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Performance, Carcass Characteristics, and Meat Quality of Broiler Chickens Fed β-Mannanase and Two Levels of Energy

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ABSTRACT

This study aimed at evaluating the response to supplementation of β-Mannanase with two levels of energy on performance, carcass yield, and meat quality of 1600 1-d-old straight run Indian River broilers which were randomly allotted to 4 dietary treatments (10 replicates/treatment, 40 chicks per replicate) in a 35-d feeding trial. The trial consisted of 2 phases, starter (0 to 14 d) and grower (15 to 35 d). The experiment was designed in a 2 x 2 factorial arrangement. The first factor was the energy level of the ration consisted of 2 levels, i.e., a low energy level with 2.900 kcal ME/kg during the starter phase and 3.000 kcal ME/kg during the grower phase; and a normal energy level with 3020 kcal ME/kg during starter phase and 3120 kcal ME/kg during grower phase, with variations based on the content of feed grade soybean oil. The second factor was the level of β-Mannanase in the diet consisted of 2 levels, i.e., ration without β-Mannanase (BETAMINUS: 0%) and ration with 0.05% β-Mannanase (BETAPLUS: 0.05%). Feeding low ME increased feed intake (p<0.05) during the overall phase (0 to 35 d) and normal ME decreased (p<0.05) feed conversion ratio during the starter and overall phases. Birds fed normal ME exhibited higher (p<0.05) hot and cold carcass weights. There was a significant interaction between dietary ME level and β-Mannanase to breast meat pH (p= 0.006), meat redness (a*) (p= 0.01), and meat yellowness (b*) (p= 0.0001). In conclusion, the results of enzyme supplementation did not elicit any noticeable response pertaining to productive performance, carcass characteristics, or meat quality (except pH and meat color). Moreover, feeding low dietary ME and β-Mannanase did not compromise overall broiler chickens performance.

Keywords: β-Mannanase; broilers; meat quality; metabolizable energy; performance

INTRODUCTION

In Jordan, corn and soybean meal (SMB) are considered the principal ingredients used in poultry diets. Their direct utilization by birds affects performance, as they are the primary sources of energy and protein in diets. As standard ingredients in Jordanian commercial broiler diets, corn and SBM contain certain dietary non-starch polysaccharides (NSPs).

Dietary NSPs are ingestible by poultry; however, they represent an untapped source of energy that can be exploited via dietary supplementation with a suitable exogenous enzyme (Shastak *et al.,* 2015; Arsenault *et al.,* 2017; Alqhtani *et al.,* 2022). Soybean meal contains 3% soluble NSPs and 16% insoluble NSPs encompassing mannans and galactomannans (Slominski, 2011). Beta-mannan (β-mannan), also termed beta-galactomannan (β-GAL), is a polysaccharide that consists of repeating units of mannose joined together via β-(1,4) glycosidic linkages and occurs as a structural component of plant cell walls (Saeed *et al.,* 2019). The negative impact of β-mannan on broiler performance is well documented and is attributed mainly to poor digestibility because

poultry lack endogenous enzymes to cleave β-(1,4) linkages (Shastak *et al.,* 2015; Kim *et al.,* 2017). Beta-mannans found in SBM can stimulate an innate immune response that can compromise bird performance and simulate nonproductive energy draining innate immune responses (Mohammadigheisar *et al.,* 2021). This unnecessary stimulation of the innate immune system expends energy futilely that can otherwise be used for growth and performance (Scapini *et al.,* 2018).

The benefits of supplementing beta-Mannanase (β-Mannanase) in broiler diets are well-documented (Mohammadigheisar *et al.,* 2021). Inclusion of β-Mannanase has been shown to increase apparent metabolizable energy corrected for nitrogen (AMEn), reduce feed conversion ratio (FCR), increase body weight (BW), improve feed intake (FI), improve carcass yield (CY) and organ weights, reduce gut viscosity, and reduce immune challenge caused by the presence of β-mannan in diets (Mussini *et al.,* 2011; Williams *et al.,* 2014; Rehman *et al.,* 2016; Latham *et al.,* 2016; Balasubramanian *et al.,* 2018; Hussein *et al.,* 2019).

Since β-Mannanase has been shown to increase metabolizable energy (ME) in broiler diets, the enzyme

may be utilized to reduce the cost of production via inclusion in low ME diets (Cho & Kim, 2013; Ferreira *et al.*, 2016; Lee *et al.*, 2018; Alqhtani *et al.*, 2022). This leads to an improvement in feed utilization and birds can compensate for the reduction of ME in the diet (Scapini *et al.*, 2018), and enzyme supplementation can help broiler producers to improve feed energetic efficiency, reduce cost, and increase revenue by utilizing lower ME diets.

There is a continuous increase in feed prices globally which is linked to the higher demand from developing countries and the competition with biofuel energy production. Corn and soybean meal (SBM) are the principal sources of energy and protein in poultry feed in most countries, especially Jordan. The continuous concerns for the poultry producers are to decrease feed cost per unit of production and increase the nutritional value of feed ingredients like corn and SBM by adjusting dietary ME through the use of enzyme activities such as *β*-Mannanase. This enzyme might enhance the apparent ME of corn and SBM based diets for broiler chickens by improving nutrient and energy availability without impacting bird performance. This will potentially lead to economic advantages for producers all over the world. In Jordan, feed constitutes 70% to 75% of the total cost of broiler production (Alhammd, 2020).

