^{a}	1.720^{a}	1.62^{b}	0.0681	0.00676
5th week	2.35^{a}	1.880^{b}	1.88^{b}	0.0318	0.00084
Overall	1.80^{a}	1.600^{b}	1.57^{b}	0.0569	0.00259
PI	110.00^{b}	131.000^{a}	135.00^{a}	15.3800	0.00100
EPEI	286.00^{b}	356.000^{a}	371.00^{a}	28.5500	0.00400

T1: Negative control (80 kcal kg^{-1}), T2: T1+250 g of β-mannanase (Lycell®), T3: T1+300 g of β-mannanase (Hemicell®), *Significant differences between rows at $p \leq 0.05$, NS: Non-significant difference, PI: Performance index, EPEI: European performance efficiency index, SEM: Standard error of mean, Sig.: Significance

Table 3: Effect of different treatments on carcass traits of broiler chicks at the marketing age

Parameters	T1	T2	T3	SEM	p-value
Live body weight (kg)	1.920^{b}	2.000^{a}	2.050^{a}	0.0781	0.0011
Dressing weight (kg)	1.251^{b}	1.424^{a}	1.455^{a}	0.1099	0.0003
Dressing (%)	65.16%	71.20%	70.980%	0.0297	0.0254
Abdominal fat (%)	2.45%^{a}	1.88%^{b}	1.760%^{b}	0.0099	0.0457
Liver (%)	4.00%	3.37%	3.230%	0.0041	0.0773
Gizzard (%)	4.09%	4.07%	3.990%	0.0005	0.0899
Heart (%)	0.912%	0.904%	0.966%	0.0003	0.0156
Spleen (%)	0.125%	0.144%	0.140%	0.0001	0.0558

T1: Negative control (80 kcal kg^{-1}), T2: T1+250 g of β-mannanase (Lycell ®), T3: T1+300 g of β-mannanase (Hemicell®), *Significant differences between rows at $p \leq 0.05$, NS: Non-significant difference, SEM: Standard error of mean

Hematological parameters: Data presented in Table 4 shows significant increases in PCV%, of chicks from T2 and T3 compared to control chicks, however, hemoglobin concentration was significantly reduced in both T1 and T3 groups. Moreover, RBCs and total leucocytes (TLC) counts were significantly higher in T1 than those of the other treatments which may affect blood indices.

Total colony count and total anaerobic count in droppings: Data presented in Table 5 shows significant increase in total colony count, total anaerobic count and in droppings of chicks from T2 and T3 compared to control chicks, whereas, total coliform count was significantly reduced in both T2 and T3, compared to control chicks. *Clostridium perfringens* isolated only from dropping of T1. Moreover, significant increases in total colony count was observed in litter of chicks from T2 and T3 compared to control chicks, however, no difference was observed in total anaerobic count of litter from the three different groups. Also, total coliform count was significantly reduced in both T2 and T3, compared to control chicks. Clostridium Perfringens isolated only from litter of T1.

Table 4: Effect of different treatments on haemogram and Serum biochemical analyses

Parameters	T1	T2	T3	p-value
Hematological parameters				
PCV (%)	23.70±0.70[b]	36.67±0.33[a]	36.330±0.88[a]	0.0004
HB concentration (g dL^{-1})	6.07±0.30[b]	7.70±0.19[a]	8.130±0.58[a]	0.0424
RBCs count ($\times 10^6$ cell μL^{-1})	1.38±0.08[a]	1.17±0.02[b]	1.107±0.035[b]	0.0435
TLC ($\times 10^3$ cell μL^{-1})	64.00±300[a]	30.33±1.20[b]	30.000±1.73[b]	0.0007
Heterophils ($\times 10^3$ cell μL^{-1})	35.19±5.33[a]	19.74±0.90[b]	16.560±1.11[b]	0.0325
Lymphocytes ($\times 10^3$ cell μL^{-1})	27.92±3.03[a]	9.66±0.61[b]	12.240±2.77[b]	0.0381
Monocytes ($\times 10^3$ cell μL^{-1})	0.89±0.45	0.52±0.05	0.600±0.03	0.5974
Eosinophils ($\times 10^3$ cell μL^{-1})	0.00±0.00	0.74±0.38	0.600±0.035	0.1308
Biochemical parameters				
Total protein (g dL^{-1})	3.42±0.06[b]	4.10±0.009[a]	3.500±0.07[b]	0.0008
Albumin (g dL^{-1})	1.88±0.01[c]	2.21±0.008[b]	1.820±0.01[a]	0.0002
Globulin (g dL^{-1})	1.54±0.05[a]	1.89±0.02[b]	1.680±0.07[c]	0.0005
A/G ratio	1.22±0.01[c]	1.17±0.003[b]	1.080±0.01[a]	0.0001
Cholesterol (mg dL^{-1})	129.70±2.95[a]	104.77±5.4[b]	110.880±4.08[b]	0.0003

