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Metabolizable energy and amino acid-deficient diets supplemented with β-mannanase in response to growth performance, intestinal health, and immune response in broilers

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ABSTRACT

The objective of this study is to investigate the effect of β-mannanase in energy and amino acid-deficient broiler diets on the growth performance, carcass yields, ileal nutrient digestibility, viscosity, intestinal morphology, and blood metabolites of broilers from hatch to day 35. A total of 168 one-day-old Ross 308 broiler chicks were randomly assigned to one of three dietary treatments in a completely randomized design, with each treatment having eight replicates and seven birds per cage. The treatments were as follows: i) positive control diet (**PC**) containing standard energy and amino acids; ii) negative control (**NC**) with 150 kcal/kg metabolizable energy (**ME**) and 4.5 % amino acids reduction compared to PC; iii) NC supplemented with 100 g/ton β-mannanase (NC + β-mannanase). Broilers fed the PC and NC + β-mannanase diets showed improved growth performance, including body weight on day 35 ($P < 0.001$), average daily gain (Day 1-35; $P = 0.001$), and feed efficiency (Day 1-35; $P < 0.001$), alongside lower ($P < 0.001$) digesta viscosity and enhanced ileal digestibility of dry matter ($P < 0.001$), crude protein ($P < 0.001$), and energy ($P < 0.001$), as well as an improved ($P < 0.001$) villus height to crypt depth ratio, compared to those on the NC diet. Regarding carcass traits, liver yield was higher ($P < 0.001$) in broilers on the NC + β-mannanase diet compared to other treatments by day 35. Additionally, the NC + β-mannanase diet lowered ($P < 0.001$) IL-1β and IFN-γ levels and increased ($P < 0.001$) IL-10 levels compared to the other groups. In conclusion, β-mannanase supplementation with 100 g/MT β-mannanase in nutrient-deficient diets improved broiler performance by reducing intestinal viscosity and enhancing nutrient utilization efficiency. It also promoted the development of metabolic organs like the liver, as well as immune organs such as the spleen and thymus, while modulating inflammatory and anti-inflammatory cytokine responses.

Introduction

The cost of ingredients for poultry feed makes up about 60-70 % of the total production cost, highlighting the critical role of feed formulation in the poultry industry (Wongnaa et al., 2023). Globally, broiler diets primarily consist of corn and soybean meal, the main feed ingredients in broiler production (Ferreira Jr et al., 2016). However, the primary matter is that corn and soybean meal-based diets contain substantial amounts of non-starch polysaccharides (**NSP**), including mannans, xylans, galactans, arabinans, and others as anti-nutritional factors (El-Wahab et al., 2022). Although dietary NSPs are indigestible by poultry, they could serve as a potential energy source when the proper enzymes are added to the diet (Latham et al., 2018).

Beta-mannan, a type of soluble NSP consisting of repeating mannose

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units connected by β−1,4-glycosidic bonds, is present in feed ingredients such as soybean meal, rapeseed meal, sesame meal, and corn (Zhang et al., 2024a). In particular, the content of β-mannan is approximately 1.3 % to 1.6 % of soybean meal, the second largest hemicellulose component in leguminous plants (Hsiao et al., 2006). Since β-mannan, also known as β-galactomannans, is not digested by monogastric endogenous enzymes, it is classified as an anti-nutritional factor due to its negative impact on nutrient absorption and digestion (Li et al., 2010). Moreover, it has been documented that β-mannan triggers an immune response, leading to increased energy expenditure to support the immune system (Nusairat et al., 2024). It also contributes to increased viscosity of the intestinal digesta, hinders intestinal peristalsis, heightens satiety, reduces feed intake, thickens the water layer on the intestinal mucosa, and ultimately results in energy loss (Zhang et al., 2024b). Therefore, the inclusion of exogenous enzymes in diets containing soybean meal could be an effective strategy to counteract a part of those adverse effects.

Mannanase breaks down β-mannans into smaller units such as mannose, mannobiose, and mannotriose by targeting the 1,4-β glycosidic bonds (Dhawan and Kaur, 2007; Ferreira Jr et al., 2016). Adding mannanase to broiler diets enhances performance and feed efficiency (Jackson et al., 2004; Zou et al., 2006) by improving apparent metabolizable energy (**ME**) levels (Li et al., 2010; Kiarie et al., 2021) and reducing intestinal viscosity (Latham et al., 2018). Additionally, mannanase supplementation improves the apparent ileal amino acid digestibility coefficients (Mussini et al., 2011). Moreover, mannanase facilitates the breakdown of β-mannan, releasing mannan-oligosaccharides (**MOS**) that reach the large intestine, where they can function as prebiotics. They support intestinal health by promoting the growth of beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, while reducing the adhesion of pathogenic bacteria like *Escherichia coli* to the gut lining (Gutierrez et al., 2008; Zhang et al., 2024b).

Mannanase has shown potential as a cost-saving measure due to its ability to be utilized in lower-nutrient broiler diets, promoting better feed energy utilization while yielding growth performance comparable to broilers receiving adequate nutrients (Ferreira Jr et al., 2016). Several studies have focused on the role of mannanase supplementation in improving growth performance, reducing digesta viscosity, optimizing energy metabolism, and enhancing the intestinal morphology in broilers fed lower-energy diets (Latham et al., 2018; Yaqoob et al., 2022; Nusairat et al., 2024; Zhang et al., 2024b). However, most of these studies have focused on energy reduction alone, and little is known about the efficacy of mannanase when both ME and amino acids are concurrently limited. Given that modern poultry production increasingly explores nutrient-restricted strategies for sustainability and cost reduction, it is critical to determine whether mannanase can maintain growth performance and physiological function under shach combined nutrient-deficient conditions. Therefore, this study aimed to examine the effects of the supplementation of endo-1,4-β-mannanase on broilers for growth performance, carcass yields, ileal nutrient digestibility, ileal digesta viscosity, ileal histomorphology, and immune marker indices when supplemented to diets reduced by 150 kcal/kg ME and 4.5 % amino acids (i.e., lysine, methionine, and threonine) compared to commercial standard mash diets. This study hypothesized that supplementing β-mannanase in a diet deficient in energy and amino acid would not impair growth performance and carcass yields and digestibility, viscosity, intestinal morphology, and immune system markers compared to those fed commercial standard diets.