At the time this trial was conducted, *β*-Mannanase had just been introduced in the Jordanian market, and a workshop was held to explain the efficacy of this enzyme and how its supplementation can provide encouraging outcomes when used with regular corn soybean diets. Therefore, the objective of this study was to investigate the effect of supplemental *β*-Mannanase with two levels of ME and their interaction on production, carcass quality, and meat quality attributes in broiler chickens fed a regular corn/soybean meal-based diet (standard diet used by broiler producers in Jordan). We hypothesized that the inclusion of *β*-Mannanase would maximize feed utilization by enhancing the energetic efficiency of digestion, which could improve the utilization of nutrients, thus positively impacting performance, carcass characteristics, and meat quality attributes.

MATERIALS AND METHODS

Ethical Approval

All procedures about bird handling were implemented following animal handling and welfare guidelines set forth by the Jordanian Society for the Protection of Animals (Approval No. 2246/37). Approval for animal handling was obtained through the Deanship of Scientific Research at The University of Jordan.

Experimental Design

A total of one thousand six hundred 1-d-old Indian River straight-run broiler chickens (BW= 43±1 g) were used in a 35-d research trial. The trial consisted of two phases, starter (0 to 14 d) and grower (15 to 35 d). Birds were randomly allotted to 4 dietary treatments with 10 replicate pens per treatment with 40 birds per pen. The

trial was a randomized complete block design with 4-floor pens representing a complete block for a total of 10 blocks. The treatments were a 2 x 2 factorial arrangement. The first factor was the ME with two levels, i.e., 1) low energy ration with 2.900 kcal ME/kg at the starter (0 to 14 d) and 3.000 kcal ME/kg at the grower (15 to 35 d) and 2) normal energy ration with 3.020 kcal ME/kg at the starter (0 to 14) and 3.120 kcal ME/kg at the grower (15 to 35 d). Variations in dietary ME levels were based on the content of feed-grade soybean oil (starter: 0% vs. 1.2%; grower: 0.40% vs. 2.1%) and corn (starter: 58.89% vs. 57.60; grower: 63.88 vs. 61.63)]. The second factor was the level of *β*-Mannanase consisted of 2 levels, i.e., 1) ration without *β*-Mannanase (BETAMINUS: 0%) and 2) ration with 0.05% *β*-Mannanase (BETAPLUS: 0.05%).

Birds and Housing

Indian River broiler chicks acquired from a local hatchery were reared from 1-d old in an open-sided house on floor pens of similar size (2.5 x 1.85 m^2) and wood shaving was used as litter material at a depth of 5 cm. Room temperature was maintained at 33 °C during the first week and gradually reduced to 21 °C until the end of the research trial. Birds were given *ad libitum* access to feed and water and fed experimental diets from 1-d old until the end of the trial (35 days old). Pens had a daily lighting regimen of 20 h of light and 4 h of dark, except for the first three days when 24 h of light was used to get birds acclimatized to the surrounding environment.

Diets

All diets were formulated according to the nutrient specifications of the breeder's manual and to meet the requirements of the National Research Council (1994) for broiler requirements for each nutrient except for energy levels in the low ME diets (Table 1). Diets were regular corn-soybean diets provided in pellet form throughout the feeding trial and fed over two phases (starter: 0 to 14 d; finisher: 15 to 35 d).

The enzyme was provided by Elanco Animal Health (Greenfield, IN) under the trademark name Hemicell™ HT. Hemicell is a dry *β*-Mannanase (EC 3.2.1.78) obtained via fermentation from *Paenibacilluslentus* (mannanolytic bacterium species) with a *β*-Mannanase no less than 160 x 10^6 units/kg. One unit of *β*-Mannanase activity is defined as the amount of enzyme which generates 0.72 micrograms of reducing sugars per minute from a mannose-containing substrate at pH 7.0 °C and 40 °C. The enzyme was added on top at a level of 0% and 0.05% (500 g/ton).

Measurements

Production performance. Production parameters were measured on a weekly basis, including feed intake (FI), feed conversion (FCR), and body weight gain (BWG). Mortality was observed and recorded daily, and FI and FCR were corrected for mortality.

Table 1. Diet and calculated nutrient composition during starter and grower phases of broiler chickens (% as-fed basis)

Dietary ingredients	Starter (0 – 14 days)		Grower (15 -35 day)	
	Normal energy	Low energy	Normal energy	Low energy
Yellow corn	57.60	58.89	63.88	61.63
Soybean Meal (48%)	37.06	37.01	32.00	32.50
Soybean oil	1.20	-------	4.00	2.10
Limestone	1.40	1.40	1.30	1.30
Monocalcium phosphate	1.04	0.95	0.80	0.80
Sodium carbonate	0.11	0.11	0.10	0.10
Sodium chloride	0.25	0.25	0.23	0.23
Choline chloride (70%)	0.10	0.10	0.10	0.10
L-Lysine-HCl	0.32	0.32	0.32	0.32
DL-Methionine	0.35	0.35	0.29	0.29
L-Threonine	0.22	0.22	0.12	0.12
Vitamin premix[1]	0.15	0.15	0.10	0.10
Mineral premix[2]	0.15	0.15	0.10	0.10
Availa – Zn120[3]	0.01	0.01	0.04	0.04
Availa – Se 1000[4]	0.04	0.04	-------	-------
Maxiban[5]	0.05	0.05	0.05	0.05
Total	100	100	100	100
Calculated nutrient Composition (%)				
ME (kcal/kg)	3,000	2,900	3,120	3020
Crude Protein	23.00	23.00	20.00	20.00
Lysine[7]	1.26	1.26	0.96	0.96
Methionine[7]	0.60	0.60	0.48	0.48
Methionine + Cystine[7]	0.91	0.91	0.91	0.91
Threonine[7]	0.82	0.82	0.65	0.65
Calcium	0.98	0.98	0.80	0.80
Available phosphorus	0.45	0.45	0.38	0.38
Sodium	0.19	0.19	0.17	0.17

