

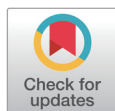
# Far-infrared ray and $\beta$ -mannanase influence on growth performance, meat quality, gut morphology, and blood parameters in nutritionally challenged broilers

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## Abstract

This study investigated the effects of far-infrared ray (FIR) radiation on broiler performance, carcass traits, meat quality, intestinal morphology, and blood metabolites in birds fed different energy and amino acid (EAA) levels with or without  $\beta$ -mannanase from hatch to day 35. A total of 384 one-day-old Ross 308 broilers were assigned to a  $2 \times 2 \times 2$  factorial design: FIR radiation (yes or no), dietary EAA level (normal or low), and  $\beta$ -mannanase supplementation (yes or no). Data were analyzed using three-way ANOVA to evaluate the main and interactive effects of these factors, with Tukey's test applied for multiple comparisons. FIR exposure improved ( $p < 0.001$ ) average daily gain (ADG) and average daily feed intake (ADFI) on days 1–35. Feed conversion ratio (FCR) was reduced ( $p < 0.05$ ) on days 1–10, 11–21, and overall. FIR combined with  $\beta$ -mannanase enhanced ( $p < 0.001$ ) ADG on days 1–10 and 11–21. Broilers fed a low EAA diet and exposed to FIR had higher ( $p < 0.01$ ) ADFI on days 1–10. On day 35, FIR increased ( $p < 0.001$ ) moisture, crude protein, and ash content in breast meat and improved ( $p < 0.05$ ) relative breast meat weight. FIR also enhanced ( $p < 0.01$ ) water-holding capacity and pH, while reducing ( $p < 0.05$ ) cooking loss. FIR improved intestinal morphology by increasing ( $p < 0.001$ ) villus height on days 21 and 35. A significant three-way interaction ( $p < 0.01$ ) among FIR, EAA level, and  $\beta$ -mannanase was observed for villus height on day 21. Immunologically, FIR reduced ( $p < 0.01$ ) blood levels of IL-1 $\beta$  and TNF- $\alpha$  on days 21 and 35, and IFN- $\gamma$  on day 35. FIR also lowered ( $p < 0.05$ ) blood glucose and lactate levels on both days. In conclusion, FIR radiation, particularly under nutrient-challenged conditions, enhanced broiler growth performance, carcass yield, meat quality, and immune status, with  $\beta$ -mannanase providing additional early growth benefits. FIR technology shows potential as a non-invasive, feed-independent strategy to improve commercial broiler productivity, warranting further field evaluation.

**Keywords:** Far-infrared ray,  $\beta$ -Mannanase, Nutrient density, Performance, Intestinal health

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## Competing interests

No potential conflict of interest relevant to this article was reported.

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## Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

## Authors' contributions

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## Ethics approval and consent to participate

The experimental protocol and procedures for the current study were reviewed and approved by the Animal Ethics Committee of Chungnam National University (Protocol Number: 202407A-CNU-127).

## INTRODUCTION

FIR with wavelengths ranging from 3–100  $\mu\text{m}$  have recently gained attention as an innovative technology in housing management systems in poultry production [1]. Birds perceive light through their eyes or the skull, allowing light to reach the pineal gland in the triangular region behind the brain or the hypothalamus [2]. It has been observed that organic compounds subjected to FIR emit heat [3]. Numerous studies demonstrated the biological effects of FIR radiation on humans [4] and rats [5,6]. It has been investigated that FIR radiation has beneficial effects on immune response [7], injury recovery [8], *in vivo* utilization of L-arginine [9], and energy metabolism in animals [10]. However, most studies investigating the effects of FIR on animals have been conducted using rats as experimental animals. Only limited studies investigating the impact of FIR radiation on chickens have been reported to date. For instance, Lim et al. [11] investigated the effects of FIR radiation on performance, blood biochemistry, and fecal microflora in laying hens. They found that LED and FIR radiation reduced the number of pathogenic bacteria (*Escherichia coli* and *Salmonella*) on farm floors and decreased concentrations of serum cholesterol and triglycerides while maintaining laying performance and egg quality. Moreover, Son et al. [12] investigated the effects of FIR radiation on broiler performance, blood biochemical profiles, and fecal gas emissions from the litter. Son et al. [12] demonstrated that exposing white and green light combined with FIR radiation for 35 days potentially improves broiler production, immune response, and farm environment. Oh et al. [13] further showed that FIR exposure improved growth performance and carcass traits and reduced circulating pro-inflammatory cytokines in broilers, plausibly via improved nutrient utilization and modulation of inflammatory pathways.

Along with advancements in lighting management, dietary strategies have also evolved to enhance production efficiency. Lately, there has been significant interest in reducing the energy and amino acid levels in poultry diets as a means of cutting feed costs and enhancing energy and nutrient utilization in poultry production [14]. While this approach offers economic advantages, it also comes with the risk of compromised growth due to limited nutrient availability. Supplementing  $\beta$ -mannanase is an option for enhancing nutrient digestion and absorption in diets with low nutrient levels by breaking down  $\beta$ -mannan, reducing intestinal viscosity, and increasing nutrient availability [15]. This enables the maintenance of optimal growth performance, even with reduced energy and amino acid levels in the feed.

To the best of our knowledge, no study has tested the effects of FIR radiation on proximate meat composition and meat quality in broilers. Moreover, while the effects of FIR in combination with  $\beta$ -mannanase supplementation or low-EAA diets remain unexplored, physiological evidence suggests that these factors act through distinct yet complementary mechanisms. FIR radiation can enhance nutrient utilization by stimulating mitochondrial energy metabolism [10] and inducing molecular vibration in water-rich digesta that loosens hydrogen bonding between water molecules and may reduce apparent digesta viscosity [11], improving enzyme access to substrates. On the nutritional side,  $\beta$ -mannanase supplementation improves nutrient digestibility by reducing the anti-nutritional effects of  $\beta$ -mannans, and dietary energy and amino acid levels determine growth performance by constraining metabolizable energy intake and the supply of amino acids for tissue synthesis. Taken together, we expected FIR to enhance  $\beta$ -mannanase access to substrates by promoting molecular vibration in water-rich digesta and modestly lowering digesta viscosity. Therefore, we expected FIR to augment  $\beta$ -mannanase effects, particularly under low-EAA diets.

This study aimed to evaluate the impact of FIR radiation on growth performance, carcass traits, proximate composition, meat quality, intestinal morphology, and blood metabolites in broilers fed diets with varying energy and amino acid levels with and without  $\beta$ -mannanase supplementation from hatch to day 35. We hypothesized that FIR radiation may enhance production performance

and health conditions, compensate for nutrient deficiencies in diets, and exert a synergistic effect with  $\beta$ -mannanase supplementation in the broiler production system.

## MATERIALS AND METHODS

### Birds and housing

A total of 384 one-day-old Ross 308 broiler chicks ( $45.16 \pm 0.022$  g) were obtained from a local hatchery (Yangji hatchery). All birds used in the study were vaccinated against Newcastle disease at the hatchery. The experiment was conducted in two experimental broiler chambers on the same farm, each containing different lighting systems (LED and LED + FIR), with both chambers having identical dimensions. The LED-exposed birds received light at a wavelength of  $450 \pm 20$  nm ( $0.45 \pm 0.02$   $\mu$ m), while the LED + FIR-exposed birds received FIR at a wavelength of 4–64  $\mu$ m in addition to LED light. An Energy Pang (Bio-energy wave) that generates FIR was placed 3.50 m in front of the cage. Birds were randomly allocated in battery cages ( $76 \times 61 \times 46$  cm) with two nipple drinkers and a metal trough for free access to fresh water and feed. Birds were provided commercial feeds throughout the experimental period on an *ad libitum* basis. In each chamber, an ambient temperature was maintained at  $30 \pm 1^\circ\text{C}$  from day 1 to day 7, then gradually decreased to  $25 \pm 1^\circ\text{C}$  until day 14. Thereafter, ambient temperature was maintained at  $25 \pm 1^\circ\text{C}$  from day 14 to 35. The birds were subjected to a daily lighting scheme with 23 hours of continuous light and 1 hour of darkness from day 1 to 7, then 18 hours of continuous light and 6 hours of darkness from day 7 to 35. The environmental conditions were monitored regularly and adjusted accordingly as per the birds' behavior and age, based on Ross broiler management handbook [16].

