

# Effects of Dietary Supplementation of Fermented Wheat Bran on Performance, Immunity, Meat Quality, and Tibia Bone Characteristics in Two Strains of Broiler Chicken

Research Article

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## ABSTRACT

The objective was to determine the effects of partial or whole replacement of wheat bran in the basal diet by the fermented wheat bran (FWB) on performance, immunity, meat quality, and tibia bone characteristics in two strains of broiler chickens. This experiment had a  $2 \times 4$  factorial design with four levels of FWB, [0, 0.05, 0.10, and 0.15 % diet] that replaced autoclaved wheat bran in a basal diet and two broiler strains (Arian and Ross 308) with six replicate cages of six female chicks each. The low (0.05%) and medium (0.10%) levels of dietary treatment significantly increased the total body weight gain (BWG) of the birds compared to the controls ( $P < 0.05$ ). The whole wheat bran replacement by FWB (0.15% dry matter (DM) basis), significantly increased thigh meat cook loss in both broiler strains ( $P < 0.05$ ). The high level of FWB substitution also increased bursa and spleen relative weight in Arian broilers compared to the corresponding control group. However, Newcastle vaccine-induced titer of Arian broilers fed a 0.15% FWB diet was lower compared to the Control-Arian group. The supplementation of 0.15% FWB also indicated better results in terms of tibia bone weight and breaking strength compared to the groups that received 0.05% of the prepared FWB, but the effect of the treatments on the chemical composition of the tibial bone was different depending on the broiler strain. It was concluded that FWB could be utilized at 0.05% in broiler diet when carcass yield and immunity were considered.

**KEY WORDS** aspergillus, broilers, immunity, meat quality, solid-state fermentation.

## INTRODUCTION

Wheat bran, an agricultural byproduct of wheat flour, is globally produced in large amounts and contains essential macronutrients and micronutrients. Wheat bran can also be used as a source of phytases, phenolic compounds, arabinoxylans, and betaine in a broiler diet (Chuang *et al.* 2019). On the other hand, undesired compounds occur in the different fractions of wheat bran, such as phytic acid and tannins, which are recognized anti-nutritive compounds (Chuang *et al.* 2019). To reduce the anti-nutritive com-

pounds in wheat bran and increase its nutritional value, approaches such as solid-state fermentation (Yang *et al.* 2021) have been suggested. Among the benefits of solid-state fermentation of food waste by *Aspergillus* fungi in animal nutrition, the following can be mentioned: Degradation of cellulose and hemicellulose contents of agricultural by-products; improves the nutrient value of some feed-stuffs, increasing the crude protein content of the diet, The reduction of anti-nutritive substances, increasing digestibility of nutrients, improve intestinal flora balance, and finally promote skeletal muscle protein metabolism (Hong *et al.*

2004; Saleh *et al.* 2015; Yu *et al.* 2015; Huang *et al.* 2021). Moreover, the ferulic acid released from wheat bran after fermentation has antioxidant effects in the broilers (Huang *et al.* 2021). In common rearing conditions, broilers have a good chance of encountering environmental microorganisms, which might create inflammatory states by inducing oxidative stress and lipid peroxidation. Fungal-derived phenolic compounds and polysaccharides have antioxidant properties that could alleviate the growth-retarding effects of oxidative stress on the broiler body (Samuel *et al.* 2017).

*Aspergillus niger* and *Aspergillus oryzae* has been broadly used as microbial substrates in SSF to improve the nutritional value of agricultural by-products (Yang *et al.* 2021). *Aspergillus niger* primarily decomposes plant material and thrives in artificial environments like bathrooms, producing enzymes and metabolites such as citric acid through fermentation. Conversely, *Aspergillus oryzae* is widely used in food fermentation, particularly in East Asia, and generates several enzymes, including amylases, proteases, lipases, and cellulases (Benoit-Gelber *et al.* 2017). These species of *Aspergillus* were also added to the broiler diet in live form as a probiotic. The beneficial probiotic effects of the probiotic form of *Aspergillus* spp. on animal health have been investigated in several studies (Saleh *et al.* 2015).

However, the advantage of prebiotic substances produced by *Aspergillus*-induced fermentation over probiotics (supplementing live cells of *Aspergillus* to the diets) may include the following items: 1- The affected bacteria are those that naturally present in the digestive system of the host animal and thus are already adapted to that environment; 2- The fermented products are more acceptable by birds; 3- They are easier to access and less expensive than probiotics (Hong *et al.* 2004). Several studies have shown the beneficial effects of *Aspergillus*-originated prebiotics on weight gain, gut health, and immunity of broiler (Fathi *et al.* 2021).

The usefulness of different prebiotics may depend on the distinct fermentation profile developed by a particular microbial species used for fermentation, dietary inclusion level, and the initial microbial population that existed in the bird's gut before supplementation (Scholz-Ahrens *et al.* 2016).

Moreover, PUFA-enriched feed could be produced by fermentation of agricultural byproducts with filamentous fungi strains, which, if fed to birds, could improve the immunity, meat quality, and blood biochemistry in broilers (Semjon *et al.* 2020). Therefore, this study aimed to determine performance, carcass traits, immunity, and tibia bone characteristics in two strains of broiler chickens (Arian and Ross 308) fed different levels of fermented wheat bran.

## MATERIALS AND METHODS

### Preparation of experimental FWB

A local isolate of *A. niger* and *A. oryzae* (strain LS1) was prepared from a fungi collection at the Pasteur Institute (Iran), then the fungi were cultured separately in wheat bran by solid-state fermentation (Subhosh Chandra and Rajasekhar Reddy, 2013).

### Experimental procedure and husbandry

A total of 576 one-day-old commercial female broiler chickens were randomly and equally assigned to 48 cages (50×60 cm) of eight treatment groups. Broiler chickens were reared under heat stress from day 21 until day 35 of the experimental period, where all broilers were exposed to  $33 \pm 1$  °C (10:00-17:00) for seven hours a day. The basal diet was formulated according to the Arian recommendation. The 0.15% autoclaved non-fermented wheat bran was included in the basal diet formulation (0% FWB), which *Aspergillus*-FWB replaced at different levels (0.05, 0.10, and 0.15% diet, DM basis). The broiler chickens were fed experimental diets until 42 days of age *ad libitum* (Table 1). Vaccination of chickens was carried out according to the routine regional vaccination program.

### Measurement of performance factors

Body weight and feed intake were recorded during the experiment. At 32 and 42 days of age, one bird from each cage was selected for killing. Abdominal organs (i.e., spleen, bursa of Fabricius, liver, proventriculus, gizzard, intestines, pancreas, and bile sac) were only removed and weighed at 32 days of age.

### Hematocrit and heterophil/lymphocyte ratio

Blood samples were collected in heparinized tubes from the branchial vein for determining PCV and heterophils to lymphocytes (H/L) ratio at days 32 and 41. Hematocrit was gauged by the microhematocrit method using capillary glass tubes. Blood smears were air-dried, fixed immediately in 100% ethanol, and stained with Wright-Giemsa stain. Heterophils and lymphocytes were identified using standard avian guidelines under an optical microscope, and the H/L ratio was determined by identifying 100 cells under  $40 \times$  power.

### Immune response tests

The cellular immune response was investigated in broilers using delayed-type phytohemagglutinin-P (PHA) and dinitrochlorobenzene (DNCB) hypersensitivity response test at the end of the experiment.