Materials and methods

Animal ethics statement

The experimental procedures and protocol were reviewed and approved by the Animal Ethics Committee of Chungnam National University (Protocol Number; 202401A-CNU-001).

Birds and housing

A total of 168 one-day-old male Ross 308 chickens (41.71 ± 0.270 g) were obtained from a local hatchery (Yangji hatchery, Pyeongtaek, Gyeonggi-do, Republic of Korea) and used in the study for 35 d. The birds were individually weighted and randomly allocated to cages (0.066 m^2 per bird) in a completely randomized design. Each raised battery cage (76 × 61 × 46 cm^3) housed seven birds, leading to 8 cage replicates per treatment (i.e., three dietary treatments applied). The cages were equipped with two nipple drinkers and a metal trough to provide water and feed efficiently. The experimental diets were offered *ad libitum*, and the birds had continuous access to clean drinking water through nipple drinkers throughout the study. The birds were vaccinated against infectious bronchitis and Newcastle disease at the hatchery. The room temperature was maintained at 30 ± 1°C from day 1 to 3 and then gradually decreased to 25 ± 1°C until d 14. Thereafter, a 24 ± 1°C was maintained throughout the experiment according to the Ross 308 broiler management guideline (Aviagen, 2018). The average relative humidity during the trial was 56 ± 14 %, and continuous lighting was provided throughout the experimental period.

Experimental design and diets

The birds were assigned to one of three dietary treatments in a completely randomized design, with eight replicate pens per treatment. The three dietary treatments included a nutritionally adequate positive control (**PC**); negative control (**NC**) with 150 kcal/kg diet reduction in ME and 4.5 % reduction in the amino acids (i.e., lysine, methionine, threonine); NC supplemented with 100 g/MT β-mannanase. Natupulse TS (BASF SE, Germany), a β-mannanase source with an activity of 8000 TMU (thermostable endo-1,4-β-mannanase units) g^{-1}, was used in this study. The basal PC diet was formulated to either meet or exceed the nutrient requirements of broilers (Aviagen, 2022). The composition, calculated values, and analyzed data for the starter (days 1-10), grower (days 11-21), and finisher (days 22-35) are presented in Tables 1 and 2. All diets contained phytase (Natuphos E 5000 G; 5000 FTU/g) and were provided in a mash form. Full matrix values for the phytase were applied based on the manufacturer's specifications. Furthermore, Cr_2O_3 (chromium oxide powder, >99.95 % purity, Sigma-Aldrich, St. Louis, MO, USA) was added to the finisher feed as an indigestible marker for digestibility analysis at a proportion of 0.3 % to all the experimental diets in the finisher period.

Growth performance measurements

On days 10, 21, and 35, all birds and leftover feed in the cages were weighed at each time point to determine the body weight (**BW**) and average daily feed intake (**ADFI**). The average daily gain (**ADG**) was calculated as the BW of the previous time point was subtracted from the BW of the current time point. Feed intake was calculated by subtracting the remaining feed amount from the initial feed amount. Finally, mortality-corrected ADFI and feed conversion ratio (**FCR**) were calculated for each cage of the experiment (Sta. Cruz et al., 2024).

Post-mortem procedure and sample collection

Eight birds per treatment (one bird per cage) that were closer to the median body weight were selected in the respective cage on day 35. Subsequently, their weights were recorded as the live body weight. Blood samples were collected from the brachial vein into a vacutainer coated with lithium heparin (BD Vacutainer, BD, Franklin Lakes, NJ, USA) before euthanizing the birds. The birds were then euthanized using carbon dioxide asphyxiation for sample collection. The dressing percentage of meat with giblets (i.e., heart, gizzard, and liver) was

Table 1
Composition (%, as-fed basis) of the experimental diets.

Items	Experimental Diets[1]					
	Starter (Day 1-10)		Grower (Day 11-21)		Finisher (Day 22-35)	
	PC	NC	PC	NC	PC	NC
Corn	55.43	58.40	58.25	60.62	61.79	64.53
Soybean meal, 44 %	28.58	30.40	24.38	27.48	22.27	24.27
Corn DDGS	2.00	2.00	6.00	6.00	6.50	6.50
Corn gluten	2.00	0.78	0.90	-	-	-
Corn carrier	0.60	0.60	0.60	0.60	0.60	0.60
Feather meal	3.00	2.19	2.95	0.90	1.99	-
Limestone	1.09	1.09	0.80	0.81	0.69	0.72
Mono-calcium phosphate	1.43	1.48	0.87	0.93	0.53	0.53
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Vegetable oil	2.33	1.30	1.92	1.14	2.12	1.10
Animal fat	1.50	-	1.50	-	1.50	-
DL-methionine, 98 %	0.41	0.38	0.36	0.33	0.34	0.30
L-lysine-Sulfate, 70 %	0.62	0.42	0.54	0.32	0.47	0.30
L-Threonine, 98 %	0.21	0.17	0.17	0.13	0.15	0.11
Choline chloride, 50 %	0.08	0.07	0.05	0.04	0.03	0.02
Vitamin-Mineral premix[2]	0.40	0.40	0.40	0.40	0.40	0.40
Phytase[3]	0.02	0.02	0.02	0.02	0.02	0.02
Cr_2O_3	-	-	-	-	0.30	0.30
Calculated values						
Metabolizable energy, kcal/kg	2,975	2,825	3,050	2,900	3,100	2,950
Crude protein, %	22.87	22.27	21.49	20.55	19.50	18.74
Crude fat, %	6.10	3.62	5.98	3.67	6.24	3.71
Calcium, %	0.95	0.95	0.75	0.75	0.65	0.65
Available phosphorus, %	0.50	0.50	0.42	0.42	0.36	0.36
SID lysine, %	1.32	1.25	1.18	1.12	1.08	1.03
SID methionine + cystine, %	1.00	0.96	0.92	0.88	0.86	0.82
SID methionine, %	0.69	0.66	0.63	0.60	0.59	0.56
SID threonine, %	0.88	0.84	0.79	0.75	0.72	0.68
Soluble Mannan, %	0.38	0.40	0.38	0.42	0.37	0.39

