Growth performance and nutrient digestibility of broilers fed low-energy corn-soybean meal-based diets supplemented with an exogenous enzyme cocktail as a combined activity

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ABSTRACT - The experiment aimed to evaluate the effects of supplementing a standard broiler diet, formulated based on corn and soybean meal (CSBM), with two levels of an exogenous enzyme (EZ) cocktail (0 and 0.05%), under two dietary metabolizable energy (ME) levels – normal (positive control, PC) and low (negative control, NC). From 0 to 35 d, 288 Ross 308 chicks were distributed across four treatments with 12 replicates of six chicks each. Growth performance was evaluated during the starter, grower, finisher, and cumulative period. At 35 d, blood samples were collected to measure serum metabolite concentrations, and birds were processed to determine carcass traits. Ileum segments were prepared for histological measurements, and excreta were collected to analyze apparent nutrient digestibility. Data were analyzed employing two-way ANOVA and Tukey's test. The results indicated no significant interaction between ME and EZ for any measured parameter. The EZ supplementation improved feed conversion rate (FCR) during the starter phase, and improved feed intake, body weight gain (BWG), FCR, and production efficiency index (PEI) during the grower phase; PEI during the finisher phase; and BWG, FCR, PEI, and final BW over the cumulative phase. Furthermore, EZ enhanced dressing percentage, breast yield, villi length, retention of crude protein, and nitrogen-corrected ME (AMEn), while also increasing glucose concentration and reducing the relative weight of the gizzard and intestine. Compared with the NC diet, the PC diet enhanced feed efficiency across the grower, finisher, and cumulative phases and increased AMEn and triglyceride levels. Supplementing ME-adequate CSBM diets with an EZ cocktail can boost the nutrient digestibility and growth efficiency of broilers.

Keywords: broiler, digestibility, energy restriction, enzyme, growth curve

1. Introduction

It has been established that diets based on corn and soybean meal (CSBM) are not fully digested by poultry. This inadequacy predominantly stems from the presence of antinutritive factors such as non-starch polysaccharides (NSP) (Olukosi et al., 2015), which have been shown to impede the normal digestion and absorption of nutrients by encapsulating them in the digestive tract (Musigwa et al., 2021).

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Additionally, Stefanello et al. (2016) demonstrated that the oligosaccharides found in soybean meal (SBM) could reduce nutrient digestion, nitrogen-corrected true metabolizable energy, and increase the rate at which feed passes through the intestines. The estimated total and water-soluble NSP contents in corn are approximately 76.3 and 6.4 mg/g, respectively, while in SBM, these contents are higher, at around 136.7 and 13.4 mg/kg, respectively (Yegani and Korver, 2013). Even though corn has a lower NSP content compared with SBM, its impact on the overall NSP level is more significant due to its high inclusion rate in poultry diets (Wealleans et al., 2017).

Extensive research has confirmed that exogenous enzymes (EZ) can enhance the nutritional value of CSBM-based rations for poultry. They achieve this by improving nutrient digestion, releasing monosaccharides, and counteracting the nutrient-encapsulating effects of cell walls (Stefanello et al., 2019; Cowieson et al., 2020). The introduction of protease and carbohydrase, either before or after feed processing, has been demonstrated to enhance the nutritional quality of CSBM-based rations for poultry (Pessôa et al., 2016). Furthermore, broilers fed CSBM-based rations fortified with α-galactosidase exhibit improved energy utilization (Mohiti-Asli et al., 2020; Llamas-Moya et al., 2021). One method to evaluate the effectiveness of these enzymes is to incorporate them into low nutrient-density diets, such as those with reduced metabolizable energy (ME). If the enzymes function effectively, they can restore the nutritional value of low-density feed, resulting in performance that matches or surpasses that of normal-density feed (Abudabos, 2014). However, current knowledge regarding the enhancement of the nutritional value of CSBM diets through EZ products is contradictory, and further research in this field is warranted (Zou et al., 2013). Consequently, this study was conducted to assess the effectiveness of an EZ cocktail containing five active substances at various dietary ME levels. The evaluation focused on growth efficiency, carcass traits, selected biochemical markers, intestinal histology, and the apparent digestibility of nutrients in broilers that received typical CSBM diets up until 35 d.