**Table 1** Composition and nutrient content of the basal diet

Ingredient (% of as fed)	1-14 days of age	15-24 days of age	25-35 days of age	35-42 days of age
Maize	54.58	62.72	66.60	68.55
Soybean meal (44% crude protein)	38.80	31.69	27.80	22.16
Autoclaved wheat bran	0.15	0.15	0.15	0.15
Corn gluten	1.00	0.00	0.00	0.00
Soybean oil	1.00	1.00	1.30	1.00
Limestone	1.16	1.07	1.00	1.01
Calcium phosphate	1.88	1.71	1.44	1.49
Vitamin premix <sup>1</sup>	0.25	0.25	0.25	0.25
Mineral premix <sup>2</sup>	0.25	0.25	0.25	0.25
DL-methionine	0.30	0.31	0.27	0.26
L-lysine	0.19	0.26	0.26	0.23
L-threonine	0.07	0.13	0.13	0.10
NaCl	0.33	0.21	0.21	0.21
Sodium bicarbonate	0.04	0.25	0.34	0.34
Sum	100	100	100.	100
<b>Calculated composition</b>				
Metabolizable energy (kcal/kg)	2871	2950	3025	3025
Crude protein (%)	22.50	19.50	18.06	17.44
Lysin (%)	1.33	1.20	1.10	1.04
Methionine (%)	0.67	0.63	0.57	0.55
Methionine + cysteine (%)	1.00	0.92	0.85	0.82
Threonine (%)	0.89	0.82	0.76	0.72
Calcium (%)	0.96	0.87	0.78	0.78
Available phosphorus (%)	0.48	0.44	0.39	0.39
Na (%)	0.17	0.18	0.20	0.20
Anion-cation balance (mEq/kg)	240	230	225	220

Each kilogram of vitamin premix contains vitamin A: 11494 IU; vitamin D: 1725 IU; vitamin E: 40 IU; vitamin K: 2.29 mg; thiamine: 1.43 mg; vitamin K: 3.44 mg; Folic acid: 0.56 mg; Biotin: 0.05 mg; Pantothenic acid: 6.46 mg; Niacin: 40.17 mg and Pyridoxine: 2.29 mg.

Each kilogram of mineral premix contains Iron: 120 mg; Manganese: 150 mg; Copper: 15 mg; Zinc: 120 mg; Iodine: 1.5 mg; Selenium: 0.3 mg and Cobalt: 0.4 mg.

The injection site of the left wattle thickness of each selected bird (10 chickens per treatment group) was marked and measured using a digital caliper before and after the intradermal injection. Then the PHA (100 µg) dissolved in 0.1 ml PBS was injected by insulin syringe into the left wattle, and the resulting swelling was quantified 24 h later. The resulting swelling was interpreted as an index of cell-mediated immunocompetence (Goto *et al.* 1978). Wattle swelling was computed as the difference between the thickness of the wattle before and after the injection of phytohemagglutinin. Cellular immunity was also assessed by skin (featherless area under the left wing of the broilers) contact with 1-chloro-2,4-dinitrobenzene (DNCB-Merck) at a dose of 0.1 mL per cm<sup>2</sup> area. The difference in skin thickness before and 24 hours after the DNCB challenge was considered a measure of cellular immunity (Cai *et al.* 2012).

Humoral immunity was determined by assaying antibody titer against sheep red blood cells (SRBC) and influenza and Newcastle vaccines as antigens. Briefly, broilers were injected intramuscularly with 2 mL of a 5% suspension (v/v) of SRBC in saline at days 30 and 36 of the rearing period. Blood samples were collected at 42 d of age, and sera were separated and frozen until assayed. The Complement in each serum was inactivated by heating to 56 °C for 30 min and then analyzed for total anti-SRBC antibody according to the micro-hemagglutination assay technique.

The 2-mercaptoethanol (2 - ME, Sigma, St. Louis Mo, USA) resistant antibody status, immunoglobulin (Ig) G, was specified by incubating the blood plasma with an equal volume (50 µL) of 1.4% (vol/vol) 2-ME in PBS at 37 °C for 30 min before the hemagglutination inhibition (HI) test. The 2-mercaptoethanol-sensitive antibody titers (1 gM) were determined by deducting the 2-ME-resistant antibody

titer from the total antibody titers (Grasman, 2010). Antibody titers against Newcastle disease virus (NDV) and avian influenza (AI) were measured in broilers through the HI test by a procedure described by Pamok *et al.* (2009).

#### Meat quality and malondialdehyde assay

After the slaughtering of 6 birds per group at 42nd days of age, individual carcasses were trimmed for breast (*Pectoralis major*) and thigh (*Biceps femoris*) muscles by removing skin, bones, and connective tissue. Following trimming, the muscle samples were vacuum packaged and stored at 4 °C until 24 h postmortem. Then, water holding capacity and cook loss of meat samples were estimated according to Castellini *et al.* (2002).

To assay meat lipid peroxidation, breast and thigh samples taken from each bird were dissected into two sub-samples one of which underwent Iron-induced lipid oxidation. Then MDA content was determined in all sub-samples according to Botsoglou *et al.* (2002).

#### Tibia bone characteristics

Tibia bones were dissected after the first slaughter and boiled for two minutes in vacuum bags to remove surrounding meat and cartilaginous caps. Tibias morphometric characteristics and tibia ash concentration (as a percentage of dry bone weight) were determined according to Mutuş *et al.* (2006). The breaking strength of tibia bones was determined by measuring the force required to break each tibia in an Allo-Kramer shearing cell (Food Technology Corporation, Rockland, MD) (Rowland *et al.* 1967).

#### Statistical analysis

A 4×2 factorial design was employed, featuring four levels of fermented wheat bran (FWB) in the diet (0, 0.5, 1, and 1.5%) and two broiler strains, Arian and Ross. Data were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS software (SAS, 2003), and the differences among treatment means were distinguished by Tukey's multiple comparison test. Differences were considered statistically different when  $P < 0.05$ .

## RESULTS AND DISCUSSION

The 0.05 and 0.10% of dietary FWB significantly increased the total body weight gain (BWG) of the birds compared to the controls (Table 2;  $P < 0.05$ ). However, total FCR was not affected by treatments. Supplementation of 0.15% FWB significantly decreased the total feed intake (FI) of the Arian broilers compared to the 0.05%FWB supplemented group during the total experimental period (Table 2;  $P < 0.05$ ). The 0.15% FWB significantly reduced the total body weight gain (BWG) of broilers during week 5 of the rearing period compared to other dietary groups ( $P < 0.01$ ).

The interaction of strain × diet on the proportional weight of the gizzard, liver, spleen, bursa, heart, and pancreas was significant ( $P < 0.01$ ; Table 3). Replacing 0.10% of the wheat bran in the basal diet with FWB increased the relative weight of the liver in Arian broilers ( $P < 0.01$ ). The relative weight of intestines was significantly lower in the FWB-supplemented groups compared to the control groups ( $P < 0.01$ ). Only in the Arian strain, replacing 0.05 and 0.10% of wheat bran in the basal diet with fermented wheat bran decreased the relative weight of gallbladders ( $P < 0.05$ ). The relative length of jejunum, ileum, and small intestine to live body weight of broilers was lower significantly at 0.10% and 0.15% FWB-Arian groups compared to those in the control-Arian group ( $P < 0.01$ ). The relative weight of the pancreas was lower in the 1.5% FWB-Arian group compared to the corresponding control group ( $P < 0.01$ ).