### Experimental design and diets

A total of 384 one-day-old Ross 308 broilers were randomly allocated in a  $2 \times 2 \times 2$  factorial arrangement with six replicates and eight birds per cage. Factors were the following: FIR radiation, yes or no; dietary energy and amino acid level, normal or low; and  $\beta$ -mannanase supplementation, yes or no. In the study, FIR was generated using the Energy Pang (Bio-energy wave) developed by Cereswaves Korea Natupulse TS (BASF SE), a  $\beta$ -mannanase source with an activity of 8000 TMU (thermostable endo-1,4- $\beta$ -mannanase units) per gram, was included at 0.10% in the diets supplemented with  $\beta$ -mannanase [17]. Corn carrier was used as finely ground corn to pre-blend  $\beta$ -mannanase before feed mixing, to improve its uniform distribution in the diet. Normal-energy and amino acid diets were formulated to meet or exceed the nutritional requirements of broilers, whereas low-energy and amino acid diets contained 150 kcal/kg less metabolizable energy and 4.5% fewer amino acids than normal-energy and amino acid diets. The feeding program comprised starter (days 1–10), grower (days 11–21), and finisher phases (days 22–35). Diets were based on corn and soybean meal and provided in mash form. The ingredients and the analyzed nutrient compositions are recorded in Table 1. All diets contained phytase (Natuphos E 5,000 G; 5,000 FTU/g) and were provided in a mash form. Full matrix values for the phytase were applied based on the manufacturer's specifications.

### Growth performance evaluation

Throughout the experiment, the average body weight (BW) and feed remains were recorded on days 10, 21, and 35 to calculate the ADG and ADFI. The FCR was also calculated using this data for the entire experimental period. In cases of bird mortalities, the mortality-corrected FCR was also calculated.

**Table 1.** Composition (g/kg, as-fed basis) of the experimental diets

Items	Experimental diets <sup>1)</sup>					
	Starter (d 1–10)		Grower (d 11–21)		Finisher (d 22–35)	
	Normal-energy and amino acid diet	Low-energy and amino acid diet	Normal-energy and amino acid diet	Low-energy and amino acid diet	Normal-energy and amino acid diet	Low-energy and amino acid diet
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 <sup>2)</sup>	0.40	0.40	0.40	0.40	0.40	0.40
Phytase <sup>3)</sup>	0.02	0.02	0.02	0.02	0.02	0.02
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
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

<sup>1)</sup>Normal-energy and amino acid diet: The diet formulated meets the standard or exceeds Ross 308 nutrition specification [16]; Low-energy and amino acid diet: Nutritionally challenged (150 kcal/kg metabolizable energy & 4.5% amino acid reduction) from Normal-energy and amino acid diet.

<sup>2)</sup>Provided per kilogram of diet: vitamin A (trans-retinyl acetate), 6,400 IU; vitamin D<sub>3</sub> (cholecalciferol), 1,760 IU; vitamin E, 12,000 IU; vitamin K<sub>3</sub>, 1,760 mg; biotin, 80 mg; thiamin, 1,600 mg; riboflavin, 3,520 mg; pyridoxine, 2,400 mg; vitamin B<sub>12</sub>, 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.

<sup>3)</sup>phytase: Natuphos E 5000G, 5000 FtU/g (200 g/metric ton).

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

### Post-mortem procedure and sample collection

On days 21 and 35, six birds per treatment (one bird per cage) with body weights closest to the mean were selected and euthanized by carbon dioxide asphyxiation for sample collection. The dressing percentage with giblets (i.e., heart, gizzard, and liver) was determined as a function of the live weight of the birds. Breast and leg meat were separated from the carcasses and weighed to measure their relative to the entire carcass weight. On day 35, both sides of the breast meat were collected for meat quality and composition analyses. The left sides were for proximate composition analysis, and the right sides were for meat quality analysis. Breast meats were extracted, deboned, and placed in resealable plastic bags and then sent to the laboratory for further analysis.

Blood samples were collected from the brachial vein on days 21 and 35 of the experiment using a vacutainer coated with lithium heparin (BD Vacutainer, Becton Dickinson) prior to the euthanasia of the birds. The collected blood samples were immediately transported to the laboratory for plasma separation.

Abdominal incisions on the bird were made on each sacrificed bird to separate the ileum sample from the gastrointestinal tract. The segment of the small intestine that extends from Meckel's diverticulum to the ileocecal junction is the ileum [18]. The removed ileal samples (3 cm piece) were flushed with ice-cold phosphate-buffered saline (PBS, pH 7.4), placed into plastic containers containing 10% formaldehyde for fixation, and stored until microscopic slide preparation.

### Sample preparation and laboratory analyses

To investigate the carcass proximate composition, the breast meat samples were analyzed for moisture, crude protein, crude fat, and crude ash using the methodology outlined by [19] and [20]. The moisture content was determined by dehydrating the meat in a drying oven. Each sample, weighing two grams, was placed in Petri dishes and then dried in an oven at 103 °C for 16 to 18 hours. Following drying, the weight of the Petri dishes with the samples was recorded after they were cooled. The moisture content was then calculated using the following formula: % Moisture =  $(W_3 - W_1) / (W_2 - W_1) \times 100$ , where  $W_1$  is the weight of the petri dish,  $W_2$  is the weight of the sample in a petri dish before drying, and  $W_3$  is the weight of the sample in a petri dish after drying. The crude protein content was determined using the Kjeldahl method [19]. The percentage of nitrogen was first obtained through titration and was then used to calculate the crude protein using the following formula: % Crude protein = % Nitrogen  $\times$  6.25. To determine crude fat, the Soxhlet extraction method in accordance with [19] standards was applied. Five grams of meat samples were extracted for 8 hours using the Soxhlet apparatus (DKZW4; Shanghai Kexing Instrument LTD) and the % Fat was then calculated using the formula: % Fat =  $(W_3 - W_2) / W_1 \times 100$ , where  $W_1$  is the weight of the dried sample,  $W_2$  is the weight of the water's volume in the flask, and  $W_3$  is the volume of water after fat extraction in the flask. To measure the ash content, 3.00 g of meat samples were placed into crucibles. The crucibles were dried and ignited in a muffle furnace (HD 230 PAD; Horno de Mufla, Tecnylab) at 550 °C for 4 hours until a light grey ash was obtained. After turning it into an ash-dried sample, the weight was recorded. The ash content was computed using the following formula: % Ash =  $(W_3 - W_1) / (W_2 - W_1) \times 100$ , where  $W_1$  was the weight of the crucible without the sample,  $W_2$  was the weight of the crucible with the dried sample before ignition, and  $W_3$  was the weight of the crucible containing the ash after ignition.

The pH values of the breast meat were monitored immediately after sample collection. An aliquot (9 mL) of distilled water was added to 1 g of muscle, followed by homogenization (T25 basis, IKA-Werke) for 30 seconds. The homogenate was centrifuged at 2,090 $\times$ g (ScanSpeed 1580R, LaboGene ApS) for 10 minutes, and the supernatant was filtered through filter paper (No. 4, Whatman). The pH of the filtrate was measured using a pH meter (SevenEasy, Mettler-Toledo). The CIE lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) of broiler breast meat were determined using a spectrophotometer (CM-3500d, Konica Minolta). Measurements were taken perpendicularly to the surface of the broiler breast meat with a 30 mm diameter illumination area at three different locations per sample. The results were analyzed using SpectraMagic software (Spectramagic<sup>TM</sup> NX, Konica Minolta). For the water-holding capacity measurements, a 2 g sample of raw broiler breast meat was precisely weighed, placed on cotton wool, and added to a centrifuge tube. The weight of the meat after centrifugation at 2,090 $\times$ g (ScanSpeed 1580R, Labogene ApS) for 10 min was measured and compared to the initial meat weight. The moisture content of meat was determined by drying 2 g of samples placed in aluminum dishes for 3 hours at

110°C. After centrifugation, the remaining moisture (%) present in the meat was expressed as the water-holding capacity. To measure the cooking loss, the breast meat of the broiler was weighed, vacuum packaged and cooked for 20 min in a water bath at 80°C until the internal temperature reached 70°C. The cooked breast meat of broilers was cooled at room temperature (20°C) for 30 min. After the vacuum bag was removed, the surface moisture of the breast meat of the broiler was removed with paper towels, and the cooked breast meat of the broiler was weighed. The cooking loss was calculated as the difference between the weight of raw breast meat and cooked breast meat. For the shear force analysis, the samples measuring cooking loss were cut into 5 cm (length)  $\times$  1.3 cm (diameter) pieces. The shear force was measured using an A-XT2 texture analyzer (Stable Micro Systems). The speed was set to 1.5 mm/sec, and the measured cutting depth was 3 cm [21].