Note: [1]Supplied per kilogram of mix: Vitamin A, 30,870,000 IU; Vitamin D3, 13,230,000 IU; Vitamin E, 66.150 IU; Vitamin K3, 6.006 mg; Thiamin 6,174 mg; Riboflavin, 26.460 mg; Pyridoxine, 11.025 mg; Pantothenic acid 39.690 mg; Niacin, 154.350 mg; Folic acid, 3.528 mg; Biotin, 264 mg; Vitamin B12, 53 mg.
[2]Supplied per kilogram of mix: Iron, 33.5 g; Zinc, 214 g; Manganese, 300 g; Copper, 3.4 g; Iodine, 2.1 g; Selenium, 500 ¼g.
[3]Availa-Zn120: Organic zinc mineral source (12% Zn); Zinpro Corporation, Eden Prairie MN, USA.
[4]Availa-Se1000: Organic selenium source (1000 mg or 0.10% Se); Zinpro Corporation, Eden Prairie, MN, USA.
[5]Maxiban: Coccidiostat (narasin and nicarbazin) added at 0.50 kg/ton; Elanco Animal Health, Greenfield, IN, USA.
[6]Phyzyme XP: bacterial phytase (750 U/kg feed); Danisco Animal Heath, Marlborough, UK.
[7]Digestible amino acid values.

Slaughter and carcass measurements. At the end of the 35 days trial, 60 birds from each treatment (15 per replicate) were randomly selected, weighed, and fasted 10 h before slaughtering and processed under standard commercial conditions. Slaughtered birds were scalded, feathers were mechanically plucked using a rotary drum picker, and eviscerated. Feet, shanks, head, and neck were removed, and carcasses were immediately weighed to obtain post-slaughter hot carcass weight (HCW) minus giblets. Giblets represent the total yield of the liver, heart, and gizzard, which were excised in addition to the abdominal fat pad and weighed relative to live body weight. Carcasses were chilled for 24 h at 2 °C to 4 °C and then weighed again to calculate cold carcass weight (CCW) and determine carcass yield as a % of live weight. Carcasses were then dissected for further processing to obtain various commercial parts (breast, thighs, wings, and neck) and abdominal fat pad to determine each part's yield. Each part was weighed separately, put in sealed plastic bags and stored at -25

°C for further chemical analyses. Cuts were related to cold carcass weight and expressed as a percentage.

Breast meat quality measurements. Meat quality characteristics were evaluated on excised breasts (15 per replicate and 60 per treatment). Frozen breasts were thawed overnight in a refrigerator at 4 °C while the muscles were still in plastic bags. Samples were removed from bags and weighed, and whole breast samples were cut up and manually deboned. The left and right *pectoralis major* muscles were excised for meat quality measurements, including pH, meat color, cooking loss, shear force, and water holding capacity (Abdullah & Matarneh, 2010). The right *pectoralis major* was used to measure pH, meat color, and water holding capacity, while the left muscle was used to measure cooking loss and shear force.

Breast meat pH values were determined by using the iodoacetate method described by (Alierzalua *et al.*, 2019). From 1.0 g to 1.5 g of raw muscle were put into a

test tube containing 10 mL of neutralized 5 mM iodoacetate reagent and 150 mM KCL and homogenized using a homogenizer (Ultra-Turrax T8, IKA Labortechnik, Janke & Kunkal GmbH & Co., Germany). Before recording the pH, the values of the solutions on a pH meter (pH spear, model 35634-40, Eurotech Instruments, Malaysia). The ultimate pH (pH_u) was measured 24 h after the slaughter at three points on the cranial area of the superficial pectoral muscle at about 5 cm from the sternum line.

Color measurements were taken on the same area as pH for each sample using a colorimeter (12 MM Aperture U 59730-30, Cole-Parameter International Inc, Pittsford, NY, USA). Three measurements were taken at each point on the medial portion of the pectoralis muscle. Colors for each sample were expressed in terms of values for lightness (L*), redness (a*), and yellowness (b*) of the breast meat.

Water holding capacity (WHC) was measured using the method described by Obeidat et al. (2016) using a sample of the initial weight of 5 g of raw meat (1 sample per replication). Each sample was cut into smaller pieces and covered with two filter papers (qualitative, 185 mm circles, fine crystalline retention) and two thin plates of quartz material, then pressed with a weight of 2500 gm for 5 min. The meat samples were then removed from filter paper and their weight was recorded (final weight). Water holding capacity was calculated as the difference between the sample's initial and final weight divided by the initial sample weight and expressed as a percentage.

Each left pectoralis major muscle was weighed and individually put in sealed polyethylene bags to measure cooking loss. The bags were cooked in a thermostatically controlled water bath for 25 min at 85 °C to achieve the maximum internal temperature of 80 °C. Samples were then removed and put under running cold water to cool down for 45 min, then well dried to remove excess surface moisture and re-weighed. Cooking loss was reported as weight lost during cooking divided by fresh sample weight and expressed as a percentage (Obiedat et al., 2016).