2. Material and Methods

Animal research was carried out in compliance with the institutional committee on animal use (KSU-SE-20-22).

2.1. Husbandry practices and trial design

The research was conducted in a broiler grow-out unit situated in Riyadh, Saudi Arabia (24°43'23" N, 46°37'25" E). In this trial, 288 Ross 308 broiler chicks, immunized and straight-run, were selected on the basis of comparable initial body weight (BW) and were accommodated in 48 battery cages at a stocking density of 30 kg/m^2 within an environmentally monitored room. The chemical composition of both corn and SBM was analyzed, and the resulting values were utilized in the formulation of diets. The diets (Table 1) were prepared in a mashed form to supply the nutritional specifications of the strain (Aviagen, 2019) for the starter (0-10 d), grower (11-25 d), and finisher (26-35 d) phases, with the exception of ME. Throughout the duration of the trial, recommended conditions pertaining to management, environment, and hygiene were meticulously adhered to, following the guidelines specified by the strain (Aviagen, 2018).

In a design utilizing a completely randomized block, four dietary treatments were administered, with each treatment being replicated 12 times, each replication housing six chicks. The treatments were structured in a 2 × 2 factorial layout in the subsequent manner: positive control (PC) diets comprised standard ME levels of 3000, 3100, and 3200 kcal/kg for each respective growth phase without the incorporation of EZ; negative control (NC) diets contained reduced ME levels by 60, 90, and 90 kcal when compared with the PC diets for each respective growth phase without the incorporation of EZ; PC diets enriched with 0.05% EZ; NC diets enriched with 0.05% EZ. The EZ cocktail (Kemzyme Plus dry, Kemin Europa N.V., Herentals, Belgium) comprises five active substances: endo-1,3(4)-β-glucanase (2350 U/g), endo-1,4-β-glucanase (cellulase) (18000 U/g), α-amylase (400 U/g), endo-1,4-β-xylanase (35000 U/g), and bacillolysin (protease) (1700 U/g). The activity levels of each enzyme were evaluated in our laboratory and were found to be aligned with the labeled enzyme activities.

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In each replication, broilers and feed were weighed every 5 d to facilitate the computation of body weight gain (BWG), feed intake (FI), feed conversion rate (FCR, adjusted for mortality), and the production efficiency index (PEI). The computation of PEI factored in BW, FCR, livability of the birds, and duration of the trial.

Table 1 - Composition of the experimental diets (on an as-is basis) with two metabolizable energy levels – normal (PC) and low (NC)

Ingredient (%)	Starter		Grower		Finisher	
	PC	NC	PC	NC	PC	NC
Yellow corn	51.6	51.6	58.5	58.8	59.8	60.6
Soybean meal	32.4	32.4	28.2	28.2	27.0	27.0
Corn oil	3.30	2.50	3.60	2.48	4.34	3.10
Corn gluten meal	6.30	6.10	4.71	4.30	5.10	4.50
Wheat bran	2.00	3.00	1.00	2.20	0.00	1.10
Dicalcium phosphate	2.05	2.05	1.82	1.82	1.68	1.67
Ground limestone	0.90	0.90	0.88	0.88	0.87	0.87
Choline chloride	0.05	0.05	0.00	0.00	0.00	0.00
DL-Methionine	0.30	0.30	0.26	0.26	0.25	0.25
L-Lysine	0.38	0.38	0.30	0.33	0.26	0.26
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Threonine	0.14	0.14	0.13	0.13	0.08	0.08
Vitamin and mineral mix[1]	0.20	0.20	0.20	0.20	0.20	0.20
Calculated analysis						
Metabolizable energy (kcal/kg)	3000	2940	3100	3010	3200	3110
Crude protein (%)	23.0	23.0	21.5	21.5	20.0	20.0
Non-phytate P (%)	0.48	0.48	0.44	0.44	0.41	0.41
Calcium (%)	0.96	0.96	0.87	0.87	0.81	0.81
Lysine (%)	1.28	1.28	1.15	1.15	1.06	1.06
Total sulfur amino acid (%)	0.95	0.95	0.85	0.85	0.83	0.83
Threonine (%)	0.86	0.86	0.77	0.77	0.71	0.71