Collected blood samples were centrifuged (LABOGENE 1248R, Gyrozen) at 3,000 $\times$ g for 10 min at 4°C, and the plasma was separated and stored at -80°C (UniFreez U 400, Daihan Scientific) until analysis. The concentrations of interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-10 (IL-10), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interferon-gamma (IFN- $\gamma$ ) in plasma were quantified using commercially available ELISA kits (MyBioSource) according to the manufacturers' instructions described by [22]. The corticosterone concentrations were determined from the plasma using a corticosterone ELISA kit (CSB-E11991C, Cusabio) following the manufacturer's instructions. Lactate concentration was determined using a lactate assay kit (MAK065, Sigma-Aldrich) following the manufacturer's instructions. Additionally, glucose levels in the collected plasma were determined using a glucose assay kit (AM201-K, Asan Pharmaceutical) following the manufacturer's instructions [23].

To analyze the ileal morphometry, we followed the method described by [24]. Ring-shaped ileal tissue samples, six diagonal histological sections (4–6  $\mu$ m), 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) and the height 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). The height of the villi was defined as the distance from their tip to the base. The depth of the crypt was defined as the distance from the top of the crypt to the muscularis mucosa [25].

### Statistical analyses

All data were checked for normal distribution and analyzed using a three-way ANOVA in SPSS software (version 26) considering the main effect of FIR radiation, dietary energy and amino acid levels,  $\beta$ -mannanase supplementation, and their interactions. A pen was used as the experimental unit for the growth performance parameters (BW, ADG, ADFI, and FCR). Selected individual birds were used for sample collection as an experimental unit for the carcass traits, meat proximate composition, meat quality, intestinal morphology, and blood metabolites. Tukey's multiple range test was performed to identify significant variations between treatments at a 95% confidence level.

## RESULTS

### Growth performance

As shown in Table 2, FIR, nutrient levels, and  $\beta$ -mannanase influenced broiler growth performance from hatching to day 35. There were no significant differences in BW on day 1 between all treatments. Notably, the BW of birds exposed to FIR was higher ( $p < 0.001$ ) than those not exposed on days 10, 21, and 35. On days 10, 21, and 35, the BW of birds fed a low-energy and amino acid diet was lower ( $p < 0.001$ ;  $p = 0.005$ ;  $p = 0.001$ , respectively) than those fed a normal-energy and

Table 2. Effects of FIR radiation, dietary energy and amino acid levels, and  $\beta$ -mannanase supplementation on growth performance in broilers<sup>1)</sup>

Item			BW (g)				ADG (g/d)				ADFI (g/d)				FCR (g/g)			
FIR	EnAA	β-man- nanase	d 1	d 10	d 21	d 35	d 1–10	d 11–21	d 22–35	d 1–35	d 1–10	d 11–21	d 22–35	d 1–35	d 1–10	d 11–21	d 22–35	d 1–35
Yes	Normal	No	45.15	327.93	1009.55	2445.33	28.28	61.97	102.56	68.58	33.18	80.97	146.33	93.46	1.17	1.31	1.43	1.36
		Yes	45.15	322.13	1023.68	2485.97	27.70	63.78	104.45	69.74	32.34	81.12	147.27	93.64	1.17	1.27	1.41	1.34
	Low	No	45.06	305.84	947.40	2347.48	26.08	58.32	100.01	65.78	33.01	87.74	151.24	97.50	1.27	1.51	1.51	1.48
		Yes	45.23	315.89	1013.13	2458.47	27.07	63.39	103.24	68.95	31.83	83.52	149.38	95.10	1.18	1.32	1.45	1.38
No	Normal	No	45.15	294.45	934.67	2228.63	24.93	58.20	92.43	62.39	30.44	77.12	134.37	86.68	1.22	1.33	1.46	1.39
		Yes	45.19	312.52	950.36	2250.49	26.73	57.99	92.87	63.01	32.41	77.25	134.03	87.15	1.21	1.33	1.44	1.38
	Low	No	45.23	275.78	901.01	2144.56	23.06	56.84	88.82	59.98	31.98	85.70	137.20	90.95	1.39	1.51	1.55	1.52
		Yes	45.13	309.04	940.82	2229.98	26.39	57.43	92.08	62.42	32.76	81.03	135.31	88.95	1.24	1.41	1.47	1.43
Main effects																		
FIR																		
Yes	No	EnAA <sup>2)</sup>	45.15	317.95 <sup>a</sup>	998.44 <sup>a</sup>	2434.31 <sup>a</sup>	27.28 <sup>a</sup>	61.86 <sup>a</sup>	102.56 <sup>a</sup>	68.26 <sup>a</sup>	32.59 <sup>a</sup>	83.34 <sup>a</sup>	148.56 <sup>a</sup>	94.93 <sup>a</sup>	1.20 <sup>b</sup>	1.35 <sup>b</sup>	1.45	1.39 <sup>b</sup>
			45.18	297.95 <sup>b</sup>	931.71 <sup>b</sup>	2213.42 <sup>b</sup>	25.28 <sup>b</sup>	57.62 <sup>b</sup>	91.55 <sup>b</sup>	61.95 <sup>b</sup>	31.90 <sup>b</sup>	80.28 <sup>b</sup>	135.23 <sup>b</sup>	88.44 <sup>b</sup>	1.27 <sup>a</sup>	1.40 <sup>a</sup>	1.48	1.43 <sup>a</sup>
Normal	Low		45.16	314.26 <sup>a</sup>	979.56 <sup>a</sup>	2352.60 <sup>a</sup>	26.91 <sup>a</sup>	60.48	98.07	65.93 <sup>a</sup>	32.09	79.12 <sup>b</sup>	140.50	90.24 <sup>b</sup>	1.20 <sup>b</sup>	1.31 <sup>b</sup>	1.44 <sup>b</sup>	1.37 <sup>b</sup>
			45.16	301.64 <sup>b</sup>	950.59 <sup>b</sup>	2295.12 <sup>b</sup>	25.65 <sup>b</sup>	59.00	96.04	64.29 <sup>b</sup>	32.40	84.50 <sup>a</sup>	143.29	93.13 <sup>a</sup>	1.27 <sup>a</sup>	1.44 <sup>a</sup>	1.50 <sup>a</sup>	1.45 <sup>a</sup>
β-mannanase																		
Yes	No		45.18	314.90 <sup>a</sup>	982.00 <sup>a</sup>	2356.23 <sup>a</sup>	26.97 <sup>a</sup>	60.65 <sup>a</sup>	98.16	66.03 <sup>a</sup>	32.34	80.73 <sup>b</sup>	141.50	91.21	1.20 <sup>b</sup>	1.33 <sup>b</sup>	1.45	1.38 <sup>b</sup>
			45.15	301.00 <sup>b</sup>	948.16 <sup>b</sup>	2291.50 <sup>b</sup>	25.59 <sup>b</sup>	58.83 <sup>b</sup>	95.95	64.18 <sup>b</sup>	32.15	82.88 <sup>a</sup>	142.29	92.15	1.26 <sup>a</sup>	1.41 <sup>a</sup>	1.49	1.44 <sup>a</sup>
SEM			0.022	1.279	4.914	8.292	0.128	0.400	0.716	0.237	0.107	0.491	0.887	0.430	0.006	0.010	0.012	0.007
p-value																		
FIR			0.563	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.003	<0.001	<0.001	<0.001	0.034	0.211	0.012
EnAA			0.908	<0.001	0.005	0.001	<0.001	0.071	0.163	0.001	0.165	<0.001	0.125	0.002	<0.001	<0.001	0.014	<0.001
β-Mannanase			0.563	<0.001	0.001	<0.001	<0.001	0.029	0.131	<0.001	0.396	0.034	0.659	0.281	<0.001	<0.001	0.074	<0.001
FIR × EnAA			0.908	0.549	0.457	0.756	0.550	0.512	0.914	0.756	0.004	0.422	0.684	0.868	0.077	0.831	0.940	0.834
FIR × β-mannanase			0.207	<0.001	0.539	0.508	<0.001	0.049	0.804	0.510	<0.001	0.904	0.854	0.842	0.262	0.110	0.883	0.668
EnAA × β-mannanase			0.908	0.004	0.061	0.050	0.004	0.212	0.472	0.050	0.081	0.025	0.544	0.150	<0.001	0.003	0.235	0.005
FIR × EnAA × β-mannanase			0.088	0.949	0.488	0.919	0.973	0.451	0.797	0.923	0.336	0.911	0.861	0.971	0.306	0.572	0.871	0.973

<sup>1)</sup>Mean values are based on 6 replicates per treatment.<sup>2)</sup>Normal, diet contained the standard or exceed Ross 308 nutrition specification [16]; Low, diet with a 150 kcal/kg metabolizable energy reduction and reducing amino acids by 4.5% from Normal.