Cooked pieces of meat were cut to obtain 6 cores (20 x 13 x 13 mm) on each breast sample using a cylindrical metal that measures 1.25 cm in diameter to determine the shear-force of meat according to Obiedat et al. (2016), (Warner-Bratzler Meat Shear Apparatus/ INSTRON, G-R manufacturing CO. 1317 Collins LN, Manhattan, KS). This apparatus measures the maximum strength in kg/cm^2.

Statistical Analysis

Data were analyzed using the mixed model procedure (PROC MIXED) of SAS® 9.4 (2013) using repeated measures analysis for a randomized complete block design with a 2 x 2 factorial arrangement of treatments. Data were analyzed using a two-way ANOVA for the main fixed effects of enzyme and/or energy level and their interaction. Block was included as a random effect in the model to minimize variation among experimental units due to air draft near the main door of the poultry barn. Adjusted Tukey's test was used to separate means. The level of significance was stated at $p \leq 0.05$; tendencies (trends) were reported where $0.05 \leq p \leq 0.10$.

RESULTS

Production Performance

The level of ME had a significant effect on FI during the 35 days trial ($p < 0.05$), where birds fed the low ME diet consumed 235 g more than those fed the normal ME diet (Table 2). No significant effects were observed about enzyme supplementation or interaction throughout the trial or during the starter and/or grower phases. Birds fed the normal ME diet were significantly more efficient and exhibited a significantly ($p < 0.05$) lower FCR value than those fed a low ME diet during the starter phase and the whole duration of the trial and were numerically lower during the grower phase (Table 3). There were no differences in FCR values vis-a-vis enzyme supplementation or interaction during starter and/or grower phases or the whole trial. Neither ME level nor β-Mannanase significantly affected the BWG of birds throughout the whole experiment or during the starter and grower phases (Table 4).

Carcass Characteristics and Relative Organ Weights

Our results (Table 5) showed that broilers fed normal ME had significantly ($p < 0.05$) greater hot (HCW) and cold (CCW) carcass weights than those fed low ME at the end of the trial. There were no significant differences pertaining to the effect of β-Mannanase or interaction with ME level on HWC, CCW, carcass yield, or relative organ weights (heart, liver, or gizzard). There were no significant effects on relative weights of cold cuts (Table 6) except for neck relative weight, which was significantly ($p < 0.05$) greater by 0.82% for broilers fed the normal ME diet in contrast to those fed a low ME diet.

Breast Meat Quality

ME level, β-Mannanase, and/or their interaction had no significant impact on breast shear force or WHC (Table 7). Breast CL was numerically ($p < 0.06$) affected by an interaction between ME level and β-Mannanase such that chicks fed normal ME and BETAMINUS exhibited the highest cooking loss of breast meat in contrast to the other diets, with those fed normal ME and BETAPLUS showing the lowest CL value among treatments. Our results (Table 7) indicated that breast meat pH was significant ($p < 0.05$) for birds fed low ME with and without β-Mannanase (BETAPLUS, and BETAMINUS) in addition to those fed normal ME without enzyme in comparison to those fed normal ME and BETAPLUS birds. Moreover, breast pH for birds fed low ME was greater than normal ME, and for BETAMINUS vs. BETAPLUS as the main effects ($p < 0.05$). A significant interaction ($p < 0.05$) between ME level and enzyme showed that birds fed normal energy and BETAPLUS exhibited lower breast pH value than the three other treatments

Table 2. Feed intake (g/bird) of broiler chickens treated with different metabolizable energy levels and supplemental β-Mannanase

Treatments		Period of trial		
ME level	With or without enzyme[1]	Starter (0-14 days)[2]	Grower (15-35 days)[2]	Total (0-35 days)[2]
Normal	BETAPLUS[3]	601.40	3037.20	3639.00
Normal	BETAMINUS[3]	613.20	3097.60	3710.80
Low	BETAPLUS	632.40	3230.80	3863.00
Low	BETAMINUS	618.00	3338.60	3956.80
SEM[4]		15.082	134.950	132.280
Main effects				
ME level				
Normal		607.30	3067.40	3674.90[a]
Low		625.20	3284.70	3909.90[b]
SEM		10.665	109.840	110.460
Enzyme				
BETAPLUS		616.90	3134.00	3751.00
BETAMINUS		615.60	3218.10	3833.80
SEM		10.662	109.840	110.84
Statistical probabilities[5]				
ME level		NS	0.07	0.04
Enzyme		NS	NS	NS
Interaction[6]		NS	NS	NS

Note: [a-b] Means with different superscripts differ significantly (p<0.05).
[1]β-Mannanase supplemented to either normal and/or low ME diets (0% or 0.05% added on top). [2]Starter phase (0-14 days of age); Grower phase (15-35 days age); Whole trial period (0-35 days of age). [3]BETAPLUS (diet with β-Mannanase); BETAMINUS: (diet without β-Mannanase). [4]SEM: Standard error of the mean. [5]Level of significance was set at (p<0.05). [6]Interaction: ME Level x Enzyme.