T1: Negative control (80 kcal kg^{-1}), T2: T1+250 g of β-mannanase (Lycell®), T3: T1+300 g of β-mannanase (Hemicell®), Data are presented as mean values±SE Significant difference at $p \leq 0.05$

Table 5: Effect of beta-mannanase addition on droppings and litter microbiology

Parameters	T1	T2	T3
Droppings			
Total colony count	4×10^8	7×10^8	6×10^8
Total anaerobic count	14×10^3	18×10^3	17×10^3
Total coliform count	40×10^5	17×10^5	10×10^5
Clostridium perfringens	+ve	-ve	-ve
Litter			
Total colony count	5×10^9	18×10^9	15×10^9
Total anaerobic count	10×10^4	9×10^4	6×10^4
Total coliform count	9×10^6	7×10^6	3×10^6
Clostridium perfringens	+ ve	- ve	- ve

T1: Negative control (80 kcal kg^{-1}), T2: T1+250 g of β-mannanase (Lycell®), T3: T1+300 g of β-mannanase (Hemicell®)

Table 6: Effect of beta-mannanase addition on digestion trial of broilers

Parameters	T1	T2	T3	Sig.
Feed intake g $chick^{-1}$	215.8[a]	199.6[b]	191.3[b]	*
Excreta output g $chick^{-1}$	39.6[a]	32.7[b]	31.0[b]	*
DMD, AD (%)	80.0	81.9	82.3	NS
OMD, AD (%)	82.8	83.9	84.4	NS
DMD, DD (%)	81.9	83.7	83.8	NS
OMD, DD (%)	84.4	85.5	85.7	NS
Primary moisture (%)	72.4	78.9	73.8	NS
Excreta percentage of feed intake	18.1	16.3	16.2	NS

T1: Negative control (80 kcal kg^{-1}), T2: T1+250 g of β-mannanase (Lycell®), T3: T1+300 g of β-mannanase (Hemicell®), DMD: Dry matter digestibility, OMD: Organic matter digestibility, AD: Air dried basis, DD: Dry matter basis, *Significant differences between rows at $p \leq 0.05$, NS: Non-significant difference, Sig.: Significance

Digestion trial: Data presented in Table 6 shows significant ($p<0.05$) increase in feed intake (g $chick^{-1}$) and excreta output (g $chick^{-1}$). However, there were non-significant differences between other digestion parameters.

Histopathology: Microscopy of the duodenum of chicken revealed partial necrosis of the lining epithelium at the tips of intestinal villi in T1 (Fig. 1a and b) and a normal long intestinal villus in T2 and T3 (Fig. 1c and d). The histology of Jejunum showed widening of the intestinal villi in T1 (Fig. 2a) and normal long intestinal villi in T2 and T3 (Fig. 2b and c). Microscopy of liver revealed mononuclear and heterophilic cells infiltration in T1 (Fig. 3a), congestion with mononuclear and heterophilic cells infiltration in T3 (Fig. 3b) and normal histological structure in T2. The length of villi of the duodenum and jejunum was the highest in T3 compared to the other groups (Fig. 3c) (Table 7).