FIR, far-infrared ray; BW, bodyweight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; EnAA, Dietary energy and amino acid level; SEM, Pooled standard error of the mean.

amino acid diet. Birds supplemented with  $\beta$ -mannanase showed higher ( $p < 0.001$ ;  $p = 0.001$ ;  $p < 0.001$ , respectively) BW than birds not supplemented with  $\beta$ -mannanase on days 10, 21, and 35. On day 10, birds exposed to FIR supplemented with  $\beta$ -mannanase exhibited higher ( $p < 0.001$ ) BW than other treatments from an interactive effect between FIR radiation and  $\beta$ -mannanase supplementation. In addition, birds fed a normal-energy and amino acid diet supplemented with  $\beta$ -mannanase showed higher ( $p = 0.004$ ) BW than other treatments from an interactive effect between dietary energy and amino acid level and  $\beta$ -mannanase supplementation on day 10.

Birds exposed to FIR showed higher ADG than those not exposed in the starter (days 1–10;  $p < 0.001$ ), grower (days 11–21;  $p < 0.001$ ), finisher phases (days 22–35;  $p < 0.001$ ), and throughout the experimental periods (days 1–35;  $p < 0.001$ ). On the other hand, birds fed a low-energy and amino acid diet showed lower ADG than those fed a normal-energy and amino acid diet in the starter phase (days 1–10;  $p < 0.001$ ) and throughout the experimental period (days 1–35;  $p = 0.001$ ). Birds supplemented with  $\beta$ -mannanase had higher ADG than those not supplemented with  $\beta$ -mannanase in the starter (days 1–10;  $p < 0.001$ ), grower (days 11–21;  $p = 0.029$ ), and finisher phases (days 1–35;  $p < 0.001$ ). Additionally, birds exposed to FIR supplemented with  $\beta$ -mannanase exhibited higher ADG than other treatments in the starter (days 1–10;  $p < 0.001$ ) and the grower phases (days 11–21;  $p = 0.049$ ) due to an interactive effect between FIR radiation and  $\beta$ -mannanase supplementation. In the starter phase (days 1–10), birds fed a low-energy and amino acid diet without  $\beta$ -mannanase supplementation had a lower ( $p = 0.004$ ) ADG than other treatments from an interactive effect between dietary energy and amino acid level and  $\beta$ -mannanase supplementation.

Notably, birds exposed to FIR exhibited a higher ADFI than those not exposed in the starter (days 1–10;  $p = 0.002$ ), grower (days 11–21;  $p = 0.003$ ), finisher phases (days 22–35;  $p < 0.001$ ) and throughout the experimental periods (days 1–35;  $p < 0.001$ ). However, birds fed a low-energy and amino acid diet exhibited a higher ADFI than those fed a normal-energy and amino acid diet in the grower phase (days 11–21;  $p < 0.001$ ) and throughout the experimental periods (days 1–35;  $p = 0.002$ ). In the grower phase (days 11–21), birds supplemented with  $\beta$ -mannanase had a lower ( $p = 0.034$ ) ADFI than those not supplemented with  $\beta$ -mannanase. Moreover, the birds exposed to FIR and fed a low-energy and amino acid diet showed a higher ( $p = 0.004$ ) ADFI than other treatments in the starter phase (days 1–10). In the starter phase (days 1–10), birds exposed to FIR and supplemented with  $\beta$ -mannanase had a lower ( $p < 0.001$ ) ADFI due to an interactive effect between FIR radiation and  $\beta$ -mannanase supplementation. Additionally, birds fed a low-energy and amino acid diet and not supplemented with  $\beta$ -mannanase had a higher ( $p = 0.025$ ) ADFI than other treatments from an interactive effect between dietary energy and amino acid level and  $\beta$ -mannanase supplementation in the grower phase (days 11–21).

Interestingly, birds exposed to FIR had a lower FCR than those not exposed in the starter (days 1–10;  $p < 0.001$ ), grower phases (days 11–21;  $p = 0.034$ ), and throughout the experimental periods (days 1–35;  $p = 0.012$ ). Birds fed a low-energy and amino acid diet showed a higher FCR than those fed a normal-energy and amino acid diet in the starter (days 1–10;  $p < 0.001$ ), grower (days 11–21;  $p < 0.001$ ), finisher phases (days 22–35;  $p = 0.014$ ) and throughout the experimental periods (days 1–35;  $p < 0.001$ ). Birds supplemented with  $\beta$ -mannanase exhibited a lower FCR than those not supplemented with  $\beta$ -mannanase in starter (days 1–10;  $p < 0.001$ ), grower phases (days 11–21;  $p < 0.001$ ), and throughout the experimental periods (days 1–35;  $p < 0.001$ ). In addition, birds fed a normal-energy and amino acid diet and supplemented with  $\beta$ -mannanase observed a lower FCR than other treatments from an interactive effect between dietary energy and amino acid levels and  $\beta$ -mannanase supplementation in the starter (days 1–10;  $p < 0.001$ ), grower phases (days 11–21;  $p = 0.003$ ), and throughout the experimental periods (days 1–35;  $p = 0.005$ ).

### Carcass traits

Table 3 presents carcass traits affected by FIR, nutrient levels, and  $\beta$ -mannanase on days 21 and 35. On day 21, FIR radiation had no effect on dressing ratio and relative breast and leg meat weights. However, FIR radiation resulted in higher ( $p = 0.016$ ) relative breast meat weights without affecting dressing ratio and relative leg meat weights on day 35. However, there were no significant differences in relative breast and leg meat weights among treatments on day 21. In addition, there were no significant differences in the dressing percentage and relative breast and leg meat weights among the treatments on day 35. On the other hand, birds fed a low-energy and amino acid diet exhibited lower dressing percentage ( $p = 0.002$ ) and relative leg meat weights ( $p = 0.009$ ) than those fed a normal-energy and amino acid diet on day 21. Moreover, the birds supplemented with  $\beta$ -mannanase showed a higher ( $p < 0.018$ ) dressing percentage than those not supplemented with  $\beta$ -mannanase on day 21.

**Table 3.** Effects of FIR radiation, dietary energy and amino acid levels, and  $\beta$ -mannanase supplementation on carcass traits in broilers<sup>1)</sup>

Item			d 21			d 35		
FIR	EnAA	$\beta$ -mannanase	Dressing (%)	Breast (%)	Leg (%)	Dressing (%)	Breast (%)	Leg (%)
Yes	Normal	No	88.44	25.53	9.40	89.84	28.25	9.88
		Yes	88.84	25.57	9.33	89.92	28.08	9.98
	Low	No	87.25	24.64	9.11	88.74	27.87	9.77
		Yes	87.81	25.01	9.15	89.06	28.16	9.83
No	Normal	No	88.43	24.62	9.38	89.36	27.44	9.84
		Yes	88.57	25.24	9.39	89.50	27.4	9.88
	Low	No	87.01	24.21	9.15	88.83	26.09	9.71
		Yes	88.64	25.54	9.13	89.76	26.96	9.83
Main effects								
FIR								
Yes			88.09	25.19	9.25	89.39	28.09 <sup>a</sup>	9.87
No			88.17	24.90	9.26	89.36	26.97 <sup>b</sup>	9.82
EnAA <sup>2)</sup>								
Normal			88.57 <sup>a</sup>	25.24	9.38 <sup>a</sup>	89.65	27.79	9.90
Low			87.68 <sup>b</sup>	24.85	9.14 <sup>b</sup>	90.00	27.27	9.78
$\beta$ -mannanase								
Yes			88.47 <sup>a</sup>	25.34	9.25	89.56	27.65	9.88
No			87.79 <sup>b</sup>	24.75	9.26	89.19	27.41	9.80
SEM			0.138	0.180	0.044	0.181	0.223	0.070
<i>p</i> -value								
FIR			0.777	0.434	0.862	0.939	0.016	0.731
EnAA			0.002	0.289	0.009	0.133	0.248	0.418
$\beta$ -mannanase			0.018	0.111	0.920	0.316	0.594	0.571
FIR $\times$ EnAA			0.436	0.360	0.970	0.254	0.406	0.894
FIR $\times$ $\beta$ -mannanase			0.466	0.298	0.935	0.647	0.689	0.986
EnAA $\times$ $\beta$ -mannanase			0.142	0.478	0.852	0.480	0.450	0.941
FIR $\times$ EnAA $\times$ $\beta$ -mannanase			0.236	0.788	0.686	0.706	0.802	0.818

<sup>1)</sup>Mean values are based on 6 replicates per treatment.