Abbreviations: DDGS, distillers dried grains with soluble; SID, standardized ileal digestible.

[1] PC: positive control diet; NC, negative control diet with a 150 kcal /kg metabolizable energy (ME) reduction and reducing amino acids by 4.5 %.

[2] Provided per kilogram of diet: vitamin A (trans-retinyl acetate), 6,400 IU; vitamin D3 (cholecalciferol), 1,760 IU; vitamin E, 12,000 IU; vitamin K3, 1,760 mg; biotin, 80 mg; thiamin, 1,600 mg; riboflavin, 3,520 mg; pyridoxine, 2,400 mg; vitamin B12, 8 mg; niacin, 28,000 mg; pantothenic acid, 10,000 mg; folic acid 480 mg; Fe (from iron sulfate), 20,000 mg; Cu (from copper sulfate), 81,000 mg; Zn (from zinc oxide), 32,500 mg; Mn (from manganese oxide), 43,800 mg; I (from potassium iodide), 750 mg; Se (from sodium selenite), 50 mg.

[3] Phytase: Natuphos E 5000 G, 5000 FTU/g (200 g/metric ton).

calculated by dividing it by the live weight of the birds. The breast and leg meats were then separated and weighed to measure them relative to the total carcass weight. Furthermore, the liver, spleen, the bursa of Fabricius, and the thymus were harvested and weighed to measure them relative to the total carcass weight.

Abdominal incisions were made on each sacrificed bird, and the ileum was separated from the gastrointestinal tract. The ileum was defined as the segment of the small intestine that extended from Meckel's diverticulum to the ileocecal junction (Yu et al., 2021). A 3 cm fragment of the ileum was collected from each sacrificed bird, rinsed with phosphate-buffered saline at pH 7.4, and stored in 10 % formaldehyde.

Three birds were selected to get digesta based on closeness to the mean body weight of the birds in the respective cage. The digesta of the ileum from birds was gently flushed with distilled water into labeled plastic containers and stored at - 80°C until further analysis for nutrient digestibility and viscosity.

Table 2
Analyzed composition of the experimental diets.

Items	Experimental Diets[1]					
	Starter (Day 1-10)		Grower (Day 11-21)		Finisher (Day 22-35)	
	PC	NC	PC	NC	PC	NC
Dry matter, %	89.28	88.44	88.82	87.62	87.38	87.96
Crude protein (Nitrogen × 6.25), %	22.08	19.96	21.44	19.39	19.68	18.52
Gross energy, kcal/kg	3,976	3,806	4,006	3,844	4,066	3,891
Crude fat, %	6.01	3.58	5.78	3.49	6.08	3.79
Crude fiber, %	2.35	2.47	2.42	2.47	2.02	2.75
Amino acids						
Lysine, %	1.43	1.38	1.26	1.21	1.18	1.12
Methionine, %	0.73	0.70	0.66	0.63	0.62	0.60
Cystine, %	0.45	0.43	0.47	0.45	0.49	0.47
Threonine, %	0.98	0.94	0.88	0.84	0.82	0.78
Arginine, %	1.55	1.55	1.44	1.45	1.33	1.33
Histidine, %	0.49	0.46	0.49	0.47	0.45	0.47
Isoleucine, %	0.96	0.95	0.89	0.86	0.82	0.82
Leucine, %	1.57	1.56	1.57	1.56	1.55	1.56
Phenylalanine, %	0.94	0.93	0.89	0.88	0.91	0.89
Tryptophan, %	0.25	0.24	0.21	0.25	0.24	0.22
Valine, %	1.11	1.05	1.01	0.95	0.92	0.89
Alanine, %	0.93	0.95	0.93	0.93	0.94	0.94
Aspartic acid, %	1.58	1.51	1.47	1.54	1.47	1.50
Glutamic acid, %	6.11	6.07	6.32	6.05	6.00	6.17
Glycine, %	0.96	0.89	0.86	0.86	0.88	0.85
Proline, %	1.23	1.27	1.27	1.27	1.27	1.27
Serine,%	0.85	0.84	0.84	0.84	0.84	0.85
Tyrosine, %	0.54	0.50	0.50	0.48	0.52	0.50

[1] PC: positive control diet; NC, negative control diet with a 150 kcal /kg metabolizable energy (ME) reduction and reducing amino acids by 4.5 %.