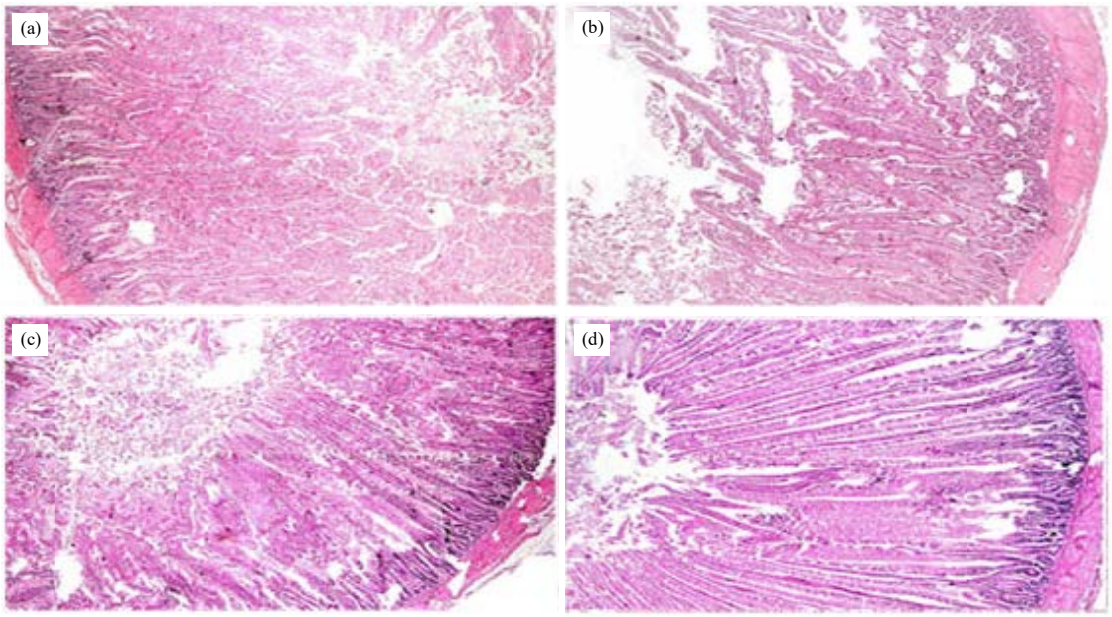

Fig. 1(a-d): Duodenum of chicken (a, b) partial necrosis of the lining epithelium at the tips of intestinal villi in T1. (c, d) normal long intestinal villi in T2 and T3 (H and E stain ×40)

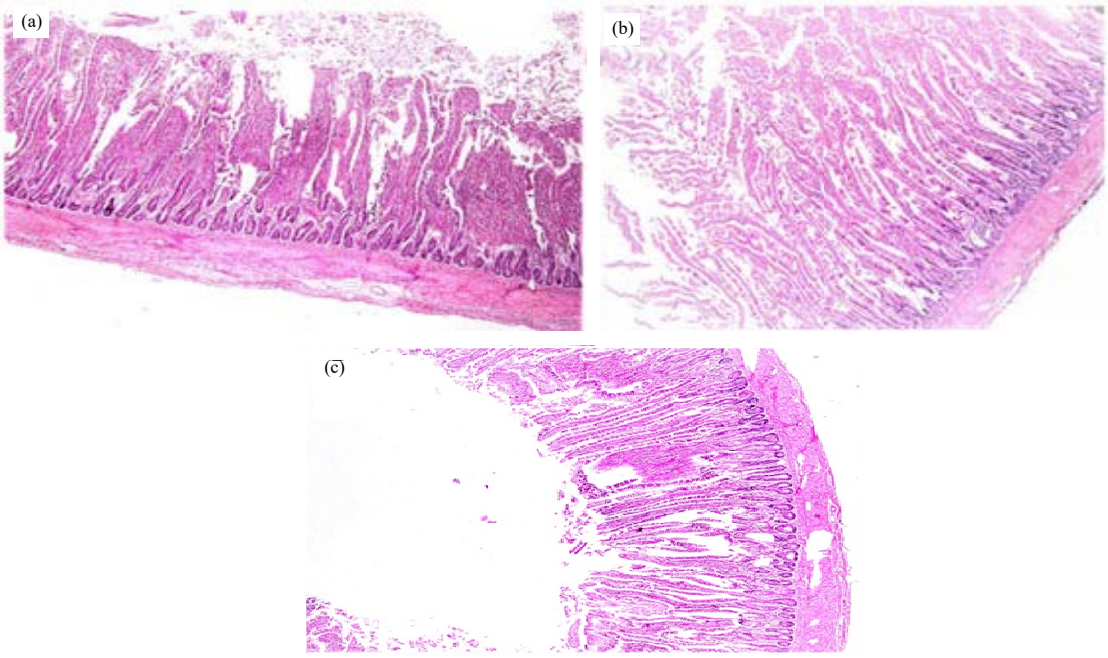

Fig. 2(a-c): Jejunum of chicken (a) widening of the intestinal villi in T1. (b, c) normal long intestinal villi in T2 and T3 (H and E stain ×40)

DISCUSSION

The obtained results revealed that broiler diets supplemented with different types of β-mannanase (Lycell® or Hemicell®) significantly ($p<0.05$) increase final live body weight, decrease feed consumption and improve feed conversion ratio. The positive effect of β-mannanase began to be clear from the third week of age, and it may be attributed