<sup>2)</sup>Normal diet contained the standard or exceed Ross 308 nutrition specification [16]; Low, diet with a 150 kcal/kg metabolizable energy reduction and reducing amino acids by 4.5% from Normal.

FIR, far-infrared ray; EnAA, Dietary energy and amino acid level; SEM, Pooled standard error of the mean.

### Meat proximate composition

Table 4 summarizes meat proximate composition as affected by FIR, nutrient levels, and  $\beta$ -mannanase on day 35. There were no significant differences in crude fat content among the treatments. FIR radiation resulted in lower ( $p < 0.001$ ) moisture, crude protein, and crude ash contents in the breast meat. In addition, birds fed low-energy and amino acid diets had lower moisture ( $p = 0.042$ ), crude protein ( $p = 0.001$ ), and crude ash contents ( $p = 0.046$ ) in the breast meat than birds fed normal-energy and amino acid diets. Interestingly, birds exposed to FIR and supplemented with  $\beta$ -mannanase showed higher ( $p = 0.011$ ) crude protein content due to the interaction between FIR radiation and  $\beta$ -mannanase supplementation. On the other hand, no difference was found in breast meat composition by supplementing  $\beta$ -mannanase in diets.

### Meat quality

As shown in Table 5, FIR, dietary nutrients, and  $\beta$ -mannanase influenced breast meat quality on day 35. As a result, FIR radiation had no effect on shear force and meat color (lightness, redness,

**Table 4.** Effects of FIR radiation, dietary energy and amino acid levels, and  $\beta$ -mannanase supplementation on breast meat composition in broilers<sup>1)</sup>

Item			Moisture (%)	Crude protein (%)	Crude fat (%)	Crude ash (%)
FIR	EnAA	$\beta$ -mannanase				
Yes	Normal	No	74.15	22.77	1.51	1.57
		Yes	74.01	22.99	1.42	1.58
	Low	No	74.27	22.73	1.51	1.50
		Yes	74.05	22.81	1.57	1.57
No	Normal	No	74.45	22.76	1.38	1.41
		Yes	74.34	22.73	1.51	1.41
	Low	No	74.75	22.63	1.24	1.37
		Yes	74.59	22.59	1.43	1.39
Main effects						
FIR						
Yes			74.12 <sup>b</sup>	22.82 <sup>a</sup>	1.50	1.56 <sup>a</sup>
No			74.53 <sup>a</sup>	22.68 <sup>b</sup>	1.39	1.40 <sup>b</sup>
EnAA <sup>2)</sup>						
Normal			74.24 <sup>b</sup>	22.81 <sup>a</sup>	1.46	1.49 <sup>a</sup>
Low			74.42 <sup>a</sup>	22.69 <sup>b</sup>	1.44	1.46 <sup>b</sup>
$\beta$ -mannanase						
Yes			74.25	22.78	1.49	1.49
No			74.40	22.72	1.49	1.49
SEM			0.043	0.018	0.034	0.009
<i>p</i> -value						
FIR			<0.001	<0.001	0.117	<0.001
EnAA			0.042	0.001	0.780	0.046
$\beta$ -mannanase			0.076	0.140	0.288	0.094
FIR $\times$ EnAA			0.260	0.742	0.188	0.785
FIR $\times$ $\beta$ -mannanase			0.774	0.011	0.221	0.404
EnAA $\times$ $\beta$ -mannanase			0.708	0.289	0.449	0.274
FIR $\times$ EnAA $\times$ $\beta$ -mannanase			0.958	0.397	0.736	0.480

<sup>1)</sup>Mean values are based on 6 replicates per treatment.

<sup>2)</sup>Normal, diet contained the standard or exceed Ross 308 nutrition specification [16]; Low, diet with a 150 kcal/kg metabolizable energy reduction and reducing amino acids by 4.5% from Normal.

FIR, far-infrared ray; EnAA, Dietary energy and amino acid level; SEM, Pooled standard error of the mean.

**Table 5.** Effects of FIR radiation, dietary energy and amino acid levels, and  $\beta$ -mannanase supplementation on meat quality in broilers<sup>1)</sup>

Item			Water-holding capacity (%)	Cooking loss (%)	pH	Shear force (N)	Meat color		
FIR	EnAA	β-mannanase					L*	a*	b*
Yes	Normal	No	72.92	20.69	5.92	96.63	53.54	5.36	11.10
		Yes	73.06	20.05	5.96	97.59	53.81	5.36	11.25
	Low	No	71.02	20.72	5.92	94.47	53.44	5.37	11.17
		Yes	71.70	20.69	5.92	97.65	53.54	5.36	11.10
No	Normal	No	68.35	22.37	5.85	95.52	53.62	5.33	11.44
		Yes	70.85	21.19	5.90	96.17	53.59	5.34	11.39
	Low	No	65.87	22.86	5.80	96.35	53.23	5.34	11.31
		Yes	67.91	22.72	5.85	95.61	53.27	5.33	11.14
Main effects									
FIR									
Yes			72.18 <sup>a</sup>	20.54 <sup>b</sup>	5.93 <sup>a</sup>	96.59	53.58	5.36	11.15
No			68.24 <sup>b</sup>	22.28 <sup>a</sup>	5.85 <sup>b</sup>	95.91	53.43	5.34	11.32
EnAA <sup>2)</sup>									
Normal			72.30	21.07	5.91	96.48	53.64	5.35	11.29
Low			69.13	21.75	5.87	96.02	53.37	5.35	11.18
β-mannanase									
Yes			70.88	21.16	5.91	96.76	53.55	5.35	11.22
No			69.54	21.66	5.87	95.74	53.46	5.35	11.25
SEM			0.582	0.359	0.013	3.179	0.270	0.034	0.187
p-value									
FIR			0.002	0.019	0.007	0.917	0.775	0.742	0.658
EnAA			0.070	0.355	0.202	0.943	0.620	0.984	0.762
β-mannanase			0.257	0.492	0.213	0.874	0.867	0.987	0.920
FIR × EnAA			0.647	0.640	0.594	0.927	0.872	0.939	0.836
FIR × β-mannanase			0.430	0.820	0.572	0.869	0.867	0.936	0.844
EnAA × β-mannanase			0.988	0.571	0.777	0.974	0.962	0.920	0.825
FIR × EnAA × β-mannanase			0.828	0.884	0.730	0.888	0.907	0.997	0.935

<sup>1)</sup>Mean values are based on 6 replicates per treatment.<sup>2)</sup>Normal, diet contained the standard or exceed Ross 308 nutrition specification [16]; Low, diet with a 150 kcal/kg metabolizable energy reduction and reducing amino acids by 4.5% from Normal.

FIR, far-infrared ray; EnAA, Dietary energy and amino acid level; SEM, Pooled standard error of the mean.

and yellowness). However, birds exposed to FIR had higher water-holding capacity ( $p = 0.002$ ) and pH ( $p = 0.007$ ) than those not exposed. On the other hand, birds exposed to FIR exhibited lower ( $p = 0.019$ ) cooking loss than those not exposed. There were no significant differences in cooking loss, pH, shear force, and meat color in the breast meat among the treatments.