Nutrient digestibility and viscosity

The collected digesta samples were dried at 55°C for 24 h, ground, and strained through a 0.75-mm sieve (ZM 200 Ultra-Centrifugal Mill, Retsch GmbH & Co., KG, Haan, Germany) as detailed by (Yu et al., 2023) for nutrient digestibility analysis. Dry matter (**DM**) was determined using the standard AOAC procedure (method 930.15; AOAC, 2005). The concentration of chromic oxide was also determined using the method of Oketch et al. (2022). The diets and digesta samples were analyzed for crude protein (method 984.13), crude fat (method 920.39), crude fiber (method 962.09), amino acids (method 982.30 E [a, b]) following the procedures by the AOAC (2005). Gross energy in digesta and diets was determined using an adiabatic bomb calorimeter (IKA Calorimeter System C 6000; IKA Works, Wilmington, NC). The apparent ileal digestibility (**AID**) of DM, energy, crude protein, crude fat, crude fiber, and amino acids were calculated as follows;

$$\text{Digestibility, } \% = 100 - \left[100 \times \frac{M_{diet} \times N_{digest}}{M_{digest} \times N_{diet}}\right]$$

M_{diet} is the marker concentration in the diet whereas N_{digest} is the nutrient concentration in ileal digesta whereas M_{digest} is the marker concentration in ileal digesta, and N_{diet} is the nutrient concentration in the diet.

Digesta viscosity was determined based on the method of Cruz et al. (2024). Collected samples of the ileal digesta were centrifuged at 4,000 rpm at 4°C for 15 mins. Each tube containing 0.5 mL supernatant was analyzed for viscosity using a viscometer (Model DV-II; Brookfield, Middleboro, MA, USA). The unit used to express viscosity measurement is mPa/s.

Intestinal morphology

To analyze the ileal morphometry, we followed the method described by Oketch et al. (2022). Ring-shaped ileal tissue samples, six diagonal histological sections (4-6 μm), were excised and dehydrated,

followed by impregnation in paraffin wax. The height of 10 well-align villi and their associated crypts were observed with an inverted microscope (Eclipse TE2000, Nikon Instruments Inc., Melville, NY 11747-3064, USA), and the height and width of the villi and the depth of the crypts were measured through the analysis of images of histological sections made from the computerized image-capture software (NIS-Elements Viewer software, Version: 4.20; NIS Elements, Nikon, USA). The height of the villi is defined as the distance from their tip to the base, and the width of the villi was measured at the half-height point. The depth of the crypt is defined as the distance from the top of the crypt to the muscularis mucosa (Seyyedin and Nazem, 2017). Moreover, the villus absorptive surface area was calculated using the collected data following the methodology outlined by Prakatur et al. (2019) as follows;

$$Villus\ absorptive\ surface\ area = 2\pi \times (villus\ width \div 2) \times villus\ height$$

Blood parameters

Collected blood samples were centrifuged (LABOGENE 1248R, Gyrozen Co., Ltd., Daejeon, Korea) at 3,000 × *g* for 10 min at 4°C and the plasma was separated and stored at −80°C (UniFreez U 400, DAIHAN Scientific Co., Ltd, Wonju, Korea) until analysis. The concentrations of interleukin-1β (**IL-1β**), interleukin-10 (**IL-10**), interferon-gamma (**IFN-γ**), tumor necrosis factor-α (**TNF-α**) in plasma were quantified using commercially available ELISA kits (MyBioSource, San Diego, CA) according to the manufacturers' instructions described by Yu et al. (2021).

Statistical analyses

All data were analyzed using the general linear model (**GLM**) procedure for one-way ANOVA in SPSS software (version 29, Armonk, NY). The pen was used as the experimental unit for all growth performance measurements. Selected individual birds were considered as the experimental unit for the proportion of carcass trait weights, nutrient digestibility, intestinal morphology, and blood metabolites. Statistical significance was determined at a significance level of $P < 0.05$. Whenever treatment effects were found to be significant ($P < 0.05$), the means were further analyzed and compared using Tukey's multiple range test procedures implemented in SPSS.

Results

Growth performance

The results for growth performance are summarized in Table 3. Broilers on the NC diet had lower ($P < 0.01$) BW compared to other treatments across all periods except on day 1. There were no differences ($P > 0.05$) in BW between broilers fed the PC diet and those fed the NC + β-mannanase diet for the entire experimental period. Broilers receiving the NC + β-mannanase diet had higher ($P < 0.05$) ADG than those on the NC diet during the starter (days 1–10), grower (days 11–21), finisher (days 22–35), and overall (days 1–35) phases. Regarding ADFI, broilers on the NC diet consumed more ($P = 0.003$) than those on the PC diet during the grower phase (days 11–21). However, there were no differences ($P > 0.05$) in ADFI between treatments during the starter, finisher, or overall periods. In terms of FCR, broilers fed the NC diet had a higher ($P < 0.001$) FCR across all measured periods. No differences in FCR were noted between the PC diet and the NC + β-mannanase diet across all phases.

Carcass traits

As presented in Table 4, there were no differences ($P > 0.05$) in the dressing ratio or the relative weights of the leg and bursa of Fabricius between the treatments. However, broilers fed the NC + β-mannanase diet had higher ($P < 0.05$) relative weights of the breast, liver, spleen, and thymus compared to those fed the NC diet. Additionally, broilers on the NC + β-mannanase diet exhibited a higher ($P < 0.001$) relative liver weight compared to those fed the PC diet.

Table 3
Effect of β-mannanase supplementation on growth performance in broilers fed energy and amino acid deficient diets[1].