Table 3. Feed conversion ratio (g feed : g body weight gain) of broiler chickens treated with different metabolizable energy level and supplemental β-Mannanase

Treatments		Period of trial		
ME level	With or without enzyme[1]	Starter (0-14 days)[2]	Grower (15-35 days)[2]	Total (0-35 days)[2]
Normal	BETAPLUS[3]	1.20	1.66	1.58
Normal	BETAMINUS[3]	1.29	1.60	1.54
Low	BETAPLUS	1.38	1.78	1.70
Low	BETAMINUS	1.38	1.76	1.69
SEM[4]		0.047	0.089	0.065
Main effects				
ME level				
Normal		1.29[a]	1.63	1.55[a]
Low		1.38[b]	1.77	1.69[b]
SEM		0.036	0.074	0.056
Enzyme				
BETAPLUS		1.33	1.71	1.64
BETAMINUS		1.33	1.68	1.61
SEM		0.036	0.074	0.056
Statistical probabilities[5]				
ME level		0.05	0.08	0.04
Enzyme		NS	NS	NS
Interaction[6]		NS	NS	NS

Note: [a-b] Means with different superscripts differ significantly (p<0.05).
[1]β-Mannanase supplemented to either normal and/or low ME diets (0% or 0.05% added on top). [2]Starter phase (0-14 days of age); Grower phase (15-35 days age); Whole trial period (0-35 days of age). [3]BETAPLUS (diet with β-Mannanase); BETAMINUS: (diet without β-Mannanase). [4]SEM: Standard error of the mean. [5]Level of significance was set at (p<0.05). [6]Interaction: ME Level x Enzyme.

with a difference of 0.07 in contrast to the highest value (5.88 vs. 5.95).

The effect of ME and β-Mannanase on breast meat color is shown in (Table 8). The L* value of broilers fed low ME was significantly greater (p<0.05) than those fed normal ME (45.51 vs. 44.4, respectively). Moreover, birds supplemented with β-mannanase exhibited a greater L* (p<0.05) value for breast meat than those

Table 4. Body weight gain (g/bird) of broiler chickens treated with different metabolizable energy level and supplemental β-Mannanase

Treatments		Period of trial		
ME level	With or without enzyme[1]	Starter (0-14 days)[2]	Grower (15-35 days)[2]	Total (0-35 days)[2]
Normal	BETAPLUS[3]	471.20	1840.40	2311.80
Normal	BETAMINUS[3]	473.80	1948.80	2422.60
Low	BETAPLUS	457.60	1826.20	2283.80
Low	BETAMINUS	448.20	1896.40	2344.60
SEM[4]		13.148	59.773	63.693
Main effects				
ME level				
Normal		464.40	1894.60	2367.80
Low		452.90	1861.40	2314.20
SEM		10.779	42.266	45.038
Enzyme				
BETAPLUS		464.40	1813.30	2297.80
BETAMINUS		461.00	1922.60	2383.60
SEM		10.779	42.266	45.038
Statistical probabilities[5]				
ME level		NS	NS	NS
Enzyme		NS	NS	NS
Interaction[6]		NS	NS	NS

Note: [1]β-Mannanase supplemented to either normal and/or low ME diets (0% or 0.05% added on top). [2]Starter phase (0-14 days of age); Grower phase (15-35 days age); Whole trial period (0-35 days of age). [3]BETAPLUS (diet with β-Mannanase); BETAMINUS: (diet without β-Mannanase). [4]SEM: Standard error of the mean. [5]Level of significance was set at ($p<0.05$). [6]Interaction: ME Level x Enzyme.

Table 5. Hot and cold carcass weights, carcass yield, and percentages of heart, liver, and gizzard of broiler chickens treated with different metabolizable energy level and supplemental β-Mannanase

Treatments		Slaughtering variables					
ME level	With or without[1] enzyme	HCW (g)[2]	CCW (g)[2]	Carcass yield (%)[3]	Heart (%)[4]	Liver (%)[4]	Gizzard (%)[4]
Normal	BETAPLUS[5]	1874.00	1871.25	76.00	0.575	2.38	1.22
Normal	BETAMINUS[5]	1864.60	1857.90	75.88	0.605	2.35	1.24
Low	BETAPLUS	1788.40	1783.30	76.24	0.598	2.30	1.24
Low	BETAMINUS	1773.50	1765.90	76.31	0.604	2.40	1.22
SEM[6]		55.221	54.371	0.854	0.021	0.152	0.056
Main effects							
ME level							
Normal		1869.30[a]	1864.57[a]	75.94	0.59	2.36	1.23
Low		1780.95[b]	1777.60[b]	75.76	0.601	2.34	1.22
SEM		46.048	45.004	0.602	0.017	0.142	0.041
Enzyme							
BETAPLUS		1831.20	1827.77	76.13	0.587	2.34	1.23
BETAMINUS		1819.05	1811.90	75.60	0.604	2.38	1.23
SEM		46.048	45.004	0.602	0.017	0.142	0.041
Statistical probabilities[7]							
ME level		0.04	0.04	NS	NS	NS	NS
Enzyme		NS	NS	NS	NS	NS	NS
Interaction[8]		NS	NS	NS	NS	NS	NS

Note: [a-b] Means with different superscripts differ significantly ($p<0.05$).
[1]β-Mannanase supplemented to either normal and/or low ME diets (0% or 0.05% added on top). [2]HCW: Hot carcass weight; CCW: Cold carcass weight. [3]Carcass yield = (cold carcass/live weight)*100%. [4]Relative organ weight= (organ weight/live weight)*100%. [5]BETAPLUS (diet with β-Mannanase); BETAMINUS: (diet without β-Mannanase). [6]SEM: Standard error of the mean. [7]Level of significance was set at $p<0.05$. [8]Interaction: ME Level x Enzyme.