### Intestinal morphology

Table 6 summarizes ileal morphology influenced by FIR, dietary nutrients, and  $\beta$ -mannanase on days 21 and 35. On day 21, FIR radiation had no effect on villus height: crypt depth ratio but resulted in higher ( $p < 0.001$ ) villus height and crypt depth. FIR radiation resulted in higher villus height ( $p = 0.040$ ) without affecting crypt depth and villus height: crypt depth ratio on day 35. However, no significant differences were observed in the villus height: crypt depth ratio among the treatments on day 21, nor in the villus height: crypt depth ratio on day 35. Meanwhile, birds fed a low-energy and amino acid diet had lower villus height ( $p < 0.001$ ) and crypt depth ( $p = 0.005$ ) than those fed a normal-energy and amino acid diet on day 21. On day 35, villus height was lower

**Table 6.** Effects of FIR radiation, dietary energy and amino acid levels, and  $\beta$ -mannanase supplementation on intestinal morphology in broilers<sup>1)</sup>

Item			d 21			d 35		
FIR	EnAA	β-mannanase	Villus height (μm)	Crypt depth (μm)	Villus height : Crypt depth ratio	Villus height (μm)	Crypt depth (μm)	Villus height : Crypt depth ratio
Yes	Normal	No	691.36	146.77	4.93	803.58	159.04	5.29
		Yes	795.07	168.79	4.93	877.77	167.36	5.49
	Low	No	622.23	132.09	4.93	765.27	151.46	5.29
		Yes	657.66	139.62	4.93	789.37	154.64	5.35
No	Normal	No	631.82	122.96	5.16	773.30	161.01	5.03
		Yes	695.00	135.26	5.16	844.70	169.43	5.22
	Low	No	505.45	98.37	5.16	736.43	153.33	5.03
		Yes	607.90	118.31	5.16	759.63	156.55	5.08
Main effects								
FIR								
Yes			691.58 <sup>a</sup>	146.82 <sup>a</sup>	4.93	809.00 <sup>a</sup>	158.13	5.36
No			610.04 <sup>b</sup>	118.73 <sup>b</sup>	5.16	778.52 <sup>b</sup>	160.08	5.09
EnAA <sup>2)</sup>								
Normal			703.31 <sup>a</sup>	143.45 <sup>a</sup>	5.05	824.84 <sup>a</sup>	164.21	5.26
Low			598.31 <sup>b</sup>	122.10 <sup>b</sup>	5.05	762.68 <sup>b</sup>	154.00	5.19
β-mannanase								
Yes			688.91 <sup>a</sup>	140.49 <sup>a</sup>	5.05	817.87 <sup>a</sup>	162.00	5.29
No			612.72 <sup>b</sup>	125.05 <sup>b</sup>	5.05	769.65 <sup>b</sup>	156.21	5.16
SEM			4.613	3.582	0.132	7.171	5.342	0.187
p-value								
FIR			<0.001	<0.001	0.397	0.040	0.856	0.484
EnAA			<0.001	0.005	1.000	<0.001	0.345	0.850
β-Mannanase			<0.001	0.037	1.000	0.002	0.591	0.741
FIR × EnAA			0.852	0.936	1.000	0.934	0.995	0.996
FIR × β-mannanase			0.477	0.925	1.000	0.949	0.997	0.993
EnAA × β-mannanase			0.436	0.812	1.000	0.094	0.810	0.850
FIR × EnAA × β-mannanase			0.006	0.444	1.000	0.974	0.999	0.996

<sup>1)</sup>Mean values are based on 6 replicates per treatment.<sup>2)</sup>Normal, diet contained the standard or exceed Ross 308 nutrition specification [16]; Low, diet with a 150 kcal/kg metabolizable energy reduction and reducing amino acids by 4.5% from Normal.

FIR, far-infrared ray; EnAA, Dietary energy and amino acid level; SEM, Pooled standard error of the mean.

( $p < 0.001$ ) in birds fed a low-energy and amino acid diet than those fed a normal-energy and amino acid diet. Additionally, birds supplemented with  $\beta$ -mannanase had higher villus height ( $p < 0.001$ ) and crypt depth ( $p = 0.037$ ) than birds not supplemented with  $\beta$ -mannanase on day 21. On day 35, birds supplemented with  $\beta$ -mannanase had higher ( $p = 0.002$ ) villus height than those not supplemented with  $\beta$ -mannanase. Villus height: crypt depth ratio was not affected by nutrient energy and amino acid levels or  $\beta$ -mannanase supplementation in the diet. Remarkably, there was an interactive effect ( $p = 0.006$ ) among FIR radiation, dietary energy and amino acid levels, and  $\beta$ -mannanase supplementation for villus height on day 21.

### Blood metabolites

As shown in Table 7, FIR, dietary nutrients, and  $\beta$ -mannanase influenced blood metabolites on days 21 and 35. Remarkably, birds exposed to FIR had lower blood IL-1 $\beta$  ( $p = 0.003$ ), TNF- $\alpha$  ( $p$

Table 7. Effects of FIR radiation, dietary energy and amino acid levels, and  $\beta$ -mannanase supplementation on blood metabolites in broilers<sup>1)</sup>

Item			d 21							d 35						
FIR	EnAA	β-man- nanase	IL-1β (pg/mL)	IL-10 (ng/mL)	TNF-α (pg/mL)	IFN-γ (ng/mL)	Glucose (mg/dL)	Lactate (nmol)	Corticos- terone (ng/mL)	IL-1β (pg/mL)	IL-10 (ng/mL)	TNF-α (pg/mL)	IFN-γ (ng/mL)	Glucose (mg/dL)	Lactate (nmol)	Corticos- terone (ng/mL)
Yes	Normal	No	673.85	3.77	484.50	2.05	155.21	196.96	11.01	868.12	3.49	560.40	3.18	307.50	217.32	10.95
		Yes	559.05	2.77	461.46	1.75	146.38	188.28	9.94	767.75	4.72	504.34	2.63	278.87	190.80	11.58
	Low	No	684.72	3.59	490.49	2.43	162.45	191.44	9.91	824.53	5.11	630.31	2.72	298.08	217.18	12.31
		Yes	556.46	2.88	491.97	2.11	159.99	183.52	11.05	783.32	5.08	548.07	2.34	287.81	226.98	11.44
No	Normal	No	813.37	3.47	669.76	2.77	165.98	207.20	10.98	962.98	5.19	746.81	3.64	311.04	239.14	12.23
		Yes	673.61	3.77	580.11	2.33	169.59	217.73	10.26	903.98	4.99	670.84	2.80	320.14	231.55	11.74
	Low	No	779.42	3.60	694.52	3.08	170.72	216.27	12.10	1037.82	5.07	743.07	4.44	315.05	234.08	12.61
		Yes	733.13	3.38	609.99	2.40	170.08	214.61	11.62	964.27	5.02	726.74	3.33	334.13	241.48	12.08
Main effects																
FIR																
Yes			618.52 <sup>b</sup>	3.25	482.11 <sup>b</sup>	2.09	156.01 <sup>b</sup>	190.05 <sup>b</sup>	10.48	810.93 <sup>b</sup>	4.60	560.78 <sup>b</sup>	2.72 <sup>b</sup>	293.06 <sup>b</sup>	213.07 <sup>b</sup>	11.57
No			749.88 <sup>a</sup>	3.56	638.59 <sup>a</sup>	2.65	169.09 <sup>a</sup>	213.95 <sup>a</sup>	11.24	967.26 <sup>a</sup>	5.07	721.86 <sup>a</sup>	3.55 <sup>a</sup>	320.09 <sup>a</sup>	236.56 <sup>a</sup>	12.16
EnAA <sup>2)</sup>																
Normal			679.97	3.45	548.96	2.23	159.29 <sup>b</sup>	202.54	10.55	875.71	4.60	620.60	3.06	304.39	219.70	11.62
Low			688.43	3.36	571.74	2.50	165.81 <sup>a</sup>	201.46	11.17	902.48	5.07	662.05	3.21	308.77	229.93	12.11
β-mannanase																
Yes			630.56 <sup>b</sup>	3.20	535.88	2.15	161.51	201.03	10.72	854.83	4.95	612.50	2.78 <sup>b</sup>	305.24	222.70	11.71
No			737.84 <sup>a</sup>	3.61	584.82	2.58	163.59	202.97	11.00	923.36	4.71	670.15	3.49 <sup>a</sup>	307.92	226.93	12.02
SEM			20.678	0.205	15.803	0.162	1.216	4.324	0.309	18.776	0.173	17.167	0.155	6.508	5.252	0.316
p-value																
FIR			0.003	0.465	< 0.001	0.092	< 0.001	0.009	0.224	< 0.001	0.183	< 0.001	0.010	0.044	0.031	0.353
EnAA			0.839	0.841	0.475	0.399	0.011	0.901	0.322	0.480	0.181	0.234	0.644	0.738	0.336	0.447
β-mannanase			0.013	0.327	0.129	0.188	0.398	0.824	0.653	0.075	0.499	0.101	0.026	0.838	0.689	0.622
FIR × EnAA			0.917	0.902	0.887	0.783	0.116	0.642	0.323	0.284	0.142	0.657	0.100	0.725	0.463	0.845
FIR × β-mannanase			0.732	0.278	0.234	0.707	0.151	0.466	0.610	0.952	0.298	0.739	0.415	0.205	0.696	0.760
EnAA × β-mannanase			0.631	0.891	0.816	0.839	0.828	0.743	0.327	0.768	0.428	0.809	0.933	0.589	0.229	0.543
FIR × EnAA × β-mannanase			0.522	0.627	0.879	0.865	0.281	0.710	0.430	0.626	0.315	0.536	0.725	0.873	0.615	0.566