Items	Dietary treatment[2]			SEM[3]	P-value
	PC	NC	NC + β-mannanase		
Body weight, g					
Day 1	41.63	41.73	41.79	0.270	0.970
Day 10	256.09[b]	231.86[a]	252.28[b]	2.596	0.002
Day 21	882.50[b]	814.14[a]	876.96[b]	8.032	0.004
Day 35	2157.43[b]	2013.04[a]	2202.13[b]	18.707	0.001
Average daily gain, g/day					
Day 1-10	21.45[b]	19.01[a]	21.05[b]	0.254	0.002
Day 11-21	56.95[b]	52.93[a]	56.79[b]	0.660	0.035
Day 22-35	91.07[ab]	85.64[a]	94.65[b]	1.129	0.013
Day 1-35	60.45[b]	56.32[a]	61.72[b]	0.537	0.001
Average daily feed intake, g/day					
Day 1-10	24.71	25.34	24.48	0.221	0.279
Day 11-21	73.28[a]	79.79[b]	75.70[ab]	0.678	0.003
Day 22-35	129.26	135.44	130.01	1.735	0.305
Day 1-35	81.80	86.49	82.79	0.781	0.055
Feed conversion ratio, g/g					
Day 1-10	1.15[a]	1.34[b]	1.16[a]	0.010	<0.001
Day 11-21	1.29[a]	1.51[b]	1.33[a]	0.011	<0.001
Day 22-35	1.42[a]	1.58[b]	1.37[a]	0.009	<0.001
Day 1-35	1.35[a]	1.54[b]	1.34[a]	0.006	<0.001

[1] Values are the mean of eight replicates per treatment.
[2] PC: positive control diet; NC, negative control diet with a 150 kcal /kg metabolizable energy (ME) reduction and reducing amino acids by 4.5 %; NC + β-mannanase, NC + Natupulse TS 100g/MT.
[3] Pooled standard error of the mean.
[a,b] Values in a row with different superscripts differ significantly ($p < 0.05$).

Table 4
Effect of β-mannanase supplementation on carcass yields in broilers fed energy and amino acid deficient diets[1].

Items, %	Dietary treatment[2]			SEM[3]	P-value
	PC	NC	NC + β-mannanase		
Dressing ratio	90.45	90.28	91.02	0.268	0.505
Breast meat	27.17[ab]	26.55[a]	28.16[b]	0.241	0.038
Leg meat	9.82	9.65	9.60	0.081	0.524
Liver	2.35[b]	1.99[a]	2.54[c]	0.025	<0.001
Spleen	0.096[ab]	0.084[a]	0.109[b]	0.003	0.006
Bursa	0.202	0.166	0.219	0.008	0.055
Thymus	0.257[ab]	0.201[a]	0.315[b]	0.010	0.001

[1] Values are the mean of eight replicates per treatment.
[2] PC: positive control diet; NC, negative control diet with a 150 kcal /kg metabolizable energy (ME) reduction and reducing amino acids by 4.5 %; NC + β-mannanase, NC + Natupulse TS 100g/MT.
[3] Pooled standard error of the mean.
[a-c] Values in a row with different superscripts differ significantly ($p < 0.05$).

Nutrient digestibility and viscosity

The AID of DM, crude protein, and energy increased ($P < 0.001$) in broilers fed the NC + β-mannanase diet compared to those fed the NC diet (Table 5). Furthermore, the AID of energy was higher ($P < 0.001$) in the NC + β-mannanase diet compared to the PC diet. No differences ($P > 0.05$) in the AID of crude fat or crude fiber were observed among the treatments. Additionally, the NC + β-mannanase treatment showed reduced ($P < 0.001$) digesta viscosity values compared to both the PC

Table 5
Effect of β-mannanase supplementation on apparent ileal digestibility of nutrients and ileal viscosity in broilers fed energy and amino acid deficient diets[1].

Items	Dietary treatment[2]			SEM[3]	P-value
	PC	NC	NC + β-mannanase		
Dry matter, %	75.59[b]	73.66[a]	76.57[b]	0.163	<0.001
Crude fat, %	77.22	77.08	78.01	0.270	0.338
Crude fiber, %	15.23	15.67	18.55	0.816	0.220
Crude protein, %	77.22[b]	74.78[a]	78.03[b]	0.286	<0.001
Energy, %	76.81[b]	74.73[a]	78.12[c]	0.172	<0.001
Viscosity, mPa/s	2.84[b]	2.97[c]	2.70[a]	0.022	<0.001

[1] Values are the mean of eight replicates per treatment.
[2] PC: positive control diet; NC, negative control diet with a 150 kcal /kg metabolizable energy (ME) reduction and reducing amino acids by 4.5 %; NC + β-mannanase, NC + Natupulse TS 100g/MT.
[3] Pooled standard error of the mean.
[a-c] Values in a row with different superscripts differ significantly ($p < 0.05$).

and NC treatments. As shown in Table 6, the AID of lysine and threonine was higher ($P < 0.05$) in the PC diet compared to the NC diet, but no differences in amino acid digestibility were noted between the PC and NC + β-mannanase diets.

Intestinal morphology

Broilers fed the PC and NC + β-mannanase diets exhibited increased ($P < 0.01$) villus height and villus height to crypt depth ratio compared to those on the NC diet (Table 7). Nevertheless, no changes ($P > 0.05$) were observed in crypt depth, villus width, or villus absorptive surface area for the NC + β-mannanase diet compared to other treatments.

Blood metabolites

Feeding the NC + β-mannanase diet reduced ($P < 0.001$) IL-1β and IFN-γ levels compared to both the PC and NC diets on day 35 (Table 8). Moreover, the NC + β-mannanase diet increased ($P < 0.001$) IL-10 levels compared to all other treatments. Broilers on the NC diet showed higher ($P < 0.001$) TNF-α levels compared to the other groups.