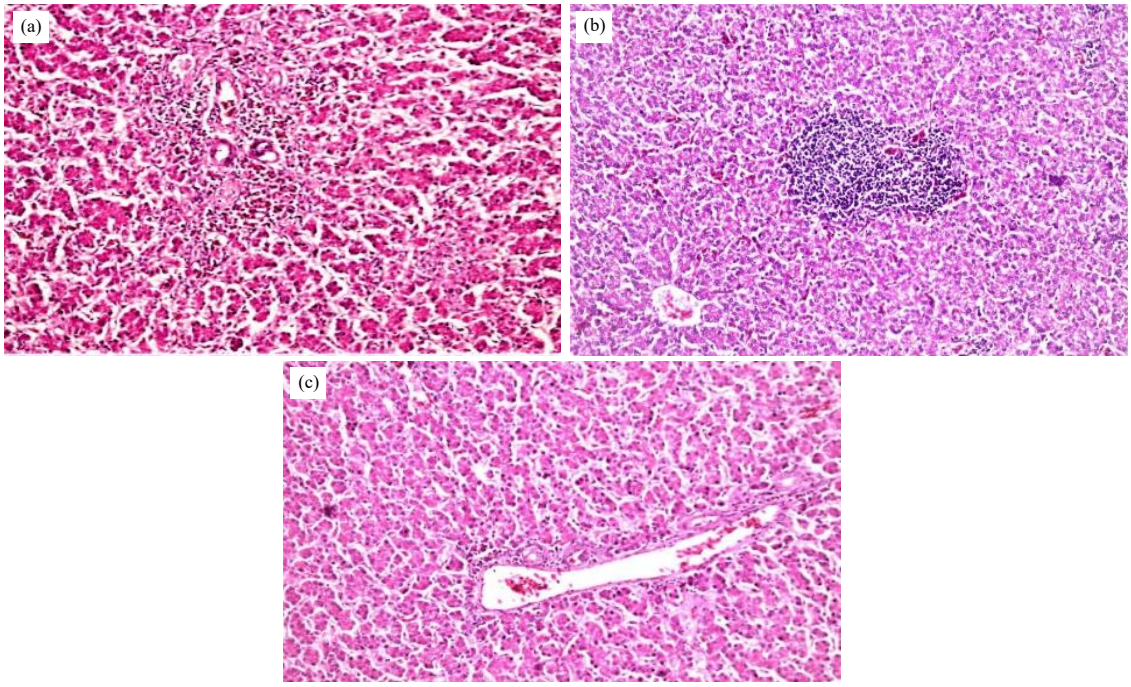

Fig. 3(a-c): Liver of chicken (a) mononuclear and heterophilic cells infiltration in T1, (b) Congestion with mononuclear and heterophilic cells infiltration in T3, (c) Apparently normal histological structure of the liver in T2 (H and E stain ×200)

Table 7: Intestinal villi length, width, crypt depth, length to crypt depth and absorbance surface of intestinal villi in the duodenum and Jejunum

Parameters	T1	T2	T3	Sig.
Duodenum				
Length	306.33±14.09^{b}	328.35±10.74^{a}	443.38±23.61^{a}	**
width	32.64±1.81	35.71±2.37	33.03±3.41	NS
crypt depth	78.57±8.92^{c}	98.00±4.32^{a}	87.31±5.13^{b}	**
L/C	3.90±0.48^{b}	3.38±0.13^{b}	5.27±0.53^{a}	*
AS (mm^{2})	9.40±0.86^{b}	9.20±0.91^{b}	13.24±1.34^{a}	*
Jejunum				
Length	280.60±12.27^{b}	290.22±6.29^{b}	313.00±23.63^{a}	*
width	40.13±1.93^{a}	41.75±1.71^{a}	29.24±3.08^{b}	*
crypt depth	88.51±7.29^{a}	63.57±4.6^{b}	59.68±8.27^{b}	*
L/C	3.17±0.28^{c}	4.57±0.38^{b}	5.24±1.13^{a}	**
AS (mm^{2})	6.99±0.65^{b}	7.08±0.47^{b}	10.70±0.80^{a}	*

T1: Negative control (80 kcal kg^{-1}), T2: T1+250 g of β-mannanase (Lycell®), T3: T1+300 g of β-mannanase (Hemicell®), L/C: Length to crypt depth, AS: Absorbance surface, *Significant differences between rows at p≤0.05, Sig.: Significance

to improvement in starch digestion and elimination of the negative effects of non-starch polysaccharides including galactomannans. These results are in agreement with those reported by Anderson *et al.*[30] and Hsiao *et al.*[31] and Zou *et al.*[32] who did not observe any improvement in broiler growth until three weeks of age. The best feed conversion ratio was obtained in β-mannanase groups (1.6 and 1.57) in T2 and T3 compared with T1 (control) birds. These results showed that β-mannanase enzyme hydrolyzes NSP class that ultimately reduces viscosity of the intestinal environment, lower water retention in smaller molecules of carbohydrate and increases the availability of carbohydrates. It is thus, making it absorbed and utilized by the birds[4,33] resulting in higher improvement in the feed/grain performance. These results disagree with Opalinski *et al.*[34] who found higher feed intake in broilers fed with a diet including an enzymatic complex containing β-mannanase.

In this study, significantly higher ($p<0.05$) PI and EPEI was observed in broilers of T2 and T3 group; however, minimum and significantly lower ($p<0.05$) feed efficiency

and performance index was observed in T1 group fed negative control diet. This result is supported by Saeed *et al.*[8] and Rehman *et al.*[35] who reported that supplementation of β-mannanase enzyme increased PI and EPEI.