Dietary replacement of the whole wheat bran by FWB (at 0.15% FWB) increased the relative weight of the spleen in both Arian and Ross strains ( $P < 0.01$ ) and Bursa relative weight only in the Arian broilers ( $P < 0.01$ ). FWB supplementation at all dietary levels decreased dramatically relative weight of the heart compared with control groups ( $P < 0.01$ ).

Carcass yield was lower in the groups that received 0.05% FWB compared to the control groups (Table 4;  $P < 0.05$ ). The mean relative weights of carcass parts, excluding breast, in the dietary-treated groups of both strains were not statistically different from their respective control groups (Table 4). In the Ross strain, the breast proportion was greater in the 0.15%FWB group compared to the 1%FWB group ( $P < 0.01$ ).

The effect of dietary treatment × strain was statistically significant on the PCV values (Table 5;  $P < 0.01$ ). The PCV values were significantly lower in the 0.15% FWB-Ross group than in the non-dietary treated Ross group. The interaction between broiler strain and dietary treatment significantly impacted the heterophil/lymphocyte ratio ( $P < 0.01$ ; Table 5), resulting in a decreased H/L ratio in Arian broilers dietary supplemented with a high level of FWB (0.15% diet) compared to the corresponding control (0% FWB).

Antibody titer response to avian influenza (AI) and sheep red blood cells (SRBC) were not affected by FWB supplementation (Table 5). However, ND vaccine-induced antibody titer was significantly lower in the 0.15%FWB-Arian group than in the control-Arian group (Table 5;  $P < 0.05$ ).

Table 6 presents the impact of the treatments on meat quality attributes. There were no significant differences among the dietary treatments concerning water-holding capacity (WHC) in breast and leg muscle samples. Our results also showed that feeding FWB (at 0.10 and 0.15% diet) had a significant effect on increasing the cook loss values of thigh meat cuts ( $P < 0.05$ ).

**Table 2** Effects of feeding fermented wheat bran (FWB) on the performance<sup>1</sup> of broiler chickens

Item	BWG1-14 (g)	BWG14-21 (g)	FCR 14-21	BWG21-28 (g)	FI21-28 (g)	FCR21-28 (g)	FI28-35 (g)	BWG 28-35 (g)	FCR 28-35	FI 35-42 (g)	BWG 1-42 (g)	FI 1-42 (g)	FCR 1-42
<b>Strain</b>													
Arian	350.53 <sup>a</sup>	313.14	1.69 <sup>a</sup>	468.47 <sup>b</sup>	770.48	1.64 <sup>a</sup>	1389.97	546.28	2.61	1043.17	2278.56	4287.32 <sup>a</sup>	1.88
Ross	326.05 <sup>b</sup>	312.92	1.61 <sup>b</sup>	531.99 <sup>a</sup>	786.70	1.48 <sup>b</sup>	1381.14	568.73	2.53	1125.05	2247.38	4137.85 <sup>b</sup>	1.85
SEM	2.96	4.78	0.01	5.93	8.27	0.007	8.35	10.71	0.09	29.04	24.00	30.10	0.02
P-value	<0.01	0.97	0.01	<0.01	0.36	<0.01	0.46	<0.01	0.12	0.054	0.36	0.001	0.15
<b>Dietary treatment</b>													
Control	346.85	313.91	1.64	511.72 <sup>a</sup>	781.89 <sup>ab</sup>	1.52 <sup>b</sup>	1366.52 <sup>b</sup>	532.39 <sup>a</sup>	2.60 <sup>ab</sup>	1169.17 <sup>a</sup>	2179.74 <sup>b</sup>	4183.89 <sup>ab</sup>	1.92
0.05% FWB	337.83	304.39	1.68	473.45 <sup>c</sup>	756.63 <sup>b</sup>	1.60 <sup>a</sup>	1413.42 <sup>a</sup>	594.26 <sup>a</sup>	2.42 <sup>b</sup>	1100.29 <sup>ab</sup>	2319.79 <sup>a</sup>	4265.65 <sup>ab</sup>	1.84
0.10% FWB	333.94	327.21	1.63	509.61 <sup>ab</sup>	807.13 <sup>a</sup>	1.53 <sup>a</sup>	1395.87 <sup>ab</sup>	601.94 <sup>a</sup>	2.28 <sup>b</sup>	1152.11 <sup>a</sup>	2323.21 <sup>a</sup>	4292.74 <sup>a</sup>	1.85
0.15% FWB	330.52	309.62	1.65	506.14 <sup>ab</sup>	768.70 <sup>ab</sup>	1.58 <sup>b</sup>	1366.38 <sup>b</sup>	501.44 <sup>b</sup>	2.96 <sup>a</sup>	914.87 <sup>b</sup>	2229.15 <sup>ab</sup>	4107.52 <sup>b</sup>	1.85
SEM	4.19	6.83	0.01	8.38	11.71	0.01	14.16	15.15	0.13	41.08	33.94	42.56	0.03
P-value	0.07	0.09	0.15	<0.01	0.014	<0.01	0.02	<0.01	0.015	<0.01	<0.01	0.016	0.14
<b>Strain × diet</b>													
Arian-Control	353.59 <sup>a</sup>	316.28 <sup>ab</sup>	1.67 <sup>abc</sup>	485.16 <sup>bc</sup>	771.25	1.59 <sup>bc</sup>	1366.57 <sup>b</sup>	2.40	2.40	1097.19	2180.70	4246.06 <sup>ab</sup>	1.95
Arian 0.05% FWB	337.70 <sup>ab</sup>	308.94 <sup>ab</sup>	1.71 <sup>ab</sup>	454.58 <sup>c</sup>	770.12	1.69 <sup>a</sup>	1460.34 <sup>a</sup>	2.65	2.65	1126.37	2411.22	4502.72 <sup>a</sup>	1.87
Arian 0.01% FWB	358.67 <sup>a</sup>	337.04 <sup>a</sup>	1.62 <sup>bc</sup>	487.89 <sup>bc</sup>	803.66	1.66 <sup>ab</sup>	1366.60 <sup>b</sup>	2.23	2.22	1091.84	2319.85	4254.88 <sup>ab</sup>	1.84
Arian 0.15% FWB	352.15 <sup>a</sup>	290.31 <sup>b</sup>	1.76 <sup>a</sup>	446.25 <sup>c</sup>	736.88	1.65 <sup>ab</sup>	1366.35 <sup>b</sup>	3.14	3.14	857.28	2202.44	4140.61 <sup>b</sup>	1.88
Ross control	340.11 <sup>ab</sup>	311.54 <sup>ab</sup>	1.60 <sup>bc</sup>	538.27 <sup>ab</sup>	792.54	1.46 <sup>d</sup>	1366.52 <sup>b</sup>	2.80	2.80	1241.16	2178.78	4121.73 <sup>b</sup>	1.89
Ross 0.05% FWB	337.96 <sup>ab</sup>	299.83 <sup>ab</sup>	1.64 <sup>abc</sup>	492.33 <sup>bc</sup>	743.13	1.51 <sup>d</sup>	1366.51 <sup>b</sup>	2.18	2.18	1074.20	2228.35	4028.58 <sup>b</sup>	1.81
Ross 0.10% FWB	317.22 <sup>b</sup>	317.39 <sup>ab</sup>	1.65 <sup>abc</sup>	531.33 <sup>ab</sup>	810.59	1.40 <sup>e</sup>	1425.12 <sup>ab</sup>	2.34	2.34	1212.37	2326.56	4325.59 <sup>ab</sup>	1.86
Ross 0.15% FWB	308.89 <sup>b</sup>	322.92 <sup>ab</sup>	1.55 <sup>c</sup>	566.02 <sup>a</sup>	800.53	1.53 <sup>cd</sup>	1366.41 <sup>b</sup>	2.79	2.79	972.46	2255.85	4074.43 <sup>b</sup>	1.81
SEM	5.92	9.56	0.02	11.82	16.55	0.014	16.70	21.43	0.19	58.09	48.00	60.19	0.04
P-value	<0.01	0.05	<0.01	0.01	0.14	<0.01	<0.01	0.07	0.11	0.31	0.09	0.0004	0.56

BWG: body weight gain; FI: feed intake and FCR: feed conversion ratio.