Table 6
Effect of β-mannanase supplementation on apparent ileal digestibility of amino acids in broilers fed energy and amino acid deficient diets[1].

Items, %	Dietary treatment[2]			SEM[3]	P-value
	PC	NC	NC + β-mannanase		
Indispensable Amino acid					
Arginine	91.34	90.46	91.01	0.217	0.273
Histidine	88.21	87.89	88.53	0.185	0.390
Isoleucine	83.81	83.05	83.43	0.153	0.149
Leucine	90.80	90.87	90.90	0.172	0.974
Lysine	90.45[b]	88.91[a]	89.53[ab]	0.207	0.020
Methionine	93.43	93.16	93.49	0.189	0.757
Phenylalanine	89.03	88.92	89.10	0.147	0.880
Threonine	85.85[b]	84.77[a]	85.13[ab]	0.149	0.024
Tryptophan	90.39[b]	87.61[a]	90.03[b]	0.198	<0.001
Valine	89.39	88.85	89.10	0.191	0.529
Dispensable Amino acid					
Alanine	84.88	84.67	84.87	0.190	0.881
Aspartic acid	83.51	82.69	82.78	0.167	0.117
Cystine	80.51	81.58	81.51	0.313	0.314
Glutamic acid	93.18	93.01	93.02	0.160	0.897
Glycine	80.01	79.61	79.59	0.169	0.531
Proline	85.76	85.77	86.36	0.203	0.405
Serine	86.63	85.78	86.24	0.175	0.168
Tyrosine	86.63	86.32	86.46	0.214	0.841

[1] Values are the mean of eight replicates per treatment.
[2] PC: positive control diet; NC, negative control diet with a 150 kcal /kg metabolizable energy (ME) reduction and reducing amino acids by 4.5 %; NC + β-mannanase, NC + Natupulse TS 100g/MT.
[3] Pooled standard error of the mean.
[a,b] Values in a row with different superscripts differ significantly ($p < 0.05$).

Table 7
Effect of β-mannanase supplementation on intestinal morphology in broilers fed energy and amino acid deficient diets[1].

Items	Dietary treatment[2]			SEM[3]	P-value
	PC	NC	NC + β-mannanase		
Villus height, μm	928.08[b]	779.36[a]	876.34[b]	14.607	0.002
Crypt depth, μm	121.15[ab]	132.80[b]	111.22[a]	2.501	0.008
Villus width, μm	102.80	100.84	105.19	2.256	0.736
Villus height: Crypt depth ratio	7.83[b]	5.87[a]	7.90[b]	0.192	<0.001
Villus absorptive surface area, mm^2	0.299[b]	0.247[a]	0.289[ab]	0.0074	0.0215

[1] Values are the mean of eight replicates per treatment.
[2] PC: positive control diet; NC, negative control diet with a 150 kcal /kg metabolizable energy (ME) reduction and reducing amino acids by 4.5 %; NC + β-mannanase, NC + Natupulse TS 100g/MT.
[3] Pooled standard error of the mean.
[a,b] Values in a row with different superscripts differ significantly ($p < 0.05$).

Discussion

The ongoing rise in feed prices presents a persistent challenge for poultry and livestock industries. In response, there is continuous research for strategies that are acceptable to consumers and practical for producers to mitigate feed and production costs. Feed additives that enhance the efficiency of feed ingredient utilization stand out as a promising solution, enabling the reduction of feed costs and the use of lower-quality ingredients in animal diets. Many feed components, particularly soybean meal, contain β-mannans, which can interfere with nutrient utilization. Consequently, using β-mannanase could be one of the strategies to improve nutrient absorption and utilization from soybean-based diets.

The findings of the present study indicate that diets with reduced ME and amino acid levels can negatively impact broiler performance for the entire experimental period. However, the addition of β-mannanase to the NC diet resulted in a 9.39 % increase in body weight on day 35 compared to the NC diet alone, though it was not significantly different from the PC diet. Likewise, regarding average daily gain, broilers fed the NC + β-mannanase diet showed increases of 10.73 %, 7.29 %, 10.52 %, and 9.59 % during the starter, grower, finisher phases and over the entire experiment, respectively, compared to those fed the NC diet

Table 8
Effect of β-mannanase supplementation on immune marker in broilers fed energy and amino acid deficient diets[1].

Items	Dietary treatment[2]			SEM[3]	P-value
	PC	NC	NC + β-mannanase		
IL-1β, pg/mL	1850.26[c]	1725.06[b]	1547.25[a]	18.778	<0.001
IL-10, pg/mL	1234.25[a]	1369.13[b]	2769.09[c]	18.678	<0.001
IFN-γ, pg/mL	453.88[b]	614.22[c]	380.96[a]	11.577	<0.001
TNF-α, pg/mL	515.93[a]	862.85[b]	521.06[a]	10.619	<0.001

[1] Values are the mean of eight replicates per treatment.
[2] PC: positive control diet; NC, negative control diet with a 150 kcal /kg metabolizable energy (ME) reduction and reducing amino acids by 4.5 %; NC + β-mannanase, NC + Natupulse TS 100g/MT.
[3] Pooled standard error of the mean.
[a-c] Values in a row with different superscripts differ significantly ($p < 0.05$).