[1] Provides per kg diet: retinol, 12,000,000 IU; cholecalciferol, 5,000,000 IU; tocopherol, 80,000 IU; menadione, 3,200 mg; thiamine, 3,200 mg; riboflavin, 8,600 mg; niacin, 65,000 mg; pantothenic acid, 20,000 mg; pyridoxine, 4,300 mg; biotin, 220 mg; folic acid, 2,200 mg; cyanocobalamin, 17 mg; antioxidants (butylated hydroxyanisole and butylated hydroxytoluene), 50,000 mg; copper, 16,000 mg; iodine, 1,250 mg; iron, 20,000 mg; manganese, 120,000 mg; selenium, 300 mg; zinc, 110,000 mg.

2.2. Sampling and analysis

One bird was randomly chosen from each replication for sampling at 35 d. Blood specimens, approximately 5 mL each, were collected from the wing veins, and after centrifuging at $3,000 \times g$ for 15 min, the resulting sera were stored in 1.5-mL centrifuge tubes at −80 °C for subsequent biochemical assessment. The concentration of various components, including total protein (TP), albumin (ALB), glucose (GLU), triglyceride (TG), as well as liver enzymes comprising alanine transaminase (ALT) and aspartate aminotransferase (AST) in the serum, were measured as per the manufacturer's guidelines (Randox Laboratories Ltd., Crumlin, UK). Additionally, the concentration of globulin (GLO) was subsequently determined by subtracting the values of TP and ALB concentrations.

Following the individual weighing, humanely euthanizing, and carcass processing of the sampled birds, the weights of the hot carcass, breast, leg quarters, abdominal fat pad, spleen, bursa of Fabricius, liver, and empty gizzard and intestine were recorded and represented as a proportion of the pre-slaughter BW.

A section of the lower ileum, approximately 2 cm in length, was excised and prepared for histological assessments. The fixed ileal segments (preserved in 10% formalin) underwent a series of procedures, including dehydration, paraffin embedding, cross-sectioning at 5 μm, mounting on slides, and dyeing with hematoxylin and eosin, as described by Abudabos et al. (2020). The dimensions of villi, including their length (VL) and width (VW), were determined employing a microscope and a PC-based image analysis

system equipped with software (Olympus NV, Aartselaar, Belgium). The villus surface area (VSA) was subsequently computed applying the previously mentioned formula (Abdelqader and Al-Fataftah, 2016).

At 35 d, one bird from each replication was placed in metabolic cages. During a 72-hour period, excreta were collected using the total collection method (Kong and Adeola, 2014) and stored at −20 °C, while FI was recorded. The pooled excreta and feed for each bird were weighed, subjected to oven-drying at 60 °C until reaching a constant weight, and subsequently milled until it could fit through a 0.5 mm screen. Both diets and excreta were subjected to analysis of dry matter (DM_{Dig}) through drying at 105 °C until a constant weight was achieved (method 930.15), crude protein (CP_{Dig}) by the Kjeldahl procedure (method 984.13), and ether extract (EE_{Dig}) by the Soxhlet procedure (method 920.39), following the guidelines set forth by AOAC (AOAC, 2019). The apparent digestibility of DM_{Dig}, CP_{Dig}, and EE_{Dig} was calculated utilizing the equation provided by De Marco et al. (2015):

$$\text{Apparent digestibility (\%)} = \frac{\text{component ingested} - \text{component emptied}}{\text{component ingested}} \times 100 \qquad (1)$$

The bomb calorimeter, standardized using benzoic acid (Parr Instruments Co., Moline, IL, USA), was employed to measure gross energy. Subsequently, apparent ME corrected for zero nitrogen retention (AMEn) was computed following the method outlined by Khalil et al. (2021).