The means within the same column with at least one common letter, do not have significant difference ( $P > 0.05$ ).

SEM: standard error of the means.

High levels of FWB in the diet decreased breast meat susceptibility to lipid peroxidation ( $P < 0.01$ ). MDA values in the thigh meat samples of Arian broilers fed 0.05% and 0.15% FWB were lower compared to the corresponding control group ( $P < 0.01$ ). After iron-induced oxidation, MDA values of thigh meat cuts decreased significantly in both Arian and Ross broilers due to FWB supplementation ( $P < 0.01$ ). MDA values in the breast meat samples, with or without oxidative stress induction, decreased in Arian broilers fed 0.10% FWB compared to Arian birds fed the control diet ( $P < 0.01$ ).

Tibia ash of broilers fed the diet containing 0.05% and 0.10% FWB were significantly greater than those fed the control diet ( $P < 0.01$ ; Table 7). The Medial and lateral wall thickness, robusticity, and tibiotarsal index of the tibiotarsi were not affected significantly by dietary treatments. The mean tibia bone weight, tibiotarsi wt/length index, and breaking strength did differ significantly in the FWB-treated groups compared to the control groups. However, the abovementioned characteristics were increased in the 0.15% FWB groups compared to 0.05% FWB groups ( $P < 0.01$ ). Diaphysis diameter decreased significantly in the birds fed 0.05% FWB compared to the control ( $P < 0.05$ ).

In agreement with our finding (Table 2), it was reported that the body weight gains of broiler chickens treated with dietary fermented rice bran (Kang *et al.* 2015) or FWB (An *et al.* 2022) were significantly greater than those receiving the basal diet. The growth-promoting effects of FWB have been attributed to the increase in the content of bioactive substances with antioxidant properties due to solid-state fermentation by microorganisms (Kang *et al.* 2016; An *et al.* 2022).

FWB supplementation at a low level (0.05% diet) improved the carcass yield of Ross broilers compared to the corresponding control group (Table 3). It is possible that the FWB at this dietary level has prevented protein degradation in the broiler body (Saleh *et al.* 2015), or with its prebiotic properties exerts a positive effect on the intestinal microbiota and gut morphology (Sayrafi *et al.* 2011; Saleh *et al.* 2015). The absence of impact from high-level FWB (0.15% diet) on carcass yield may be due to the increased levels of compounds like ochratoxin-A, nucleic acids, and certain metabolites in the diet (Chiou *et al.* 2001).

In our experiment, feeding the medium level of FWB (0.10%) increased the liver relative weights of Arian chicken (Table 3).

**Table 3** Effects of fermented wheat bran (FWB) on carcass yield, abdominal fat (% of live body weight), relative length of different segments of small intestine, and relative weights of internal organs of broilers at 32 days of age

Item	Car- cass %	Length of different segments of the small intestine (cm per 100 g of live weight)				Relative digestive and immune organ weights of broiler chicks (% of live body weight)									A. Fat
		Duode- num	Jeju- num	Ilium	Cecum	Gut	Proven- tricu- lus	Gizzard	Liver	Spleen	Bursa	Heart	Pancreas	Bile sac	
<b>Strain*</b>															
A	57.56 <sup>b</sup>	1.96	4.91 <sup>b</sup>	4.84 <sup>b</sup>	1.11	7.13	0.42	1.71 <sup>a</sup>	2.48	0.08	0.17 <sup>a</sup>	0.59 <sup>a</sup>	0.28	0.044	1.38 <sup>a</sup>
R	60.81 <sup>a</sup>	2.01	5.24 <sup>a</sup>	5.30 <sup>a</sup>	1.16	7.14	0.41	1.56 <sup>b</sup>	2.47	0.07	0.24 <sup>b</sup>	0.56 <sup>b</sup>	0.27	0.040	1.02 <sup>b</sup>
SEM	0.21	0.03	0.09	0.08	0.02	0.12	0.009	0.03	0.03	0.003	0.004	0.009	0.006	0.002	0.05
P-value	<0.01	0.28	0.02	<0.01	0.16	0.95	0.38	<0.01	0.96	0.09	<0.01	0.01	0.39	0.19	<0.01
<b>Diet</b>															
Control	57.72 <sup>c</sup>	1.97 <sup>b</sup>	5.27 <sup>a</sup>	5.54 <sup>a</sup>	1.19 <sup>a</sup>	8.07 <sup>a</sup>	0.41 <sup>ab</sup>	1.53 <sup>b</sup>	2.56	0.06 <sup>b</sup>	0.20	0.66 <sup>a</sup>	0.30 <sup>a</sup>	0.049	1.04 <sup>b</sup>
0.05% FWB	60.13 <sup>ab</sup>	2.20 <sup>a</sup>	5.49 <sup>a</sup>	5.29 <sup>ab</sup>	1.21 <sup>a</sup>	7.16 <sup>b</sup>	0.44 <sup>a</sup>	1.70 <sup>a</sup>	2.39	0.07 <sup>b</sup>	0.21	0.58 <sup>b</sup>	0.30 <sup>a</sup>	0.039	1.33 <sup>a</sup>
0.10% FWB	60.15 <sup>a</sup>	1.81 <sup>b</sup>	4.59 <sup>b</sup>	4.57 <sup>c</sup>	1.10 <sup>ab</sup>	6.49 <sup>b</sup>	0.42 <sup>ab</sup>	1.65 <sup>ab</sup>	2.53	0.08 <sup>b</sup>	0.20	0.56 <sup>bc</sup>	0.26 <sup>ab</sup>	0.038	1.22 <sup>ab</sup>
0.15% FWB	58.75 <sup>bc</sup>	1.97 <sup>b</sup>	4.97 <sup>ab</sup>	4.89 <sup>bc</sup>	1.07 <sup>b</sup>	6.80 <sup>b</sup>	0.39 <sup>b</sup>	1.65 <sup>ab</sup>	2.42	0.10 <sup>a</sup>	0.22	0.50 <sup>c</sup>	0.25 <sup>b</sup>	0.043	1.21 <sup>ab</sup>
SEM	0.30	0.04	0.13	0.11	0.03	0.17	0.01	0.04	0.05	0.004	0.006	0.01	0.009	0.003	0.07
P-value	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	0.04	0.02	0.03	<0.01	0.17	<0.01	<0.01	0.06	0.04
<b>Interaction</b>															
A-Control	56.83 <sup>c</sup>	2.00 <sup>bcd</sup>	5.60 <sup>ab</sup>	5.86 <sup>a</sup>	1.22 <sup>ab</sup>	8.32	0.40	1.62 <sup>abc</sup>	2.44 <sup>bc</sup>	0.07 <sup>c</sup>	0.15 <sup>c</sup>	0.65 <sup>a</sup>	0.34 <sup>a</sup>	0.06 <sup>a</sup>	1.13
A-0.05% FWB	58.51 <sup>bc</sup>	2.16 <sup>ab</sup>	5.12 <sup>abcd</sup>	4.45 <sup>dc</sup>	1.10 <sup>bc</sup>	6.93	0.45	1.75 <sup>ab</sup>	2.41 <sup>bc</sup>	0.08 <sup>bc</sup>	0.18 <sup>bc</sup>	0.64 <sup>ab</sup>	0.30 <sup>ab</sup>	0.04 <sup>ab</sup>	1.51
A-0.10% FWB	58.20 <sup>c</sup>	1.85 <sup>ed</sup>	4.40 <sup>d</sup>	4.11 <sup>e</sup>	1.11 <sup>bc</sup>	6.63	0.43	1.83 <sup>a</sup>	2.80 <sup>a</sup>	0.09 <sup>ab</sup>	0.17 <sup>bc</sup>	0.57 <sup>abc</sup>	0.27 <sup>bc</sup>	0.04 <sup>b</sup>	1.50
A-0.15% FWB	56.72 <sup>c</sup>	1.86 <sup>ed</sup>	4.55 <sup>d</sup>	4.45 <sup>ed</sup>	1.02 <sup>c</sup>	6.58	0.38	1.62 <sup>abc</sup>	2.26 <sup>c</sup>	0.09 <sup>ab</sup>	0.21 <sup>ab</sup>	0.51 <sup>c</sup>	0.21 <sup>c</sup>	0.03 <sup>b</sup>	1.36
R-Control	58.61 <sup>bc</sup>	1.95 <sup>cde</sup>	4.95 <sup>bcd</sup>	5.21 <sup>abc</sup>	1.16 <sup>bc</sup>	7.82	0.41	1.44 <sup>c</sup>	2.69 <sup>ab</sup>	0.06 <sup>c</sup>	0.25 <sup>a</sup>	0.66 <sup>a</sup>	0.26 <sup>bc</sup>	0.03 <sup>b</sup>	0.94
R-0.05% FWB	61.75 <sup>a</sup>	2.34 <sup>a</sup>	5.68 <sup>a</sup>	5.64 <sup>ab</sup>	1.31 <sup>a</sup>	7.40	0.42	1.65 <sup>abc</sup>	2.37 <sup>bc</sup>	0.07 <sup>c</sup>	0.25 <sup>a</sup>	0.53 <sup>c</sup>	0.29 <sup>ab</sup>	0.04 <sup>b</sup>	1.15
R-0.10% FWB	62.10 <sup>a</sup>	1.78 <sup>e</sup>	4.79 <sup>dc</sup>	5.03 <sup>bcd</sup>	1.08 <sup>bc</sup>	6.31	0.40	1.48 <sup>bc</sup>	2.26 <sup>c</sup>	0.06 <sup>c</sup>	0.23 <sup>a</sup>	0.54 <sup>bc</sup>	0.25 <sup>bc</sup>	0.04 <sup>b</sup>	0.94
R-0.15% FWB	60.77 <sup>ab</sup>	2.09 <sup>abc</sup>	5.40 <sup>abc</sup>	5.32 <sup>abc</sup>	1.11 <sup>bc</sup>	7.02	0.39	1.67 <sup>abc</sup>	2.58 <sup>abc</sup>	0.12 <sup>a</sup>	0.23 <sup>a</sup>	0.49 <sup>c</sup>	0.29 <sup>ab</sup>	0.05 <sup>ab</sup>	1.05
SEM	0.18	0.06	0.19	0.16	0.04	0.24	0.02	0.06	0.07	0.006	0.009	0.02	0.01	0.004	0.10
P-value	0.04	0.04	<0.01	<0.01	0.04	0.10	0.49	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	0.31