Table 6. Percent cold cuts of broiler chickens treated with different metabolizable energy level and supplemental β-Mannanase

Treatments		Slaughtering variables					
ME level	With or without[1] enzyme	Fat pad (%)[2]	Wings (%)[2]	Thigh (%)[2]	Breast (%)[2]	Back (%)[2]	Neck (%)[2]
Normal	BETAPLUS[3]	0.924	9.70	26.84	37.23	12.19	12.93
Normal	BETAMINUS[3]	0.920	9.45	26.56	37.42	12.35	13.12
Low	BETAPLUS	0.928	9.74	26.52	38.20	12.50	12.11
Low	BETAMINUS	0.978	9.64	26.99	37.14	12.53	12.43
SEM[4]		0.087	0.197	0.401	0.576	0.341	0.494
Main effects							
ME level							
Normal		0.922	9.58	26.70	37.52	12.27	13.02[a]
Low		0.953	9.69	26.66	37.67	12.51	12.27[b]
SEM		0.074	0.146	0.284	0.422	0.252	0.447
Enzyme							
BETAPLUS		0.926	9.72	26.58	37.71	12.34	12.52
BETAMINUS		0.949	9.55	26.78	37.28	12.44	12.77
SEM		0.074	0.146	0.284	0.422	0.252	0.447
Statistical probabilities[5]							
ME level		NS	NS	NS	NS	NS	0.01
Enzyme		NS	NS	NS	NS	NS	NS
Interaction[6]		NS	NS	NS	NS	NS	NS

Note: [a-b] Means with different superscripts differ significantly (p<0.05).
[1]β-Mannanase supplemented to either normal and/or low ME diets (0% or 0.05% added on top). [2]Cuts Relative Weight= (cut weight/cold carcass weight)*100%. [3]BETAPLUS (diet with β-Mannanase); BETAMINUS: (diet without β-Mannanase). [4]SEM: Standard error of the mean. [5]Level of significance was set at p<0.05. [6]Interaction: ME Level x Enzyme.

Table 7. Breast meat quality of broiler chickens treated with different metabolizable energy level and supplemental β-mannanase

Treatments		Meat quality attributes			
ME level	With or without enzyme[1]	Breast SF (kg/cm²)[2]	Breast WHC (%)[2]	Breast CL (%)[2]	Breast pH
Normal	BETAPLUS[3]	2.41	39.12	26.49	5.88[b]
Normal	BETAMINUS[3]	2.39	38.42	29.23	5.94[a]
Low	BETAPLUS	2.03	37.76	28.87	5.94[a]
Low	BETAMINUS	2.27	38.47	27.25	5.95[a]
SEM[4]		0.183	1.634	1.086	0.009
Main effects					
ME level					
Normal		2.4	38.77	27.86	5.91[b]
Low		2.15	38.11	28.06	5.95[a]
SEM		0.145	1.153	0.767	0.006
Enzyme					
BETAPLUS		2.22	38.44	27.68	5.90[b]
BETAMINUS		2.33	38.44	28.24	5.95[a]
SEM		0.145	1.153	0.767	0.006
Statistical probabilities[5]					
ME level		NS	NS	NS	0.002
Enzyme		NS	NS	NS	0.002
Interaction[6]		NS	NS	0.06	0.006

Note: [a-b] Means with different superscripts differ significantly (p<0.05).
[1]β-Mannanase supplemented to either normal and/or low ME diets (0% or 0.05% added on top). [2]Breast SF: Shear force; Breast WHC: Water holding capacity; Breast CL: Cooking loss. [3]BETAPLUS (diet with β-Mannanase); BETAMINUS: (diet without β-Mannanase). [4]SEM: Standard error of the mean. [5]Level of significance was set at p<0.05. [6]Interaction: ME Level x Enzyme.

without supplementation. The a* value showed an interaction between ME and enzyme as values for broiler fed normal ME and BETAMINUS were significantly lower (p<0.05) in contrast to the three other treatments. Also, birds fed a low ME had greater (p<0.05) value of a* in contrast to normal ME (3.25 vs. 3.12, respectively).

Table 8. Effects of metabolizable energy level and supplemental β-mannanase on meal color

Treatments		Meat color variables		
ME level	With or without enzyme[1]	L[2]	a[2]	b[2]
Normal	BETAPLUS[3]	45.18	3.33[a]	17.99[b]
Normal	BETAMINUS[3]	44.31	2.91[b]	16.66[c]
Low	BETAPLUS	45.66	3.23[a]	17.94[b]
Low	BETAMINUS	45.74	3.27[a]	18.58[a]
SEM[4]		0.386	0.128	0.477
Main effects				
ME Level				
Normal		44.74[b]	3.12[b]	17.28[b]
Low		45.51[a]	3.25[a]	18.26[a]
SEM		0.288	0.111	0..451
Enzyme				
BETAPLUS		45.22	3.09[b]	17.92
BETAMINUS		45.03	3.28[a]	17.62
SEM		0.288	0.111	0.451
Statistical probabilities[5]				
ME level		NS	0.03	0.000
Enzyme		0.04	0.01	0.000
Interaction[6]		0.07	0.01	NS

Note:[a-c] Means with different superscripts differ significantly (p<0.05).
[1]β-Mannanase supplemented to either normal and/or low ME diets (0% or 0.05% added on top). [2]L*: Meat lightness; a*: Meat redness; b*: Meat yellowness. [3]BETAPLUS (diet with β-Mannanase); BETAMINUS: (diet without β-Mannanase). [4]SEM: Standard error of the mean. [5]Level of significance was set at p<0.05. [6]Interaction: ME Level x Enzyme.