alone. There was also no significant difference observed in ADG between the NC + β-mannanase group and the PC group. Furthermore, the NC + β-mannanase diet improved feed efficiency for the starter, grower, finisher, and over the entire period by 13.43 %, 11.92 %, 13.29 %, and 12.99 %, respectively, compared to the NC diet. These findings align with the results of Ferreira Jr et al. (2016), who reported a decrease in ADG and an increase in FCR in Cobb 500 male broilers fed a corn-soybean-based diet with 100 kcal lower ME and 3 % reduced total amino acids, specifically lysine, methionine + cysteine, arginine, threonine, tryptophan, and valine, compared to broilers fed nutritionally adequate control diets. Furthermore, numerous studies (Ferreira Jr et al., 2016; Latham et al., 2018; Yaqoob et al., 2022; Nusairat et al., 2024) have shown that β-mannanase supplementation in a reduced energy diet enhances the growth performance of broilers, which aligns with the findings of our present results. Notably, while other experiments typically reduced ME by 100 kcal/kg, our study applied a more stringent reduction of 150 kcal/kg along with a 4.5 % reduction in standardized ileal digestible amino acids, including lysine, methionine + cystine, threonine, yet β-mannanase supplementation still yielded substantial positive effects on growth performance. The observed improvement in nutrient utilization can be attributed to the ability of β-mannanase to hydrolyze β-mannans, a key anti-nutritional factor in corn-soybean-based poultry diets. This enzyme breaks down these complex polysaccharides into smaller oligosaccharides, such as mannobiose and mannotriose, which are more easily absorbed in the gastrointestinal tract (Dhawan and Kaur, 2007). By reducing molecular size and disrupting the nutrient-encapsulating effects of NSP, β-mannanase enhances the digestibility of DM (Balasubramanian et al., 2018), crude protein, and crude fiber (Li et al., 2010). Additionally, β-mannanase supplementation improves ME utilization (Kong et al., 2011; Nusairat et al., 2024) and ileal amino acid digestibility (Mussini et al., 2011; Ferreira Jr et al., 2016), contributing to better overall nutrient absorption and growth performance in broilers.

This current study found that supplementing feed, deficient in energy and amino acids, with β-mannanase improved the breast muscle yield in broilers. This outcome suggests that β-mannanase enhances nutrient absorption even under nutrient-deficient conditions, thereby facilitating muscle growth. The improved protein digestibility likely leads to a greater availability of amino acids, which are essential substrates for muscle protein synthesis. Specifically, absorbed amino acids activate the mammalian target of rapamycin (**mTOR**) signaling pathway, a crucial regulator of muscle protein synthesis and hypertrophy (Kimball and Jefferson, 2006). While this mechanism was not directly analyzed in our study, it provides a potential explanation for the observed improvements in growth performance. These findings align with the studies (Cho and Kim, 2013; Yaqoob et al., 2022) in which β-mannanase-supplemented diets were shown to improve protein digestibility and promote muscle development in broilers. The study also revealed that broilers fed a diet deficient in energy and amino acids exhibited the lowest relative liver weight. This suggests that developing crucial metabolic organs like the liver can be hindered when essential nutrients are lacking. The liver is central to metabolic processes, including gluconeogenesis, lipid metabolism, and detoxification. A lack of energy and amino acids can hinder hepatocyte proliferation and protein synthesis, both of which are essential for liver growth and function. These results are in line with the findings that nutrient deficiencies like methionine (Peng et al., 2018) and energy (Mohammadigheisar et al., 2018) were also shown to impair liver development and metabolic function. Energy deprivation may also limit adenosine triphosphate (**ATP**) availability, compromising biosynthetic pathways and organ development. On the other hand, the study showed that the relative liver weight of broilers improved when mannanase was added to a diet deficient in energy and amino acids. This improvement may result from the enzymatic breakdown of NSP, which encapsulates nutrients and impedes digestion (Singh et al., 2018). By hydrolyzing β-mannans into smaller oligosaccharides, β-mannanase reduces intestinal viscosity, enhances nutrient release, and improves the digestibility of amino acids, lipids, and energy sources (Dhawan and Kaur, 2007; Balasubramanian et al., 2018). Beyond NSP degradation, β-mannanase may also influence gut health by modulating the intestinal microbiota, promoting beneficial bacteria like *Lactobacillus*, spp., which can enhance nutrient absorption and metabolic efficiency (Zhang et al., 2024b). This multifaceted action likely contributes to compensating for nutrient deficiencies and supporting liver growth. However, some studies have reported that adding mannanase led to no significant change or reduced liver weight (Li et al., 2010; Mohammadigheisar et al., 2021; Yaqoob et al., 2022). These discrepancies may be due to differences in feed composition, broiler breeds, growth stages, and the nutrient levels of the diets used in the experiments. In particular, this study utilized a diet with a 150 kcal/kg energy reduction and a 4.5 % decrease in key essential amino acids (lysine, methionine + cysteine, and threonine), which may have sensitized the broilers to the positive effects of β-mannanase. A more detailed comparative analysis of diet compositions across studies is necessary to resolve these differences. In this present study, adding mannanase to a diet deficient in energy and amino acids improved the relative weights of the immune organs, saying the spleen and thymus in broilers. These results indicate that mannanase may promote the development of immune organs under specific nutrient-deficient conditions. The likely reason for this outcome is that mannanase breaks down β-mannans in the feed into MOS (Zou et al., 2006). These oligosaccharides are known to have prebiotic effects, as they modulate the gut microbiota, enhance the proliferation of beneficial bacteria, and indirectly stimulate the immune system by improving gut health and reducing the burden of pathogenic bacteria. Enhanced immune activity driven by these mechanisms may, in turn, contribute to the growth and functional development of immune organs such as the spleen and thymus. However, these findings differ from several studies (Li et al., 2010; Cho and Kim, 2013; Ferreira Jr et al., 2016), where the addition of mannanase showed little to no effect on immune organ development or weight reduction. While the current study highlights a potential link between MOS-mediated immune modulation and immune organ development, further research is needed to confirm these effects and explore the optimal conditions for mannanase efficacy in nutrient-deficient diets.