\* A: Arian and R: Ross 308.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

The increment of liver relative weight in broilers may reflect an acute phase of an inflammatory response in broilers because this organ is the synthesis site for the acute-phase proteins (Xie *et al.* 2000).

Moreover, the change in liver weight can be caused by the changing the fat content in the liver tissue (Abdulkarimi *et al.* 2011). The differences in intestinal microbial flora and immune competence between the Arian and Ross broilers may explain the varying effects of the treatment on the relative weight of the liver in two strains of broilers (Samadian *et al.* 2023).

Gut relative weights were decreased due to feeding dietary treatments at the 32<sup>nd</sup> day of age (Table 3). This may be comparable with the findings of Brenes *et al.* (1993). These authors observed that the dietary addition of crude fungal enzyme preparation (Roxazyme<sup>®</sup>G and Avizyme SX) to a barley-based broiler diet decreased the relative weight of the total gut. Intestinal weight reduction was attributed in previous work to the reduced Peyer's patches surface in the intestines of birds fed *A. niger* mycelium (Zyla *et al.* 2000).

The decrease in the relative weight of the gall bladder in Arian chickens due to eating FWB (Table 2) may have occurred due to the possible influence of the fermented product on the bile compounds in the digestive system (Ashayerizadeh *et al.* 2018). A high level of FWB supplementation (0.15%) also decreased pancreas-relative weight in Arian broilers (Table 3). This may be attributed to the decreased beta-glucan contents of wheat bran due to *Aspergillus* enzyme activities, which negate the effects of beta-glucans on increasing the weight of digestive organ weights (Brenes *et al.* 1993). Our results indicated that feeding a diet containing 0.15% FWB increased the relative weight of the spleen and bursa (only in the Arian strain), and reduced the heart's relative weight in both broiler strains at 32 days of age (Table 3). In a previous study, the supplementation of *A. niger* mycelium to the broiler diet increased the relative weight of the bursa and spleen (Zyla *et al.* 2000). The decrease in heart weight may be attributable to the decreased hematocrit value in Ross308 broilers due to feeding a diet containing 0.15% FWB (Table 5), which would put less stress on the heart muscles.

**Table 4** Effect of chicken strain and dietary *Aspergillus* doses on carcass efficiency and relative weight of carcass and body components at 42 d of age