2.3. Statistical analysis

The data underwent analysis through a two-way ANOVA applying the GLM procedure within SAS software (Statistical Analysis System, version 9.4). This analysis aimed to investigate the primary impacts of dietary ME and EZ levels, as well as the interaction between these two factors, which were treated as fixed variables, while considering block as a random variable. The statistical model used was as follows:

$$Y_{ijk} = \mu + (ME)_i + (EZ)_j + (ME \times EZ)_{ij} + e_{ijk} \qquad (2)$$

In this context, Y represents the dependent variable, μ is the overall mean, ME stands for dietary metabolizable energy, EZ represents the exogenous enzymes, ME × EZ indicates the interaction between ME and EZ, and e_{ijk} is denoted as the residual error.

For performance data, the replicate was considered the experimental unit, while individual animals were utilized as the experimental units for other data. A Tukey's test was employed to compare means at a significance level of 5%. The results are presented as means with pooled standard error of the mean.

3. Results

3.1. Growth performance

During both the starter (0-10 d) and grower (11-25 d) periods, there was no interaction observed between ME and EZ for any performance parameters (P>0.05) (Table 2). For the starter period, neither ME nor EZ had an impact on FI, BWG, or PEI (P>0.05). Nevertheless, it is important to mention that EZ did lead to an improvement in FCR when compared with the unsupplemented control (UNSUP-CON) (P<0.05). In the grower period, EZ demonstrated enhancements in FI, BWG, FCR, and PEI, resulting in increases of 50 g, 65 g, 5 points, and 25 points, respectively, over the UNSUP-CON (P<0.05). Additionally, the PC group exhibited more efficient feed conversion compared with the NC group (P<0.05).

There was no interaction observed between ME and EZ for any of the performance parameters during the finisher (26-35 d) and cumulative (0-35 d) periods (P>0.05) (Table 3). During the finisher period, birds fed the PC diet demonstrated more efficient feed conversion compared with those on the NC diet, while EZ also led to an improvement in PEI over the UNSUP-CON (P<0.05). In the cumulative growth period, birds receiving the PC diet outperformed those on the NC diet in terms of FCR (1.41 vs. 1.48 g:g, respectively, P<0.01). Additionally, EZ had a positive impact on BWG (P<0.05), FCR (P<0.01), PEI (P<0.01), and final BW (P<0.05) when compared with the UNSUP-CON.

Table 2 - Impacts of exogenous enzymes (EZ) addition, metabolizable energy (ME) content, and the interaction between them on bird performance during the starter and grower periods

Group		Parameter							
		0-10 d				11-25 d			
ME	EZ (%)	FI (g)	BWG (g)	FCR (g:g)	PEI (%)	FI (g)	BWG (g)	FCR (g:g)	PEI (%)
PC	0	177	163	1.09	167	1084	807	1.34	308
NC	0	181	155	1.17	145	1100	753	1.46	272
PC	0.05	178	164	1.09	171	1139	844	1.35	314
NC	0.05	184	169	1.09	175	1145	845	1.36	315
SEM		5.44	5.95	0.016	7.80	20.9	26.5	0.029	12.9
Main effects									
ME level									
PC		178	164	1.09	169	1112	826	1.35b	311
NC		183	162	1.13	160	1123	799	1.41a	294
EZ (%)									
0		179	159	1.13a	156	1092b	780b	1.40a	290b
0.05		181	167	1.09b	173	1142a	845a	1.35b	315a
P-value									
ME		NS	NS	NS	NS	NS	NS	0.05	NS
EZ		NS	NS	0.05	NS	0.05	0.05	0.05	0.05
ME × EZ		NS	NS	NS	NS	NS	NS	NS	NS

FI - feed intake; BWG - body weight gain; FCR - feed conversion ratio; PEI - production efficiency index; SEM - pooled standard error of the mean; NS - non-significant.
PC - positive control with standard levels of dietary ME according to Ross recommendations; NC - negative control with reduced levels of dietary ME (– 60 and 90 kcal/kg for the starter and grower/finisher diets, respectively).
a,b - Means with different letters differ significantly.