The b* value was significantly (p<0.05) affected by an ME level x enzyme interaction with birds fed normal ME and BETAMINUS showed the greatest b* value in contrast to the other treatments, with normal ME and BETAMINUS being the lowest. The b* value for low ME was significantly (p<0.05) greater than normal ME (18.26 vs. 17.28, respectively). No differences were observed with β-Mannanase supplementation on meat color yellowness (b*).

DISCUSSION

Effect of ME Level

In the current research trial, birds on the low ME (100 kcal ME/kg reduction) consumed more feed, were less efficient, and had comparable BWG compared to normal ME level. Results reported herein agree with Zou et al. (2013), who reported that broilers on a lower ME energy (100 kcal reduction) consumed more feed and were less efficient in feed utilization during a 42-d trial. Moreover, Attia et al. (2021) observed poorer FCR in broilers fed 100 kcal and 150 kcal less ME than in a standard energy diet. Inconsistent with our results, several previous trials (Kong et al., 2011; Rehman et al., 2016; Hussein et al., 2020) found that FI was not affected by reductions in dietary ME ranging from 100 kcal ME/kg to 135 kcal ME/kg. It is well documented that there is a negative correlation between FI and ME levels in the diet, such that birds tend to consume more feed to accommodate low ME diets to satisfy their energy requirements. Hence, because FI was higher for birds fed low energy, BWG was not affected by a 100-kcal difference

between normal and low ME, which is consistent with previous studies (Cho & Kim, 2013), which reported that BWG was not influenced by energy in broilers between 0 to 35 d of age. A Difference of 100 kcal in metabolizable energy might not have been sufficient to elicit a marked response with regard to BWG.

Our results show that the low ME diet caused a reduction in both hot and carcass weights. Similar findings have been reported by others (William et al., 2014; Klein et al., 2015). Attia et al. (2021) attributed the decreases in carcass yield and relative weight of the liver to lower energy ME levels fed to the bird. In disagreement, we observed no differences in carcass yield, the relative weight of heart, liver, or gizzard due to varying dietary energy levels and in agreement with others (Rehman et al., 2016; Abouelezz et al., 2019; Hussein et al., 2020). This difference in response may be attributed to the difference in reduction between ME levels and/or commercial strain of broilers used (Attia et al., 2021).

The relative weights of cold cuts (breast, abdominal fat pad, wings, and thigh) were not affected by ME level in the diet except for the back, which is consistent with the findings of Hussein et al. (2020). In contrast, other researchers observed higher values of breast meat and abdominal fat pad yields when the dietary ME level was at least 100 kcal/kg greater in broiler diets (Cho & Kim, 2013; Rehman et al., 2016; Attia et al., 2021). Barbour et al. (2006) proposed that a higher ME level (2% or 3% soybean oil, 2940 kcal ME/kg or 3040 kcal ME/kg feed) with different fat sources impacted breast meat yield, and cited that a higher ME level elevated abdominal fat content. In the current study, even though soybean oil was used to establish the 100-kcal variation between

normal and low ME when formulating diets, no detectable effects were observed on breast meat or abdominal fat pad. Energy sources and levels in diet can affect breast meat and abdominal fat yields. Therefore, we postulate that the discrepancy observed vis-a-vis breast and abdominal fat results may be explained by the type and level of fat used in the current study.

Except for breast meat pH_u, meat quality values were not statistically discernible among normal and low ME diets for shear force, WHC, and CL. In our results, concerning the pH_u it was lower in the normal ME diet in contrast to the low ME diet, which is consistent with findings reported by Abouelezz *et al*. (2019), who cited a drop in ultimate breast meat pH as ME level in the diet was gradually elevated from 2.805 to 3.236 kcal ME/kg. However, these findings contradict those of Hussein *et al*. (2019), who found no effect of ME level in the diet on pH_u in breast meat. The differences in pH_u might be explained by differences in pre-slaughter response to stress, slaughter weight, and temperature, which impact glycogen reserves at slaughter, as explained by Uhlirova *et al*. (2018).

Results of the present study concerning lightness, yellowness, and redness in meat increased with low ME in comparison to normal ME after 24 h of slaughter. Our results agree with those of Cho & Kim (2013) and Mohammadigheisar *et al*. (2018), who observed that breast broilers fed a low ME diet exhibited greater lightness (L*). Moreover, Hussein *et al.* (2019) reported that yellowness (a*) of breast meat increased when birds were fed a lower ME diet. Abouelelzz *et al.* (2019) reported no effect of varying ME levels ranging from 2805 kcal ME/kg to 3236 kcal ME/kg on any of the meat color attributes. Different meat quality parameters, such as pH and meat colors are related to each other and are mainly affected by heme concentrations (Abdullah & Matarneh, 2010). Low pH in meat usually increases redness (a*) which might be due to the action of antioxidant enzymes (catalase and superoxide dismutase) that eliminate free radicals and inhibit oxidase metabolism (Mir *et al., 2017*).