This study demonstrated that supplementing a diet deficient in energy and amino acids with mannanase significantly reduced the ileal digesta viscosity and enhanced the apparent ileal digestibility of DM, crude protein, energy, and specifically tryptophan among essential amino acids. These improvements in nutrient digestibility translated into better growth performance in broilers. The reduction in intestinal viscosity facilitated better nutrient absorption, contributing to enhanced growth performance. Elevated intestinal viscosity is known to hinder nutrient utilization in diets with high-viscosity ingredients (Latham et al., 2016, 2018). β-mannans are notably viscous and may negatively affect digestion; however, this can be addressed with enzyme supplementation. β-mannanase acts by lowering digesta viscosity through the formation of mannan oligosaccharides (Saenphoom et al., 2013; Balasubramanian et al., 2018), and this enzyme-induced reduction in viscosity is thought to influence digesta DM (Tahir et al., 2005; Mehri et al., 2010). The enhancement in nutrient digestibility seen in this study resulted in more efficient feed utilization, leading to greater body weight gain and improved feed efficiency. These outcomes align with previous studies (Ferreira Jr et al., 2016; Balasubramanian et al., 2018; Mohammadigheisar et al., 2021), which similarly demonstrated that β-mannanase supplementation boosts nutrient digestibility and growth performance in broilers. For instance, Balasubramanian et al. (2018) showed that the reduction in digesta viscosity via mannanase supplementation led to significant improvements in nutrient absorption and growth rates. Additionally, Mohammadigheisar et al. (2021) reported improved feed efficiency and body weight gain, while Ferreira Jr et al. (2016) observed higher digestibility of amino acids like lysine and threonine, leading to better growth. The results from this study suggest that the improvements in nutrient digestibility, particularly the

enhanced absorption of essential amino acids, are major contributors to the growth performance gains observed. β-mannanase enables better utilization of crucial nutrients such as DM (Cho and Kim, 2013), crude protein (Li et al., 2010), energy (Daskiran et al., 2004; Kong et al., 2011), and amino acids (Mussini et al., 2011), allowing broilers to convert feed into body mass more efficiently. This is especially beneficial in diets deficient in energy and amino acids. Thus, the improved growth performance can be attributed to β-mannanase's ability to hydrolyze NSPs, reducing intestinal viscosity and enhancing the availability of nutrients critical for growth. Given the significant impact on both nutrient digestibility and growth performance, future research should focus on optimizing β-mannanase supplementation levels in broiler diets, particularly those with varying energy and amino acid compositions.

In this study, it was found that supplementing a diet deficient in energy and amino acids with mannanase increased the villus height of the ileum, decreased crypt depth, and improved the villus height to crypt depth ratio in broilers. These results align with the finding of Zhang et al. (2024b), who reported that β-mannanase enhances growth performance by alleviating intestinal inflammation and improving the intestinal microbiota. Zhang et al. (2024b) demonstrated that β-mannanase contributes to maintaining microbial balance in the gut, reduces inflammatory responses, and improves nutrient digestibility, thereby supporting broiler growth performance. The likely reason for these outcomes is that β-mannanase reduces the viscosity of intestinal mucus and breaks down anti-nutritional factors that hinder nutrient absorption, thereby enhancing the structural integrity of the gut and mitigating inflammation.

The inflammatory response is regulated by a balance between pro-inflammatory and regulatory cytokines. IL-1β and TNF-α are key mediators of inflammation, initiating immune activation and the acute-phase response, while IL-10 plays a regulatory role by limiting excessive inflammation and supporting intestinal barrier function (Kogut et al., 2018; Bradley, 2008; Jarry et al., 2008). In this study, supplementation with mannanase in a diet deficient in energy and amino acids significantly reduced the pro-inflammatory cytokines (i.e., IL-1β, IFN-γ, and TNF-α) while increasing the regulatory cytokine IL-10 in the plasma of broilers, indicating its potential to mitigate inflammatory responses and improve intestinal health. Liu et al. (2024) demonstrated similar effects in energy-deficient diets, showing that mannanase reduced the levels of IL-1β and increased the concentration of IL-10 in the jejunum mucosa, thus supporting immune modulation. These results suggest that mannanase can effectively regulate immune responses in broilers by reducing inflammation, even under nutrient-deficient conditions. This highlights its role in promoting gut health and maintaining immune stability. The reduction in inflammatory cytokines and the concurrent increase in IL-10 can be attributed to mannanase's ability to break down β-mannan, a component that negatively affects nutrient absorption and gut integrity. By reducing the viscosity of intestinal contents and improving nutrient utilization, mannanase helps enhance the growth performance in broilers (Yaqoob et al., 2022).

Conclusion

In conclusion, supplementing β-mannanase to a diet deficient in ME and amino acids improved growth performance, nutrient digestibility, and immune responses in broilers, restoring performance metrics comparable to a nutritionally adequate control diet. These findings suggest that β-mannanase may help mitigate the negative effects of nutrient-restricted diets in poultry production. However, this study was conducted under controlled conditions and with a specific reduction level of nutrients (150 kcal/kg ME and 4.5 % amino acids). Therefore, caution is needed when generalizing these findings to commercial field settings or to other nutrient reduction strategies. Future studies should incorporate a broader range of immune and intestinal health parameters to better understand the underlying mechanisms of β-mannanase action.

Declaration of competing interest

All the listed authors reviewed and approved the submission of the current manuscript to Poultry Science. The authors declare no competing interests.

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