β-mannanase enzyme supplementation improved the carcass traits, there was significant difference ($p<0.05$) in dressing weight, dressing percentage and abdominal fat; as the higher dressing weight was obtained in T2 (1.424 kg) and T3 (1.455 kg) followed by T1 (1.251 kg) in low energy diet group. In the same time, high dressing percentage was obtained in both β-mannanase supplemented groups (71%) followed by low energy diet group (65%). There was no significance difference between groups in liver, gizzard, heart and spleen weight; these results confirmed by previous results obtained by Hajati[36] who reported that enzyme supplementation increased carcass weight, dressing percentage and decrease abdominal fat but had no effect on heart, liver, proventriculus and gizzard percentages of broiler.

β-mannanase supplementation in both groups showed decrease in leukogram and globulins concentration compared with broiler fed on energy deficient diet (80 kcal kg^{-1}), which showed increase in total leukocyte count, heterophils, lymphocytes count and globulin concentration. These results agree with the findings of Mehri *et al.*[37], Incharoen *et al*[25] and Saeed *et al.*[8,25,37] who proved that β mannan present in poultry diet stimulate the innate immunity through improvement in the proliferation of monocytes and macrophages as well as resultant cytokine production. As β-mannans resemble carbohydrate structures found on the surface of pathogenic bacteria, it might induce an innate immune response (feed induced immune response) resulting in a nonproductive, energy-draining and inflammatory immune response[29,33,38] which also leads to an increase in acute phase proteins as globulins. Thus β-mannanase supplementation reduces β-mannan levels in the gut, which may lead to a depression in the stimulation of innate immunity[7]. This is not so bad as Li *et al.*[39] indicated that the decrease in immunoglobulin levels due to β-mannanase supplementation is suggestive of a down-regulated immune system that might have allowed nutrients to be redirected towards optimum performance which is also recorded in our result showing an increase in final live body weight. Regarding erythrogram results, there's significant increase in PCV% and hemoglobin concentration in both β-mannanase groups, which may be related to the improved general health status of broilers as mannanase improved morphological status in the gut[37,40] and minimized the viscosity of the intestine, allowing better absorption of nutrient[8], this also proved by our Histopathological results which revealed increased villus length, villus width, crypt depth and absorbance surface area at duodenum and jejunum in both T2 (Lycell® group) and T3 (Hemicell® group), which reflect increase in albumin concentration in both beta mannanase groups. The increase in A/G ratio in both β-mannanase groups may be due to increased albumin concentration. While increased cholesterol level in group one is agreed by Lee *et al.*[41] and Mohayayee and Karimi[42] who found that β-mannan increase feed:gain and produce higher plasma lipids for day 1-42 broilers fed guar meal. While Sundu *et al.*[43] found that diet enriched with β-mannanase statistically enhanced lipid utilization. As β- mannanase might have increased digesta flow rate leading to improved nutrient utilization[44], this agree with our result which showed significant decrease in cholesterol level in both β-mannanase groups.

The microbiological examination of droppings and litter material: T2 (Lycell® group) and T3 (Hemicell® group) revealed higher total colony count, lower coliform and anaerobic count and negative *clostridium perfringens* in both droppings and litter. These results may be due to the hydrolysis of β-mannan into β-galacto-mannan and production of mannooligosaccharides that improve chicken health, by interfering with pathogenic bacterial attachment to the epithelial cell as described by Spring *et al.*[45]. While in the energy deficient diet (80 kcal kg^{-1} group), due to the adverse effects β-mannans and their highly viscous nature, gastric emptying rate became slower and ingesta became a suitable media for growth and multiplication of pathogenic bacteria either coliform, anaerobes or *Clostridium perferingens*[46].

Effect of β-mannanase supplementation on the digestion trial of broiler: It was observed that the only differences between, energy deficient diet (80 kcal kg^{-1} group), T2 (Lycell® group) and T3 (Hemicell® group) were the feed intake $chick^{-1}$ day^{-1} and excreta output $chick^{-1}$ day^{-1}; the lower feed intake and excreta output was recorded in β-mannanase supplemented groups. While no significant differences ($p>0.05$) were recorded in dry matter digestibility, and organic matter digestibility; these results agree with some previous studies conducted by Hosseindoust[47] Tarachai and Yamauchi[48] and Rehman *et al.*[35] who stated that beta mannanase supplementation had no effect on digestibility and reported non-significant effect of beta mannanase on the dry matter and gross energy digestibility of the broilers.