<sup>1)</sup>Mean values are based on 6 replicates per treatment.<sup>2)</sup>Normal, diet contained the standard or exceed Ross 308 nutrition specification [16]; Low, diet with a 150 kcal/kg metabolizable energy reduction and reducing amino acids by 4.5% from Normal.FIR, far-infrared ray; IL-1 $\beta$ , interleukin-1 $\beta$ ; IL-10, interleukin-10; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IFN- $\gamma$ , interferon- $\gamma$ ; EnAA, Dietary energy and amino acid level; SEM, Pooled standard error of the mean.

< 0.001), glucose ( $p < 0.001$ ), and lactate ( $p = 0.009$ ) levels than those not exposed on day 21. On day 35, birds exposed to FIR exhibited lower blood IL-1 $\beta$  ( $p < 0.001$ ), TNF- $\alpha$  ( $p < 0.001$ ), IFN- $\gamma$  ( $p = 0.010$ ), glucose ( $p = 0.044$ ), and lactate ( $p = 0.031$ ) levels than those not exposed. FIR radiation had no effect on blood IL-10 and corticosterone levels in birds. However, there were no significant differences in blood IL-10, IFN- $\gamma$ , lactate, and corticosterone levels among the treatments on day 21. In addition, there were no significant differences in blood IL-10, glucose, lactate, and corticosterone levels among the treatments on day 35. Meanwhile, low-energy and amino acid levels had no effect on blood IL-1 $\beta$ , IL-10, TNF- $\alpha$ , IFN- $\gamma$ , lactate, and corticosterone levels but resulted in higher ( $p = 0.011$ ) blood glucose levels than feeding normal-energy and amino acid levels on day 21. On the other hand,  $\beta$ -mannanase supplementation resulted in lower ( $p = 0.013$ ) blood IL-1 $\beta$  levels without affecting blood IL-10, TNF- $\alpha$ , IFN- $\gamma$ , glucose, lactate, and corticosterone levels on day 21.  $\beta$ -mannanase supplementation had no effect on blood IL-1 $\beta$ , IL-10, TNF- $\alpha$ , glucose, lactate, and corticosterone levels but resulted in lower ( $p = 0.026$ ) blood IFN- $\gamma$  levels on day 35.

## DISCUSSION

This research aimed to explore the effects of FIR radiation on growth performance, carcass traits, proximate composition, meat quality, intestinal morphology, and blood metabolites in broilers fed different energy and amino acid levels with and without  $\beta$ -mannanase supplementation from hatch to day 35. Mortality rate was equal between the birds exposed to FIR and those not exposed, with three birds dying in each group throughout the experimental period. To date, the direct effects and underlying mechanisms of FIR in animal production remain insufficiently studied, making direct comparisons with previous findings difficult. Nevertheless, evidence from other animals indicates that FIR (4–20  $\mu$ m) can stimulate energy metabolism, improve blood circulation, and modulate immune responses, which may underlie the physiological changes observed in this study.

Our findings showed that FIR improved growth performance over the 35-day period. Similar to Son et al. [12], body weight gain and feed efficiency were higher in broilers exposed to FIR than those under LED light alone. In previous studies using rats, FIR radiation enhanced the bioavailability of L-arginine by expanding capillaries [9], and promoting mitochondrial energy metabolism [10]. These mechanisms may explain the improved amino acid utilization and energy metabolism observed here, contributing to growth and feed intake. Broiler fed low-energy and amino acid diets exhibited reduced growth performance, consistent with nutrient-deficient effects [26], although feed intake increased as a compensatory mechanism [27]. Conversely, dietary  $\beta$ -mannanase supplementation enhanced the growth performance of broilers in the study, including birds fed low-energy and amino acid diets. Adding  $\beta$ -mannanase in feed may increase feed efficiency by breaking down non-starch polysaccharides that are otherwise difficult to digest with endogenous enzymes, thereby promoting nutrient absorption in broilers [15]. It has been demonstrated that supplementing  $\beta$ -mannanase in broilers fed nutrient-deficient diets may compensate for poor growth performance [17,28,29]. ADG was increased in both starter (days 1–10) and grower phases (days 11–21) due to the interactive between FIR and  $\beta$ -mannanase. This suggests complementary mechanisms: FIR enhances nutrient absorption through improved circulation and metabolism, while  $\beta$ -mannanase increases nutrient availability by degrading  $\beta$ -mannans. Together, these processes may synergistically enhance growth in early development.

The yield of preferable cut-up parts, such as breast and leg meats, indicates farm productivity in the poultry sector. In the study, FIR radiation in broilers increased the relative breast meat weights. FIR increased relative breast meat weight, likely by improving amino acid utilization [9], thereby increasing the portion of muscles in broilers. In the meantime, reduced dressing percentages

and relative leg meat weights in birds fed low-energy and amino acid levels may be attributed to potential deficiencies in digestible lysine and threonine in diets [30], which are the two most important amino acids for optimal breast and leg meat yield in broilers [31]. On the other hand, previous studies have suggested that enzyme supplementation in broiler diets does not significantly affect carcass traits [32,33]. However, the present study found that  $\beta$ -mannanase increased dressing percentages. Although the exact mechanism of  $\beta$ -mannanase supplementation on carcass traits has not yet been elucidated, it may act by degrading non-starch polysaccharides and releasing bound amino acids [34,35].

In the study, moisture, crude protein, and crude ash content in the breast meat were higher in broilers exposed to FIR radiation. The FIR radiation also increased water-holding capacity and pH with decreased cooking loss in the breast meat, indicating improved meat quality. It is speculated that FIR improved meat composition by enhancing amino acids availability [9], building blocks for proteins in meat [35]. These effects may be explained by the ability of FIR to promote capillary dilation, improve peripheral blood flow, and stimulate tissue metabolism, thereby enhancing nutrient transport and utilization [4]. As a result, more amino acids may be directed toward muscle accretion, contributing to the observed increases in carcass yield and breast development. This physiological mechanism supports the idea that FIR not only improves meat quality but also contributes to overall growth efficiency [1]. Broilers fed low-energy and amino acid diets showed reduced dressing percentage and altered breast composition (higher moisture, lower protein and ash), reflecting amino acid deficiency. This aligns with Zuidhof et al. [36], who reported that higher dietary protein levels result in higher protein accretion in muscle tissue. Furthermore, the reduced leg yield and dressing percentage observed in the low-nutrient group support the notion that inadequate nutrient intake restricts both muscle deposition and overall carcass development. These findings reinforce previous research indicating that modern broilers with high genetic potential require precisely formulated diets to meet their rapid growth and tissue accretion demands [36]. Moreover, FIR exposure significantly increased percentage of breast meat on day 35. These results suggest that FIR may enhance carcass performance by improving nutrient absorption and partitioning toward lean tissue development. Similar outcomes have been reported in other studies, where FIR improved carcass characteristics through enhanced metabolic function and tissue oxygenation [1,4].  $\beta$ -mannanase supplementation significantly improved dressing percentage at day 21, implying that the enzyme may enhance nutrient digestibility and mitigate the antinutritional effects of  $\beta$ -mannans in corn-soybean-based diets. However, its effect on breast and leg yields was not statistically significant. While some studies suggest that enzyme supplementation may not consistently alter the proportional yields of breast and leg meat despite improving overall carcass traits [17,37], the observed increase in dressing percentage indicates a net gain in edible portions. This suggests that  $\beta$ -mannanase contributed to balanced muscle growth, as evidenced by increased absolute muscle weights without altering their relative distribution [17]. Such outcomes align with previous reports where carcass weight increased while muscle part ratios remained stable [37]. Conversely, Jalal et al. [38] observed that  $\beta$ -mannanase supplementation did not significantly influence carcass traits or meat yield, even when broilers were fed diets with varying energy levels, including those deficient in energy. This variation in finding highlights that the effects of  $\beta$ -mannanase can be inconsistent, potentially due to difference in factors such as experimental protocols, bird genetics, enzyme characteristics, and the nutritional composition of the basal diets. Variability in study outcomes may therefore stem from differences in diet formulation, bird strain, enzyme activity levels, and experimental conditions [39].