Item	Carcass (% of LBW)	Breast (% of carcass)	Thighs & Drumsticks (% of carcass)	Wings (% of carcass)	Neck & Back (% of carcass)	Feet (% of LBW)
<b>Strain</b>						
Arian	70.53 <sup>b</sup>	31.19 <sup>b</sup>	31.50 <sup>a</sup>	11.05 <sup>a</sup>	26.25 <sup>a</sup>	4.11 <sup>a</sup>
Ross	72.81 <sup>a</sup>	39.43 <sup>a</sup>	28.92 <sup>b</sup>	9.36 <sup>b</sup>	22.29 <sup>b</sup>	3.87 <sup>b</sup>
SEM	0.44	0.34	0.21	0.10	0.24	0.22
P-value	<0.01	<0.01	<0.01	<0.01	<0.01	0.02
<b>Dietary treatment</b>						
Control	70.33 <sup>b</sup>	35.06 <sup>ab</sup>	30.24 <sup>ab</sup>	10.26	24.43 <sup>ab</sup>	4.13
0.5% FWB <sup>1</sup> (Low level)	73.02 <sup>a</sup>	34.68 <sup>b</sup>	29.95 <sup>ab</sup>	10.28	25.08 <sup>a</sup>	3.85
1.0% FWB (Medium level)	71.85 <sup>ab</sup>	34.89 <sup>ab</sup>	31.07 <sup>a</sup>	10.22	23.81 <sup>ab</sup>	4.14
1.5% FWB (High level)	71.47 <sup>ab</sup>	36.60 <sup>a</sup>	29.58 <sup>b</sup>	10.04	23.77 <sup>b</sup>	3.84
SEM	0.62	0.49	0.29	0.14	0.34	0.31
P-value	0.03	0.03	<0.01	0.64	0.03	0.06
<b>Strain × dietary treatment</b>						
Arian-Control diet	70.44	30.46 <sup>c</sup>	31.21 <sup>ab</sup>	11.12	27.21 <sup>a</sup>	4.02 <sup>ab</sup>
Arian-0.05% FWB	70.84	31.53 <sup>c</sup>	30.79 <sup>ab</sup>	11.11	26.56 <sup>a</sup>	3.93 <sup>ab</sup>
Arian-0.10% FWB	70.36	31.49 <sup>c</sup>	32.14 <sup>a</sup>	10.99	25.37 <sup>ab</sup>	4.38 <sup>a</sup>
Arian-0.15% FWB	70.46	31.28 <sup>c</sup>	31.86 <sup>a</sup>	10.98	25.87 <sup>ab</sup>	4.13 <sup>ab</sup>
Ross-Control diet	70.21	39.67 <sup>ab</sup>	29.28 <sup>bc</sup>	9.41	21.65 <sup>c</sup>	4.24 <sup>ab</sup>
Ross-0.05% FWB	75.20	37.83 <sup>b</sup>	29.10 <sup>bc</sup>	9.46	23.60 <sup>bc</sup>	3.78 <sup>ab</sup>
Ross-0.10% FWB	73.34	38.30 <sup>ab</sup>	30.00 <sup>ab</sup>	9.46	22.24 <sup>c</sup>	3.91 <sup>ab</sup>
Ross-0.15% FWB	72.48	41.92 <sup>a</sup>	27.29 <sup>c</sup>	9.10	21.68 <sup>c</sup>	3.55 <sup>b</sup>
SEM	0.88	0.69	0.42	0.20	0.48	0.43
P-value	0.08	<0.01	<0.01	0.86	0.04	0.04

FWB: fermented what bran.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

**Table 5** Effect of chicken strain and dietary fermented wheat bran (FWB) levels on hematocrit and immunity of broilers

	PCV (%) 32 d	H/L	Total and 2-mercaptoethanol-resistant antibody titers (IgY) against sheep red blood cells, and Hemagglutinin inhibition titer to Newcastle disease (NDV) and avian influenza (AI) virus (Log2)					Increase in wattle and skin thickness (mm) in response to phytohemagglutinin-P (PHA) and 1-chloro-2,4-dinitrobenzene (DNCP) respectively	
			SRBC total titer	IgY	IgM	AI	NDV	PHA	DNCP
<b>Strain</b>									
Arian	34.84 <sup>a</sup>	0.29	5.67 <sup>a</sup>	3.25	2.42 <sup>b</sup>	8.04 <sup>a</sup>	4.54	2.00	0.81 <sup>a</sup>
Ross	32.41 <sup>b</sup>	0.29	6.71 <sup>b</sup>	3.33	3.37 <sup>a</sup>	6.92 <sup>b</sup>	4.25	2.11	0.62 <sup>b</sup>
SEM	0.44	0.005	0.27	0.30	0.15	0.25	0.20	0.14	0.06
P-value	<0.01	0.82	<0.01	0.84	<0.01	<0.01	0.31	0.59	0.04
<b>Dietary treatment</b>									
Control	34.15	0.31	6.58	3.75	2.83	8.00	4.58	1.80	0.76
0.05% FWB	34.36	0.29	5.75	3.17	2.58	7.50	4.50	2.18	0.60
0.10% FWB	33.28	0.30	6.17	3.33	2.83	7.50	4.42	2.29	0.83
0.15% FWB	32.72	0.28	6.25	2.92	3.33	6.92	4.08	1.94	0.68
SEM	0.63	0.008	0.38	0.43	0.21	0.36	0.29	0.21	0.09
P-value	0.20	0.09	0.49	0.58	0.11	0.22	0.63	0.34	0.29
<b>Strain × dietary treatment</b>									
Arian-Control diet	32.78 <sup>abc</sup>	0.34 <sup>a</sup>	6.16	3.66	2.50	9.30	5.33 <sup>a</sup>	1.54	0.62
Arian-0.05% FWB	36.72 <sup>a</sup>	0.29 <sup>ab</sup>	4.67	2.50	2.17	8.17	4.83 <sup>ab</sup>	2.24	0.79
Arian-0.10% FWB	34.64 <sup>abc</sup>	0.27 <sup>ab</sup>	5.67	3.50	2.17	7.50	4.17 <sup>ab</sup>	2.00	0.91
Arian-0.15% FWB	35.22 <sup>ab</sup>	0.26 <sup>b</sup>	6.17	3.33	2.83	7.17	3.83 <sup>b</sup>	2.22	0.93
Ross-Control diet	35.53 <sup>ab</sup>	0.27 <sup>ab</sup>	7.00	3.83	3.17	6.66	3.83 <sup>b</sup>	2.06	0.90
Ross-0.05% FWB	32.00 <sup>abc</sup>	0.29 <sup>ab</sup>	6.83	3.83	3.00	6.83	4.16 <sup>ab</sup>	2.13	0.41
Ross-0.10% FWB	31.92 <sup>bc</sup>	0.33 <sup>ab</sup>	6.66	3.17	3.50	7.50	4.00 <sup>b</sup>	2.58	0.74
Ross-0.15% FWB	30.22 <sup>c</sup>	0.28 <sup>ab</sup>	6.33	2.50	3.83	6.67	5.00 <sup>ab</sup>	1.68	0.44
SEM	0.88	0.01	0.53	0.61	0.30	0.51	0.41	0.29	0.12
P-value	<0.01	<0.01	0.32	0.34	0.73	0.06	0.02	0.18	0.02

PVC: packed cell volume and H/L: heterophil/lymphocyte ratio.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