Histopathological results in Table 7, Fig. 1-3, revealed that β-mannanase supplementation, increase the villus length, villus width, crypt depth and absorbance surface area of duodenum and jejunum in both T2 (Lycell® group) and T3 (Hemicell® group), while the energy deficient diet (80 kcal kg^{-1}) showed negative impact on intestinal

histomorphology. These results are similar to those reported by Spring *et al.*[45] who observed a greater crypt depth in duodenum, jejunum, and ileum of broiler chickens fed a barley-based diet supplemented with β-mannanase. In contrast, Saeed *et al.*[8,47] and Hosseindoust[47] stated that crypt depth and villus height to crypt depth ratio were unaffected by supplementation of β-mannanase in diets.

The morphology of intestinal villi and crypt can be related to the function of intestine[48]. The increase in villus height indicates an increase in the absorptive surface area[49]. The addition of exogenous enzyme to the diet results in the decrease of digesta viscosity which is thought to be the reason behind the improvement of intestinal morphology[50] Lowering the stressors imposed on the intestinal mucosa could significantly enhance the morphology of intestine[51]. On the other hand, it was reported that crypt depth and villus height to crypt depth ratio were unaffected by supplementation of β-mannanase in diets[47]. However, large crypt depth can lead to poor nutrients absorption and lower performance of boiler[51]. Results of Chichlowski *et al.*[52], Yang[53] and Olnood *et al.*[54] agree with the present study; they observed significant increment in villus height and villus height:crypt depth ratio and non-significant effect on crypt depth as affected by probiotics administration.

CONCLUSION

This study demonstrated that the reduction of -80 kcal kg^{-1} in the energy level negatively affected broiler performance and β-mannanase either Lycell® or Hemicell® supplementation to low energy diet improved the final weight, weight gain, FCR, carcass traits, chicken health and intestinal histomorphology. However, there was no significant effect of β-mannanase on the DMD and OMD digestibility in broilers. Therefore, β-mannanase supplementation should be considered when low energy diets are formulated.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Applied Feed Research House (AFRH), Orabi Community, Cairo, Egypt. and Alivira; animal health company from India for their financial and technical support of this study

REFERENCES

1. Caldas, J.V., K. Vignale, N. Boonsinchai, J. Wang, M. Putsakum, J.A. England and C.N. Coon 2018. The effect of β-mannanase on nutrient utilization and blood parameters in chicks fed diets containing soybean meal and guar gum. Poult. Sci., 97: 2807-2817.
2. De Barros, V.R.S.M., G.R.Q. Lana, S.R.V. Lana, A.M.Q. Lana, F.S.A. Cunha and J.V.E. Neto, 2015. β-mannanase and mannan oligosaccharides in broiler chicken feed. Ciencia Rural, 45: 111-117.
3. Graham, K.K., M.S. Kerley, J.D. Firman and G.L. Allee, 2002. The effect of enzyme treatment of soybean meal on oligosaccharide disappearance and chick growth performance. Poult. Sci., 81: 1014-1019.
4. Lee, J.T., C.A. Bailey and A.L. Cartwright, 2003. β-mannanase ameliorates viscosity-associated depression of growth in broiler chickens fed guar germ and hull fractions. Poult. Sci., 82: 1925-1931.
5. Bedford, M.R., 2000. Exogenous enzymes in monogastric nutrition-their current value and future benefits. Anim. Feed Sci. Technol., 86: 1-13.
6. Daskiran, M., R.G. Teeter, D. Fodge and H.Y. Hsiao, 2004. An evaluation of endo-β-D-mannanase (Hemicell) effects on broiler performance and energy use in diets varying in β-mannan content. Poult. Sci., 83: 662-668.
7. Jackson, M.E., K. Geronian, A. Knox, J. McNab and E. McCartney, 2004. A dose-response study with the feed enzyme beta-mannanase in broilers provided with corn-soybean meal based diets in the absence of antibiotic growth promoters. Poult. Sci., 83: 1992-1996.
8. Saeed, M., T. Ayaşan, M. Alagawany, M.E.A. El-Hack, M.A. Abdel-Latif and A.K. Patra, 2019. The role of β-mannanase (Hemicell) in improving poultry productivity, health and environment. Braz. J. Poult. Sci., Vol. 21, No. 3 10.1590/1806-9061-2019-1001
9. North, M.O., 1981. Commercial Chicken Production. 2nd Edn., AVI Publication Co. Inc., Westpost Connecticut, USA.
10. Harrison, G.J. and L.R. Harrison, 1986. Clinical Avian Medicine and Surgery: Including Aviculture. WB Saunder Company, Philadelphia, London, Toronto, ISBN-13:978-0721612416, Pages: 717.
11. Coles, E.H., 1967. Veterinary Clinical Pathology. 2nd Edn., W.B. Saunders Company, Philadelphia, USA.
12. Ross, J.G., G. Christie, W.G. Halliday and R.M. Jones 1976. Determination of haematology and blood chemistry values in healthy six week old broiler hybrids. Avian Pathol., 5: 273-281.
13. Weiss, D.J. and K.J. Wardrop, 2011. Schalm's Veterinary Hematology. 6th Edn., John Wiley & Sons, New York, ISBN: 9780470961834, Pages: 1232.
14. Henry, R.J., D.C. Cannon and W. Winkleman, 1974. Clinical Chemistry: Principles and Techniques. 1st Edn., Harper and Row Publishers, New York, ISBN: 0061411817.
15. Doumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta, 31: 87-96.
16. Vermelho, A.B. and S. Couri, 2013. Methods to Determine Enzymatic Activity. Bentham Science Publishers, Rio de Janeiro, Brazil, Pages: 322.