Table 3 - Impacts of exogenous enzymes (EZ) addition, metabolizable energy (ME) content, and the interaction between them on bird performance during the finisher and cumulative periods

Group		Parameter								
		26-35 d				0-35 d				
ME	EZ (%)	FI (g)	BWG (g)	FCR (g:g)	PEI (%)	FI (g)	BWG (g)	FCR (g:g)	PEI (%)	FBW (kg)
PC	0	1290	820	1.57	334	2551	1790	1.43	369	1.83
NC	0	1340	813	1.65	313	2621	1721	1.52	340	1.80
PC	0.05	1315	876	1.50	371	2633	1884	1.40	398	1.95
NC	0.05	1327	838	1.59	348	2655	1853	1.43	384	1.93
SEM		33.8	30.0	0.036	13.5	46.0	44.1	0.021	13.4	0.051
Main effects										
ME level										
PC		1303	848	1.54b	353	2592	1837	1.41b	384	1.89
NC		1334	826	1.62a	331	2638	1787	1.48a	362	1.87
EZ (%)										
0		1315	817	1.61	324b	2586	1756b	1.47a	355b	1.82b
0.05		1321	857	1.55	360a	2644	1869a	1.42b	391a	1.94a
P-value										
ME		NS	NS	0.05	NS	NS	NS	0.01	NS	NS
EZ		NS	NS	NS	0.05	NS	0.05	0.01	0.01	0.05
ME × EZ		NS	NS	NS	NS	NS	NS	NS	NS	NS

FI - feed intake; BWG - body weight gain; FCR - feed conversion ratio; PEI - production efficiency index; FBW - final body weight; SEM - pooled standard error of the mean; NS - non-significant.
PC - positive control with standard levels of dietary ME according to Ross recommendations; NC - negative control with reduced levels of dietary ME (– 60 and 90 kcal/kg for the starter and grower/finisher diets, respectively).
a,b - Means with different letters differ significantly.

3.2. Carcass characteristics

At 35 days, no differences in carcass dressing percentage and the yield of parts (cut-up parts and internal organs) were observed, irrespective of variations in ME levels or the interaction between ME and EZ (P>0.05) (Table 4). Conversely, EZ resulted in a 1.4% increase in dressing yield and a 1.8% increase in breast muscle yield when compared with the UNSUP-CON (P<0.01). The relative weights of the gizzard and intestine were lower in the EZ group compared with the UNSUP-CON (P<0.05). However, the relative weights of leg quarters, fat pad, spleen, bursa, and liver were not influenced by the EZ treatment (P>0.05).

Table 4 - Impacts of exogenous enzymes (EZ) addition, metabolizable energy (ME) content, and the interaction between them on carcass yields as a % of pre-slaughter weight in birds at 35 d

Group		Parameter								
ME	EZ (%)	Dressing	Breast	Leg	Fat	Spleen	Bursa	Liver	Gizzard	Intestine
PC	0	71.0	26.9	21.5	0.84	0.117	0.138	3.38	2.34	2.80
NC	0	70.4	25.3	22.0	1.20	0.112	0.153	3.29	2.49	2.75
PC	0.05	72.4	27.9	22.3	0.87	0.098	0.161	3.28	2.42	2.73
NC	0.05	72.6	28.0	22.4	0.81	0.119	0.127	3.40	2.40	2.55
SEM		0.53	0.45	0.39	0.13	0.013	0.021	0.14	0.11	0.11
Main effects										
ME level										
PC		71.7	27.4	22.0	1.00	0.117	0.080	2.07	2.49	2.77
NC		71.1	26.7	22.0	0.85	0.114	0.070	2.03	2.34	2.65
EZ (%)										
0		70.7b	26.1b	21.8	1.00	0.114	0.080	2.08	2.59a	2.78a
0.05		72.1a	27.9a	22.4	0.84	0.117	0.060	2.02	2.24b	2.64b
P-value										
ME		NS	NS	NS	NS	NS	NS	NS	NS	NS
EZ		0.01	0.01	NS	NS	NS	NS	NS	0.01	0.05
ME × EZ		NS	NS	NS	NS	NS	NS	NS	NS	NS

SEM - pooled standard error of the mean (n = 12); NS - non-significant.
PC - positive control with standard levels of dietary ME according to Ross recommendations; NC - negative control with reduced levels of dietary ME (– 60 and 90 kcal/kg for the starter and grower/finisher diets, respectively).
a,b - Means with different letters differ significantly.