**Table 6** Effects of chicken hybrid and *Aspergillus* supplementation levels on broiler meat quality and TBARS test

Item	WHC (%) in breast meat	Cook loss (%) in breast meat	WHC in thigh meat (%)	Cook loss in thigh meat (%)	MDA in breast meat (ng/g)	Induced MDA in breast muscle (ng/g)	MDA in thigh muscle (ng/g)	Induced MDA in thigh meat (ng/g)
<b>Strain</b>								
Arian	53.49 <sup>b</sup>	27.60 <sup>b</sup>	62.00	30.58 <sup>b</sup>	0.14 <sup>a</sup>	0.86	0.16 <sup>a</sup>	0.90 <sup>a</sup>
Ross	57.56 <sup>a</sup>	31.83 <sup>a</sup>	61.25	34.48 <sup>a</sup>	0.11 <sup>b</sup>	0.85	0.14 <sup>b</sup>	0.81 <sup>b</sup>
SEM	0.99	0.84	1.61	0.97	0.006	0.01	0.003	0.007
P-value	<0.01	<0.01	0.74	<0.01	<0.01	0.87	<0.01	<0.01
<b>Diet</b>								
Control	56.83	28.23	62.70	28.55 <sup>b</sup>	0.16 <sup>a</sup>	0.98 <sup>a</sup>	0.17 <sup>a</sup>	0.99 <sup>a</sup>
0.05% FWB	53.27	30.90	59.17	33.48 <sup>ab</sup>	0.12 <sup>ab</sup>	0.83 <sup>bc</sup>	0.13 <sup>b</sup>	0.81 <sup>b</sup>
0.10% FWB	55.14	28.34	60.61	34.06 <sup>a</sup>	0.12 <sup>ab</sup>	0.75 <sup>c</sup>	0.16 <sup>a</sup>	0.84 <sup>b</sup>
0.15% FWB	56.87	31.39	64.03	34.03 <sup>a</sup>	0.11 <sup>b</sup>	0.85 <sup>b</sup>	0.13 <sup>b</sup>	0.77 <sup>c</sup>
SEM	1.41	1.18	2.28	1.37	0.009	0.02	0.005	0.01
P-value	0.24	0.13	0.45	0.02	<0.01	<0.01	<0.01	<0.01
<b>Strain × diet</b>								
Arian-Control diet	57.06	25.43	63.21	23.65	0.17	1.01 <sup>a</sup>	0.19 <sup>a</sup>	1.10 <sup>a</sup>
Arian-0.05% FWB	51.63	29.25	61.71	32.86	0.14	0.90 <sup>abc</sup>	0.12 <sup>b</sup>	0.82 <sup>cd</sup>
Arian-0.10% FWB	52.15	26.00	61.27	33.91	0.14	0.60 <sup>d</sup>	0.18 <sup>a</sup>	0.88 <sup>cb</sup>
Arian-0.15% FWB	53.13	29.73	61.82	31.91	0.12	0.91 <sup>abc</sup>	0.13 <sup>b</sup>	0.79 <sup>ed</sup>
Ross-Control diet	56.59	31.03	62.19	33.45	0.15	0.95 <sup>ab</sup>	0.14 <sup>b</sup>	0.89 <sup>b</sup>
Ross-0.05% FWB	54.93	32.56	56.63	34.10	0.10	0.75 <sup>dc</sup>	0.14 <sup>b</sup>	0.79 <sup>ed</sup>
Ross-0.10% FWB	58.13	30.67	59.94	34.21	0.09	0.90 <sup>abc</sup>	0.14 <sup>b</sup>	0.80 <sup>ed</sup>
Ross-0.15% FWB	60.61	33.05	66.24	36.15	0.10	0.80 <sup>bc</sup>	0.13 <sup>b</sup>	0.74 <sup>c</sup>
SEM	1.99	1.67	3.23	1.94	0.01	0.03	0.006	0.01
P-value	0.22	0.88	0.54	0.08	0.41	<0.01	<0.01	<0.01

FWB: fermented wheat bran; WHC: water-holding capacity and MDA: Malondialdehyde.

The means within the same column with at least one common letter, do not have significant difference ( $P>0.05$ ).

SEM: standard error of the means.

However, we should note that the hematocrit values were in the normal physiological range (between 22 and 35%) in all experimental groups (Sugiharto *et al.* 2018). Increasing the PCV values in the physiological range can lead to better oxygenation of tissues and may provide an additional mechanism for growth promotion (Pavlidis *et al.* 2007). Therefore, the observed decrease in hematocrit values of Ross chickens treated with high levels of FWB compared to the corresponding control group may be an unfavorable physiological response.

Contrary to our expectation, the prebiotic supplementation at the high dietary level hurt the antibody titer of Arian chickens against Newcastle, which may be due to the possible presence of ochratoxin-A in the diet. Although the possible concentrations of ochratoxin-A in the FWB were not measured in the present study, however, the findings of our recent study (unpublished) indicated that ochratoxin-A present in the fermented oak acorn at 2.55  $\mu\text{g}/\text{kg}$  after solid-state fermentation by *Aspergillus niger*. Sugiharto (2016) reviewed that prebiotics would enhance the immune response of broiler chickens but noted that the mechanism of the immune response augmentation by *A. oryzae* is ambiguous. However, Shahir *et al.* (2014) did not observe any

significant effect of dietary-supplemented mannan-oligosaccharides on the avian influenza (AI) antibody titer in broiler chickens.

Our results indicated that feeding FWB (at 0.10 and 0.15%) increased significantly the cook loss percentages of thigh meat cuts (Table 5). Cook loss specifies raw muscle protein features and refers to the reduced moisture content of the thermally treated meat in the form of liquid or steam (Vujadinović *et al.* 2014). Meat cooking loss is a determinant of meat WHC, nutritional value, and tenderness of cooked animal meats. Factors such as the energy content of the consumed feed and meat pH value cause differences in the cooking loss values (Watanabe *et al.* 2018). If the percentage of cook loss in a sampled piece of meat is less than 35%, the meat quality is in satisfactory condition, and the possible release of meat juice during cooking would be less (Vujadinović *et al.* 2014). Therefore, considering that the mean cook loss value in the meat cuts of FWB-treated chickens was less than 35% (Table 6) which probably would not affect the meat tenderness, a little more reduction of the meat water content during the thermal processing would reduce the growth of microorganisms in the processed poultry meat products.



**Table 7** Effect of strain and dietary fermented wheat bran (FWB) levels on morphometric parameters, mineral content, and bone strength measurements of tibia at 32 d of age