Improving the structure of the gastrointestinal tract leads to more efficient feed utilization and strengthens the barrier against intestinal infections by reinforcing epithelial cells, reducing

endotoxin permeability, and lowering the risk of pathogen invasion [40,41]. In the study, FIR radiation increased villus height and crypt depth in the ileum without significantly altering the villus height to crypt depth ratio in broilers. Montanari et al. and Okabe et al. [42,43] reported that FIR radiation reduces the number of intestinal pathogenic microbes due to its antibacterial effects. FIR radiation may cause the vibration of organic matter, which can deactivate pathogenic microflora in the gut [11,44]. The reduction in colonization of pathogenic bacteria, which cause intestinal infection or mucosa inflammation, may increase the growth of villus, the surface area for nutrient absorption [45]. It is possible that alterations in gut microflora may lead to changes in intestinal morphology in birds [46], resulting in increases in both villus height and crypt depth in the ileum. Increased crypt depth reflects higher tissue turnover [47], which maintains epithelial structure but requires more energy. Thus, FIR-induced increases in villus height and crypt depth may not necessarily improve nutrient absorption efficiency. Meanwhile, birds fed low-energy and amino acid diets exhibited lower villus height and crypt depth in the ileum during the study. Feeding a energy-deficient diet to birds may reduce villus height [48], as well as crypt depth as an adaptation mechanism for energy conservation, thereby lowering the demand for maintenance energy for tissue turnover [49]. Also, insufficient dietary crude protein levels may hinder tissue synthesis, particularly in the intestines, and negatively impact intestinal morphology [50]. On the other hand,  $\beta$ -mannanase supplementation increased both villus height and crypt depth, likely due to its influence on intestinal morphology through exogenous enzyme supplementation in birds [51].

Inflammatory cytokines levels in the blood were investigated to assess the immune status of broilers in the study. As a result, FIR radiation reduced the blood inflammatory cytokines (IL-1 $\beta$ , TNF- $\alpha$ , and IFN- $\gamma$ ) levels, indicating enhanced immune status in broilers. Yoon et al. [52] demonstrated that irradiating FIR appeared to reduce the concentration of TNF- $\alpha$  and IL-1 $\beta$  in mouse models of autoimmune arthritis, which is beneficial for relieving rheumatoid arthritis. Moreover, FIR radiation decreased blood IFN- $\gamma$  levels by mitigating CD8+ or CD4+ T lymphocyte-associated vascular endothelial cell injury in orthotopic allograft transplantation (OAT), with high-intensity FIR therapy even reducing IFN- $\gamma$  expression to near basal levels. Furthermore, Kim et al. [53] demonstrated that FIR radiation may induce the production of nitric oxide and inducible nitric oxide synthetase in pigs, which are critical for enhancing the immune system and increasing antibody titers against vaccines. The reduction in inflammatory cytokine expression due to FIR radiation could prevent energy loss, potentially contributing to improved production performance on broilers. Although previous studies have shown that the radiation of FIR may enhance the immune status in animals, no study has directly investigated the immune response in broilers, so further study is needed.  $\beta$ -mannanase supplementation also reduced blood pro-inflammatory cytokines such as IL-1 $\beta$  and IFN- $\gamma$ . Reduced blood pro-inflammatory cytokines levels are likely due to the impact of  $\beta$ -mannanase supplementation, reducing the ability of mannan to stimulate the immune responses in the intestinal mucosa [54]. Moreover, supplementing  $\beta$ -mannanase may decrease the blood IL-1 $\beta$  and IL-6 levels by mitigating intestinal inflammation in chickens [55].

Several plasma metabolites, such as glucose, lactate, and corticosterone, have been suggested as potential markers for measuring animal stress levels [56,57]. Various types of stress can cause increases in metabolites such as glucose and lactate in broilers [58]. Interestingly, FIR radiation reduced blood glucose and lactate levels in the study, indicating that FIR radiation may alleviate physiological stress levels in broiler production. Leung et al. [59] demonstrated that FIR radiation reduced heart rates, as well as systolic and mean blood pressure, in rat models with stress-induced high heart rates. Tran et al. [60] reported that exposing mice to FIR radiation during acute restraint stress (ARS) prevents the ARS-induced elevation of c-Fos-IR and oxidative stress by suppressing

JAK2/STAT3 signaling through the upregulation of GPx-1. In addition, FIR radiation has anti-apoptotic effects and enhances the scavenging activity of hydrogen peroxide, thereby reducing the production of reactive oxygen species generated from macrophages in the human body [61]. Since reactive oxygen species play a significant role in pathological processes such as heart and lung toxicity, aging, and neurodegeneration, reducing reactive oxygen species in the body is deeply associated with alleviating oxidative stress levels. Meanwhile, broilers fed low-energy and amino acid diets exhibited increased blood glucose levels in the study. Generally, blood glucose levels in broilers remain consistent regardless of dietary energy or crude protein levels in the diets [62]. Strict regulation of carbohydrate metabolism maintains consistent blood glucose levels, even during fasting [63]. On the other hand, feeding diets with high energy levels may upregulate blood glucose levels in broilers, which may then be used for energy and fatty acid synthesis [64]. Studies reported that increased blood glucose levels in birds fed low-energy and amino acid diets are rare, warranting further research on this matter.

In this study,  $\beta$ -mannanase supplementation significantly reduced the concentration of proinflammatory cytokine IL-1 $\beta$  at day 21 and IFN- $\gamma$  at day 35. These cytokines are known indicators of inflammatory immune responses in broilers, and their reduction suggests that  $\beta$ -mannanase may contribute to alleviating systemic or intestinal inflammation. This observation agrees with the findings of Yu et al. [17], who reported that  $\beta$ -mannanase supplementation improved immune responses and reduced inflammatory signaling in broilers fed metabolizable energy- and amino acid-deficient diets. The authors attributed these effects to improved gut integrity, reduced gut permeability, and suppression of cytokine expression levels, including IL-1 $\beta$  and IFN- $\gamma$ , in intestinal tissues.  $\beta$ -mannan components in soybean meal are known to stimulate innate immune responses by activating macrophages and dendritic cells, which leads to increased expression of proinflammatory cytokines. By hydrolyzing  $\beta$ -mannans and reducing digesta viscosity,  $\beta$ -mannanase likely attenuates this immune stimulation, thereby lowering systemic inflammatory markers in circulation. The reductions in IL-1 $\beta$  and IFN- $\gamma$  observed in the present study support this mechanism and indicate that  $\beta$ -mannanase may play a role in immunomodulation, particularly under dietary stress conditions.

## CONCLUSION

This study demonstrated that FIR radiation may improve growth performance, carcass traits, meat quality, immune status, and stress levels in broilers, while its impact on intestinal morphology is limited. The findings suggest that FIR radiation, combined with  $\beta$ -mannanase supplementation in the diet, can potentially increase weight gain in broiler production. While the potential for FIR radiation to raise body temperature is minimal, further investigation into its effects on body temperature during exposure is recommended to ensure its safe application. Additionally, understanding the antibacterial effects of FIR on gut microbiota is essential for uncovering the mechanisms behind its positive impact on broiler production and its influence on intestinal health and overall performance. Despite these promising results, the application of FIR in broiler production warrants further exploration. Future research should explore the effects of FIR in combination with various production factors to better understand its potential benefits in broiler production systems.

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