3.3. Intestinal morphology and nutrient digestibility

The interaction of ME and EZ had no impact on ileal histological changes or apparent nutrient digestibility (P>0.05) (Table 5). A disparity in VL emerged due to the addition of EZ (P<0.05); the birds receiving EZ exhibited a greater length (532 μm) in comparison with the birds that did not receive the supplement (505 μm). Furthermore, there was an increase in CP_{Dig} and AMEn in the EZ group compared with the UNSUP-CON (68.2 vs. 67.3% for CP and 3121 vs. 3096 kcal/kg for AMEn; P<0.05). However, ileal VW and VSA, as well as the digestibility of DM_{Dig} and EE_{Dig} were unaffected by EZ (P>0.05). Birds that received the PC diet also exhibited higher AMEn when compared with those on the NC diet (3156 vs. 3062 kcal/kg, respectively, P<0.01). The dietary ME level did not have any influence on VL, VW, VSA, or the digestibility of nutrients (DM_{Dig}, CP_{Dig}, and EE_{Dig}).

3.4. Blood biochemical parameters

Serum levels of TP, ALB, GLO, ALT, and AST exhibited no significant differences across all groups and were not influenced by EZ, ME, or their interaction (P>0.05) (Table 6). However, the GLU level was solely impacted by EZ (P<0.05); birds on the control diet had a lower GLU concentration (223 mg/dL)

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Table 5 - Impacts of exogenous enzymes (EZ) addition, metabolizable energy (ME) content, and the interaction between them on intestinal morphology and nutrient digestibility of birds

Group		Parameter						
		Ileum histology (35 d)			Apparent digestibility (35-38 d)			
ME	EZ (%)	VL (μm)	VW (μm)	VSA (μm²)	DM_{Dig} (%)	CP_{Dig} (%)	EE_{Dig} (%)	AMEn (kcal/kg)
PC	0	501	91.3	0.145	78.2	67.9	80.2	3140
NC	0	508	88.7	0.131	77.7	66.6	80.0	3052
PC	0.05	542	91.5	0.152	78.3	68.4	80.9	3171
NC	0.05	521	90.3	0.147	78.1	67.9	81.4	3072
SEM		9.56	4.42	0.008	0.24	0.43	0.64	6.81
Main effects								
ME level								
PC		522	91.4	0.149	78.3	68.2	80.6	3156a
NC		515	89.5	0.139	77.9	67.3	80.7	3062b
EZ (%)								
0		505b	90.0	0.138	78.0	67.3b	80.1	3096b
0.05		532a	90.1	0.150	78.2	68.2a	81.1	3121a
P-value								
ME		NS	NS	NS	NS	NS	NS	0.01
EZ		0.05	NS	NS	NS	0.05	NS	0.05
ME × EZ		NS	NS	NS	NS	NS	NS	NS

VL - villus length; VW - villus width; VSA - villus surface area; DM_{Dig} - dry matter digestibility; CP_{Dig} - crude protein digestibility; EE_{Dig} - ether extract digestibility; AMEn - nitrogen-corrected apparent ME; NS - non-significant; SEM - pooled standard error of the mean (n = 12).
PC - positive control with standard levels of dietary ME according to Ross recommendations; NC - negative control with reduced levels of dietary ME (– 60 and 90 kcal/kg for the starter and grower/finisher diets, respectively).
a,b - Means with different letters differ significantly.

Table 6 - Impacts of exogenous enzymes (EZ) addition, metabolizable energy (ME) content, and the interaction between them on serum biochemical indicators and liver function of birds at 35 d

Group		Parameter						
ME	EZ (%)	TP (g/dL)	ALB (g dL)	GLO (g/dL)	GLU (mg/dL)	TG (mg/dL)	ALT (IU/L)	AST (IU/L)
PC	0	2.68	1.59	1.10	223	51.0	18.6	310
NC	0	2.59	1.54	1.06	222	48.3	20.2	298
PC	0.05	2.75	1.63	1.12	250	59.6	17.9	277
NC	0.05	2.75	1.58	1.17	237	49.3	16.3	313
SEM		0.15	0.09	0.10	7.47	2.95	2.38	16.4
Main effects								
ME level								
PC		2.72	1.61	1.11	237	55.3a	18.3	294
NC		2.67	1.56	1.12	230	48.8b	19.4	306
EZ (%)								
0		2.64	1.57	1.08	223b	49.7	19.4	304
0.05		2.74	1.61	1.14	244a	54.5	17.2	295
P-value								
ME		NS	NS	NS	NS	0.01	NS	NS
EZ		NS	NS	NS	0.05	NS	NS	NS
ME × EZ		NS	NS	NS	NS	NS	NS	NS