Item	Tibia weight (g)	Tibia length (mm)	Long diameter (mm)	Short diameter (mm)	Strength (kg)	Lateral wall thickness (mm)	Medial thickness (mm)	Diaphysis diameter (mm)	Tibiotarsi wt/length index	Robusticity index	Medullary canal diameter (mm)	Tibiotarsal index	EE (%)	Ash (%)
<b>Strain</b>														
Arian	4.16 <sup>a</sup>	80.70 <sup>a</sup>	8.06 <sup>a</sup>	7.10 <sup>a</sup>	4.81	1.10	1.84 <sup>a</sup>	7.58 <sup>a</sup>	51.27 <sup>a</sup>	5.19 <sup>b</sup>	6.11 <sup>a</sup>	19.61	5.52 <sup>a</sup>	46.04
Ross	3.45 <sup>b</sup>	79.04 <sup>b</sup>	6.96 <sup>b</sup>	6.21 <sup>b</sup>	4.48	0.98	1.51 <sup>b</sup>	6.59 <sup>b</sup>	43.61 <sup>b</sup>	5.39 <sup>a</sup>	5.34 <sup>b</sup>	18.97	6.03 <sup>b</sup>	46.07
SEM	0.12	0.46	0.12	0.14	0.40	0.05	0.06	0.12	1.37	0.04	0.13	0.70	0.04	0.15
P-value	<0.01	0.015	<0.01	<0.01	0.55	0.07	<0.01	<0.01	<0.01	<0.01	<0.01	0.52	<0.01	0.89
<b>Diet</b>														
Control	3.96 <sup>ab</sup>	80.85 <sup>a</sup>	7.75 <sup>a</sup>	6.90 <sup>ab</sup>	4.34 <sup>ab</sup>	1.08	1.66	7.33 <sup>a</sup>	48.82 <sup>ab</sup>	5.28	5.96 <sup>ab</sup>	18.67	5.87 <sup>b</sup>	44.81 <sup>c</sup>
0.05% FWB	3.23 <sup>b</sup>	76.78 <sup>b</sup>	6.88 <sup>b</sup>	6.03 <sup>b</sup>	3.35 <sup>b</sup>	1.00	1.55	6.45 <sup>b</sup>	42.06 <sup>b</sup>	5.35	5.18 <sup>b</sup>	19.73	6.19 <sup>a</sup>	46.34 <sup>ab</sup>
0.10% FWB	3.88 <sup>ab</sup>	80.33 <sup>a</sup>	7.95 <sup>a</sup>	6.98 <sup>a</sup>	4.23 <sup>ab</sup>	0.99	1.77	7.47 <sup>a</sup>	48.24 <sup>ab</sup>	5.27	6.09 <sup>a</sup>	18.55	5.40 <sup>c</sup>	47.71 <sup>a</sup>
0.15% FWB	4.15 <sup>a</sup>	81.50 <sup>a</sup>	7.45 <sup>ab</sup>	6.72 <sup>ab</sup>	6.46 <sup>a</sup>	1.10	1.73	7.09 <sup>ab</sup>	50.64 <sup>a</sup>	5.26	5.67 <sup>ab</sup>	20.20	5.63 <sup>bc</sup>	45.38 <sup>bc</sup>
SEM	0.17	0.65	0.18	0.20	0.56	0.0	0.09	0.17	1.94	0.05	0.18	0.99	0.05	0.23
P-value	<0.01	<0.01	<0.01	<0.01	<0.01	0.57	0.36	<0.01	0.02	0.65	<0.01	0.57	<0.01	<0.01
<b>Strain × diet</b>														
Arian-Control diet	4.19	80.69 <sup>ab</sup>	8.36	7.36	4.56	1.16	1.70 <sup>ab</sup>	7.86	51.79	5.17	6.43	18.21	5.82 <sup>c</sup>	44.68 <sup>ed</sup>
Arian-0.05% FWB	3.49	76.76 <sup>b</sup>	7.53	6.40	3.39	1.06	1.76 <sup>ab</sup>	6.97	45.64	5.21	5.55	20.35	5.14 <sup>d</sup>	46.47 <sup>cb</sup>
Arian-0.10% FWB	4.20	81.66 <sup>ab</sup>	8.40	7.49	4.93	1.06	2.13 <sup>a</sup>	7.94	51.49	5.21	6.35	20.37	4.59 <sup>e</sup>	49.03 <sup>a</sup>
Arian-0.15% FWB	4.74	83.66 <sup>a</sup>	7.94	7.16	6.36	1.14	1.78 <sup>ab</sup>	7.55	56.37	5.17	6.09	19.49	6.51 <sup>b</sup>	43.98 <sup>d</sup>
Ross-Control diet	3.72	81.01 <sup>ab</sup>	7.15	6.44	4.12	0.99	1.62 <sup>ab</sup>	6.79	45.85	5.38	5.49	19.12	5.93 <sup>c</sup>	44.95 <sup>cd</sup>
Ross-0.05% FWB	2.97	76.80 <sup>b</sup>	6.23	5.65	3.30	0.94	1.33 <sup>b</sup>	5.94	38.68	5.49	4.80	19.11	7.23 <sup>a</sup>	46.21 <sup>cb</sup>
Ross-0.10% FWB	3.56	78.99 <sup>ab</sup>	7.51	6.48	3.93	0.93	1.40 <sup>b</sup>	6.99	44.99	5.34	5.83	16.73	6.21 <sup>bc</sup>	46.38 <sup>cb</sup>
Ross-0.15% FWB	3.56	79.34 <sup>ab</sup>	6.96	6.28	6.56	1.06	1.69 <sup>ab</sup>	6.62	44.90	5.34	5.24	20.91	4.75 <sup>ed</sup>	46.77 <sup>b</sup>
SEM	0.25	0.92	0.25	0.29	0.80	0.09	0.13	0.25	2.74	0.076	0.25	1.40	0.08	0.30
P-value	0.48	0.04	0.82	0.97	0.89	0.97	0.05	0.99	0.73	0.81	0.86	0.27	<0.01	<0.01

EE: ether extract.

The means within the same column with at least one common letter, do not have significant difference ( $P > 0.05$ ).

SEM: standard error of the means.

Fermented wheat bran was found to retard lipid peroxidation of cooked broiler meat samples as measured by TBARS number during refrigerated storage. Yu *et al.* (2015) indicated that fermented material of *Aureobasidium pullulans* on wheat bran improved the serum MDA content of S180 tumor-bearing mice. Moreover, the antioxidant effects and attenuating effects of *Aspergillus* probiotics on MDA values induced by environmental stressors have been reported in many studies (Saleh *et al.* 2015). Mahmoud *et al.* (2014) indicated that phenolic and anthocyanin compounds resulting from carbohydrate hydrolyzing enzymes produced by *Aspergillus fungi* during fermentation may exert potent antioxidant activity. On the other hand, Kanauchi *et al.* (2008) indicated that feruloyl esterase pro-

duced by *A. awamori* may take part in the antioxidative properties of this fungus.

According to our findings, fermented wheat bran supplementation at 0.15% of the diet intensely reduced the amount of tibial fat in the Ross strain (Table 7). Other characteristics of the tibia, except the diameter of the diaphysis and ash, were not significantly affected by the feeding of fermented wheat bran. In the Arian strain, the percent of tibia ash was significantly greater in the broilers-fed diets containing 0.05 and 0.10% of FWB in comparison with that in the control and 0.15%FWB fed groups ( $P < 0.01$ ). This confirmed the results obtained by Zyla *et al.* (2000), where adding 4% *A. niger* to the broiler diet increased toe ash and improved the effect of dietary enzymes in absorbing miner-

als and increasing bone ash. In contrast, Hajimohammadi *et al.* (2020) reported that fermented sesame substituted with raw sesame in a broiler diet had no significant effect on the relative weight, length, density, and ash percentage of the tibia bone.

Fermentation by modulating fiber structure in wheat bran may contribute to bone health by providing more calcium and available phosphorus (Hajimohammadi *et al.* 2020). In wheat bran, over 90% of total phosphorus is in phytate form that cannot be utilized in monogastric animals due to the lack of endogenous phytase (Prueckler *et al.* 2014). Schons *et al.* (2012) found that the release of inorganic phosphorus from sorghum is enhanced when this grain is co-fermented by *Paecilomyces variotii* and phytase compared to enzymatic sorghum treatment alone. This is attributed to the phytate released from complex fiber, which subsequently degraded via the activities of enzymes secreted by probiotic microorganisms (Schons *et al.* 2012). It was reported that tibia weight, diameter, breaking strength, ash, calcium, and phosphorus were higher in turkey poults that fed *Aspergillus* meal when compared with neonatal poults that received the basal diet (Reginatto *et al.* 2011). Prebiotics such as fructooligosaccharides in broiler diets stimulate calcium and magnesium absorption in the small intestine, improve calcium homeostasis, and reduce bone loss (Scholz-Ahrens *et al.* 2016).

In the comparison between the two broiler strains, Ross 308 was superior to Arian based on observed better performance, carcass yield, the weight of valuable carcass cuts, meat quality, and plasma IgM content. However, some assessed tibial characteristics, including length, weight, and strength of the tibial bone, the cellular immune response to phytohemagglutinin and dinitrochlorobenzene, and the titer of antibodies to sheep red blood cells, were higher in the Arian broilers compared to Ross 308.

## CONCLUSION

Dietary supplementing of FWB at 0.05% diet improved total weight gain, carcass yield, and meat antioxidant status. According to our NDV immune test findings, fermented wheat bran supplementation at 0.15% of broilers' diet may not be recommendable in the Arian strain. Dietary treatment exerts different effects on examined parameters, especially tibial bone chemical composition depending on broiler strain.

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