Effect of Enzyme (*β*-Mannanse)

Supplementation of *β*-Mannanase to normal and/or low ME diets revealed no significance in the production performance parameters (FI, FCR, and BWG) between BETAPLUS and BETAMINUS diets. These results agree with previous studies (Kong *et al.,* 2011; Azarfar *et al.,* 2013; Hussein *et al.,* 2020), which also reported no differences in growth performance parameters with *β*-Mannanase supplementation. Contrary to our results, other researchers observed improvements in production parameters with supplemental *β*-Mannanase (Barros *et al.,* 2015; Balasubramanian *et al.,* 2018). Kong *et al.* (2011) cited that the calculated value of *β*-Mannan content of SBM in adequate energy and low energy diets used in their feeding trial was 0.64% and 0.44%, respectively, such that released simple carbohydrates would be enough to account for any improvement in productive performance. In the current study, the calculated *β*-Mannan content SBM in normal and low ME diets

was 0.46% and 0.68%, respectively, which is close to the values reported by Kong *et al*. (2011), and, therefore, may account for the lack of significant change observed in growth performance parameters. *β*-Mannanase is mainly found in the hull and fiber fraction of SBM and corn. The simple sugars released from this amount would not improve performance (Kong *et al.,* 2011; Mohammaddigheisar *et al.,* 2021).

Moreover, these results nullify the hypothesis that AME content of the diet should increase by the release of simple sugars due to *β*-Mannanase supplementation. It is important to keep in mind factors other than *β*-Mannan content of the diet, such as the physiological state of the birds, supplemental dose of *β*-Mannanase, its source, mode of application, and more importantly, the stability of the enzyme under high temperature of feed processing or low pH of proventriculus and gizzard (Hosseindoust *et al.,* 2019).

No significant differences were observed in carcass measurements (HCW, CCW), carcass yield, heart (%), liver (%), and gizzard (%) between BETAPLUS and BETAMINUS diets. In congruence with our data, Attia *et al*. (2021) reported no differences in carcass traits between diets with and without exogenous enzymes, including *β*-Mannanase and noted that the increased nutrient and energy availability due to exogenous enzymes did not elicit a response in carcass traits. Similarly, Hussein *et al*. (2019) reported no effect of *β*-Mannanase supplementation on liver, heart, and gizzard weights in broiler chickens. Other researchers (Azarfar, 2013; Hussein *et al.,* 2019) observed significant differences in the relative weights of the heart and liver due to enzyme supplementation. Hu *et al*. (2018) reported an increased liver weight in broiler chickens supplemented with lipase and attributed this to higher metabolic activity due to the utilization of lipids.

β-Mannanase supplementation had no impact on the relative weights of cold cuts which is in line with results reported by other researchers (Azarfar, 2013; Hussein *et al.,* 2019; Hussein *et al.,* 2020; Attia *et al.,* 2021). Moreover, Mohammaddigheisar *et al.* (2021) reported that adding *β*-Mannanase to diets did not affect the relative weights of abdominal fat, liver, gizzard, and breast meat and meat quality parameters in broiler chickens. Contrary to these findings, Rehman *et al*. (2016) reported an improvement in breast muscle relative weight due to *β*-Mannanase. This difference has been attributed to the nature of breast muscle fibers in contrast to the other muscles in terms of the type of muscle fibers, such as thigh and wings (Ibuki *et al.,* 2013).

Except for breast meat pH, the values of breast meat quality attributes were statistically non-significant between BETAPLUS and BETAMINUS diets. Low pH in meat usually increases redness (a*) which might be due to the action of an enzyme as an antioxidant that removes free radicals and inhibits oxidase metabolism (Mir *et al.,* 2017).

Consistent with our results, Habib *et al.* (2016) noted no correlation between the physical properties of broiler chicken breast meat (pH and water holding capacity) and exogenous enzyme supplementation. The

effect on breast muscle pH was explained earlier and mainly attributed to ME level in the diet. The addition of the enzyme lowered the pH since it relates to glycolysis and quantities of lactic acid accumulation (Abdullah & Matarneh, 2010).

The inclusion of β-Mannanase only significantly affected meat lightness (L*) and redness (a*), such that BETAPLUS diets exhibited greater lightness and redness than BETAMINUS diets. These results disagree with those reported by Hussein *et al.* (2020), who found no effect of β-Mannanase supplementation on the redness of breast meat. Besides the effect of the diet, meat color is affected by other factors such as myoglobin content, muscle pH, age, and the sex of birds (Hussein *et al.*, 2020). Zakaria *et al.* (2010) reported that supplementing broiler chickens with dietary enzymes did not affect meat quality parameters.

Effect of Interaction Between ME Level and Enzyme

In the current study, the interaction between ME and β-Mannanase had no impact on production parameters, carcass traits, or meat quality attributes in the trial (except meat pH and breast meat redness and yellowness). Similarly, Kong *et al.* (2011), Zou *et al.* (2013), and Attia *et al.* (2021) reported no interaction effect between energy level and β-Mannanase on growth performance or carcass traits. On the other hand, Cho & Kim (2013) found improvement in FCR and BWG due to the interaction between dietary energy and β-Mannanase. The positive interaction effect has been attributed to β-Mannanase supplementation, which improved energy availability in the diets and enhanced performance.

CONCLUSION

In conclusion, results obtained in the present study revealed no effect of β-Mannanase supplementation on broiler performance, carcass characteristics, or meat quality in general. Low ME levels in the diet did not compromise productive performance, as birds on low ME consumed more feed despite being less efficient (higher FCR). No interaction effects were found except for breast meat pH and meat redness and yellowness, which were attributed to variations in caloric content and β-Mannanase supplementation. Further research is probably needed to thoroughly investigate any potential effects of β-Mannanase on carcass quality and meat quality attributes and if any improvement can correlate to exogenous enzymes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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