17. Bartels, H., M. Bohmer and C. Heierli, 1972. [Serum creatinine determination without protein precipitation]. Clinica Chimica Acta, 37: 193-197, (In German).
18. Allain, C.C., L.S. Poon, C.S.G. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.
19. Barker, K.J., J.L. Purswell, J.D. Davis, H.M. Parker, M.T. Kidd, C.D. McDaniel and A.S. Kiess, 2010. Distribution of bacteria at different poultry litter depths[1]. Int. J. Poult. Sci., 9: 10-13.
20. Shaltout, F.A., I.M. Zakaria and M.E. Nabil, 2017. Incidence of some anaerobic bacteria isolated from chicken meat products with special reference to *Clostridium perfringens*. Nutr. Food Toxicol., 33: 292-304.
21. Ceuickahnk, R., J.P. Duguid, B.P. Marimann and R.H.A. Swain, 1975. Medical Microbiology. 12th Edn., Churchill Livingston, Edinburgh.
22. Brewer, J.H. and D.L. Allgeier, 1966. Safe self-contained carbon dioxide-hydrogen anaerobic system. Applied Microbiol., 14: 985-988.
23. Suvarna, K.S., C. Layton and J.D. Bancroft, 2018. Bancroft's Theory and Practice of Histological Techniques. 8th Edn., Elsevier Health Sciences United States Page: 672.
24. Iji, P.A., A. Saki and D.R. Tivey, 2001. Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. Br. Poult. Sci., 42: 505-513.
25. Incharoen, T., K. Yamauchi, T. Erikawa and H. Gotoh, 2010. Histology of intestinal villi and epithelial cells in chickens fed low-crude protein or low-crude fat diets. Ital. J. Anim. Sci., 9: 429-434.
26. Oliveira, M.C.D., D.M.D. Silva and D.M.B. Dias, 2013. Effect of feed restriction on organs and intestinal mucosa of growing rabbits. Rev. Bras. Zootec., 42: 530-534.
27. AOAC., 2002. Official Methods of Analysis of the Association of Official Analytical Chemists. 17th Edn., Association of Official Analytical Chemists Inc., USA.
28. SAS, 1999. SAS/STAT User's Guide, Version 8. Vol. 2, SAS Institute, Inc., Cary, NC Pages: 2552.
29. Duncan, C.J.G., N. Pugh, D.S. Pasco and S.A. Ross, 2002. Isolation of a galactomannan that enhances macrophage activation from the edible fungus *Morchella esculenta*. J. Agric. Food Chem., 50: 5683-5685.
30. Anderson, D.M., J.L. McNaughton, H.Y. Hsiao and D.W. Fodge, 2001. Improvement of body weight uniformity in broilers, turkeys, ducks and pigs by use of the *Bacillus lentus* mannanase (Hemicell®). Paper 36 (Abstr.) in International Poultry Scientific Forum. Southern Poultry Science Society and Southern Conference on Avian Diseases, Athens, GA.
31. Hsiao, H.Y., D.M. Anderson and N.M. Dale, 2006. Levels of β-mannan in soybean meal. Poult. Sci., 85: 1430-1432.
32. Zou, X.T., X.J. Qiao and Z.R. Xu, 2006. Effect of β-mannanase (Hemicell) on growth performance and immunity of broilers. Poult. Sci., 85: 2176-2179.
33. Alagawany, M., A.I. Attia, Z.A. Ibrahim, R.A. Mahmoud and S.A. El-Sayed, 2017. The effectiveness of dietary sunflower meal and exogenous enzyme on growth, digestive enzymes, carcass traits and blood chemistry of broilers. Environ. Sci. Pollut. Res., 24: 12319-12327.
34. Opalinski, M., A. Maiorka, F.d. Cunha, C.d Rocha and S.A. Borges, 2010. Adição de complexo enzimático e da granulometria da soja integral desativada melhora desempenho de frangos de corte. Ciência Rural, 40: 628-632.
35. Zaib ur Rehman, T. Aziz, S.A. Bhatti, G. Ahmad and J. Kamran *et al*., 2016. Effect of β-mannanase on the performance and digestibility of broilers. Asian J. Anim. Vet. Adv., 11: 393-398.
36. Hajati, H., 2010. Effects of enzyme supplementation on performance, carcass characteristics, carcass composition and some blood parameters of broiler chicken. Am. J. Anim. Vet. Sci., 5: 221-227.
37. Mehri, M., M. Adibmoradi, A. Samie and M. Shivazad, 2010. Effects of β-Mannanase on broiler performance, gut morphology and immune system. Afr. J. Biotechnol., 9: 6221-6228.
38. Zhang, L. and I.R. Tizard, 1996. Activation of a mouse macrophage cell line by acemannan: The major carbohydrate fraction from *Aloe vera* gel. Immunopharmacology, 35: I19-128.
39. Li, Y.H., X. Chen, Y.Q. Chen, Z.M. Li and Y.H. Cao, 2010. Effects of β-mannanase expressed by *Pichia pastoris* in corn-soybean meal diets on broiler performance, nutrient digestibility, energy utilization and immunoglobulin levels. Anim. Feed Sci. Technol., 159: 59-67.
40. Duncan, C.J.G., N. Pugh, D.S. Pasco and S.A. Ross, 2002. Isolation of a galactomannan that enhances macrophage activation from the edible fungus *Morchella esculenta*. J. Agric. Food Chem., 50: 5683-5685.
41. Lee, J.T., S. Connor-Appleton, C.A. Bailey and A.L. Cartwright, 2005. Effects of guar meal by-product with and without β-mannanase hemicell on broiler performance. Poult. Sci., 84: 1261-1267.
42. Mohayayee, M. and K. Karimi, 2012. The effect of guar meal (germ fraction) and β-mannanase enzyme on growth performance and plasma lipids in broiler chickens. Afr. J. Biotechnol., 11: 8767-8773.
43. Sundu, B., A. Kumar and J. Dingle, 2006. Response of broiler chicks fed increasing levels of copra meal and enzymes. Int. J. Poult. Sci., 5: 13-18.
44. Shastak, Y., P. Ader, D. Feuerstein, R. Ruehle, and M. Matuschek, 2019. β-Mannan and mannanase in poultry nutrition. World's Poult. Sci. J., 71: 161-174.
45. Spring, P., C. Wenk, K.A. Dawson and K.E. Newman, 2000. The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. Poult. Sci., 79: 205-211.