TP - total protein; ALB - albumin; GLO - globulin; GLU - glucose; TG - triglycerides; ALT - alanine transaminase; AST - aspartate aminotransferase; NS - non-significant; SEM - pooled standard error of the mean (n = 12).
PC - positive control with standard levels of dietary ME according to Ross recommendations; NC - negative control with reduced levels of dietary ME (– 60 and 90 kcal/kg for the starter and grower/finisher diets, respectively).
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compared with those receiving the diet with EZ (244 mg/dL). Furthermore, TG concentration was affected by the dietary ME level (P<0.01); birds on the PC diet had higher TG levels compared with those on the NC diet (55.3 vs. 48.8 mg/dL, respectively).

4. Discussion

Dietary ME levels have an impact on the intake of various nutrients. However, broilers have a remarkable ability to adjust their energy intake by regulating their feed intake in response to changes in diet energy concentration (Lopez and Leeson, 2008). The data from our study indicates that FI and BWG were not significantly influenced by the dietary ME level. This suggests that the energy level in the NC diet may not have been low enough to have a significant effect on FI and BWG. In contrast, other studies such as Coppedge et al. (2012) and Williams et al. (2014) reported reduced BW in broilers fed a low ME diet (a reduction of 132 kcal/kg). Saleh et al. (2004) also found that a substantial decrease in dietary ME by 270 kcal/kg led to reduced performance. However, their study involved a much larger reduction in ME compared with our study, which applied a reduction of 60, 90, and 90 kcal/kg for each respective phase. Despite the lack of impact on FI and BWG, FCR was negatively affected by the dietary ME level. This indicates that even a relatively small reduction in ME led to broilers being unable to meet their energy requirements efficiently, resulting in suboptimal FCR. During the grower phase, broilers fed the PC diet showed slightly lower FI and slightly higher BWG, leading to a 4.3% improvement in FCR compared with the NC group. Similarly, in the finisher period, the PC group exhibited a 4.9% better FCR compared to the NC group, and this improvement in FCR was also observed for the cumulative phase, with a 4.7% enhancement in the PC group. Our results align with the findings of O'Neill et al. (2012), who observed the negative impact of reducing dietary ME on FCR over a 42-d period. Surprisingly, the reduction of dietary ME in the NC diet did not significantly affect carcass yield parameters in our study, in contrast with the results of Williams et al. (2014), who found that reducing ME in a broiler ration led to lower carcass and breast meat yields.

It has been suggested that an EZ cocktail with multiple enzymatic activities can effectively address a wide range of feed substrates, potentially yielding more significant outcomes compared with individual enzymes, which focus on a single substrate (Olukosi et al., 2007; Coppedge et al., 2012; Williams et al., 2014; Amerah et al., 2017). Performance enhancements have been reported due to the inclusion of cocktails of NSP-degrading enzymes (NSPase). For instance, Slominski (2011) reported 3.9 and 3.2% enhancements in BWG and FCR when broilers received CSBM rations with NSPase. Similar improvements in BWG and FCR were observed in reduced energy diets with the addition of NSPase (Coppedge et al., 2012; Williams et al., 2014; Amerah et al., 2017). In the current study, the EZ preparation, which includes five active substances (β-glucanase, cellulase, α-amylase, protease, and xylanase), improved BWG, FCR, PEI, and final BW by 6.0, 3.4, 9.2, and 6.2%, respectively, over the cumulative growth period. The improvement in FCR observed with EZ supplementation can be partly attributed to the enhancement in BWG; the BWG of birds fed the NC diet with EZ was numerically better than that of those fed the PC diet without EZ. These results align with other studies that have reported improved BWG and FCR in broilers fed diets containing multiple enzyme complexes (Farran et al., 2010; Tiwari et al., 2010; Campasino et al., 2015; Pessôa et al., 2016). However, broilers fed diets supplemented with EZ consumed a similar cumulative amount of feed to those fed the control diet, which contradicts the findings of Ranade and Rajmane (1992), who reported lower FI in broilers fed diets supplemented with an EZ cocktail containing cellulase, protease, xylanase, β-glucanase, and α-amylase activities. Additionally, EZ supplementation increased dressing and breast muscle yield, which contradicts the findings of Farran et al. (2010), who observed that *pectoralis major* muscle, thigh, and drum yields were not affected by the inclusion of EZ.