46. Read, N.W., 1986. Dietary Fiber and Bowel Transit. In: Dietary Fiber. Vahouny, G.V. and D. Kritchevsky (Eds.). Springer, Boston, MA United States pp: 81-100.
47. Hosseindoust, A., S. Lee, W.G. Nho, Y.H. Song and J.S. Shin *et al*., 2019. A dose–response study to evaluate the effects of pH-stable β-mannanase derived from *Trichoderma citrinoviride* on growth performance, nutrient retention, and intestine morphology in broiler chickens. Ital. J. Anim. Sci., 18: 147-154.
48. Tarachai, P. and K. Yamauchi, 2000. Effects of luminal nutrient absorption, intraluminal physical stimulation and intravenous parenteral alimentation on the recovery responses of duodenal villus morphology following feed withdrawal in chickens. Poult. Sci., 79: 1578-1585.
49. Zulkifli, I., H.S.I. Rahayu, A.R. Alimon, M.K. Vidyadaran and S.A. Babjee, 2009. Gut microflora and intestinal morphology of commercial broiler chickens and red jungle fowl fed diets containing palm kernel meal. Arch. Geflugelk., 73: 49-55.
50. Yasar, S. and J.M. Forbes, 2000. Enzyme supplementation of dry and wet wheat-based feeds for broiler chickens: Performance and gut responses. Br. J. Nutr., 84: 297-307.
51. Xu, Z.R., C.H. Hu, M.S. Xia, X.A. Zhan and M.Q. Wang, 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult. Sci., 82: 1030-1036.
52. Chichlowski, M., W.J. Croom, F.W. Edens, B.W. McBride and R. Qiu *et al*., 2007. Microarchitecture and spatial relationship between bacteria and ileal, cecal and colonic epithelium in chicks fed a direct-fed microbial, PrimaLac and salinomycin. Poult. Sci., 86: 1121-1132.
53. Yang, Y., P.A. Iji and M. Choct, 2009. Dietary modulation of gut microflora in broiler chickens: A review of the role of six kinds of alternatives to in-feed antibiotics. World's Poult. Sci. J., 65: 97-114.
54. Olnood, C.G., S.S.M. Beski, M. Choct and P.A. Ijia, 2015. Novel probiotics: Their effects on growth performance, gut development, microbial community and activity of broiler chickens. Anim. Nutr., 1: 184-191.