The development of intestinal morphology serves as a significant indicator reflecting the productivity and welfare of broiler birds (Shang et al., 2015). In the present study, a notable increase in ileal VL was observed in broilers fed diets containing EZ, suggesting improved nutrient absorption. This observation aligns with recent findings that also showed a significant increase in ileal VL with the inclusion of a blend of NSPase (Alqhtani et al., 2022). Comparable results were documented in broilers supplemented

with exogenous xylanase and β-glucanase enzymes (Zarghi et al., 2016). The enhancement in intestinal structure due to the addition of EZ can be ascribed to its mitigating influences on the adverse influences of dietary NSP level on the mucosal surface of the intestine. Furthermore, CP_{Dig} increased as a result of EZ supplementation. This is in line with findings of Gallardo et al. (2018), who observed improved nitrogen retention with the inclusion of a multi-carbohydrase. Similarly, Cowieson and Ravindran (2008) documented a significant enhancement in nitrogen retention in CSBM-based diets formulated with xylanase, protease, and amylase. The improvement in retention is likely associated with increased protein hydrolysis and reduced endogenous and exogenous nitrogen losses facilitated by the action of the EZ (Adeola and Cowieson, 2011). Moreover, the current study revealed an improved AMEn retention with the addition of EZ. In agreement with these findings, Amerah et al. (2017) concluded that a mixture of xylanase, amylase, and protease improved AMEn when compared with a diet formulated with lower ME. Similarly, Ao et al. (2009) observed that the inclusion of α-galactosidase in a CSBM-based diet led to an increase in AMEn. Graham et al. (2002) also reported a 350 kcal/kg augmentation in true ME in a diet comprising α-galactosidase-treated SBM, which was attributed to the enhanced breakdown of raffinose and stachyose in SBM.

5. Conclusions

Our results highlight the potential for enhancing the utilization of standard CSBM-based diets in poultry farming. This improvement encompasses a range of performance metrics, including BWG, FCR, PEI, and final BW over the cumulative period, as well as carcass and breast yields, VL, AMEn, and CP_{Dig}, alongside seral GLU concentration in birds raised until 35 d of age. These improvements are attainable through the incorporation of the EZ combination, comprising β-glucanase, cellulase, α-amylase, protease, and β-xylanase into the standard CSBM-based feed. Furthermore, the formulation of CSBM-based feeds with sufficient ME levels can lead to reduced FCR across all growth phases and increased AMEn retention. The beneficial impact of including the EZ mixture in broiler feed might potentially be correlated with the breakdown of various components within NSP present in CSBM, which may well result in profitable advantages for the broiler chicken sector.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Abudabos, A. E. **Data curation:** Alqhtani, A. H.; Alharthi, A. S. and Abudabos, A. E. **Formal analysis:** Al Sulaiman, A. R.; Alharthi, A. S. and Abudabos, A. E. **Funding acquisition:** Alqhtani, A. H. and Abudabos, A. E. **Investigation:** Al Sulaiman, A. R. and Abudabos, A. E. **Methodology:** Abudabos, A. E. **Project administration:** Alqhtani, A. H. and Abudabos, A. E. **Resources:** Alqhtani, A. H. and Abudabos, A. E. **Supervision:** Abudabos, A. E. **Validation:** Al Sulaiman, A. R. and Abudabos, A. E. **Writing – original draft:** Abudabos, A. E. **Writing – review & editing:** Al Sulaiman, A. R. and Abudabos, A. E.

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