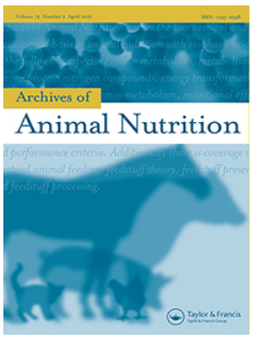

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Effect of raw and fermented pomegranate pomace on performance, antioxidant activity, intestinal microbiota and morphology in broiler chickens

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ABSTRACT

The present study was conducted to investigate the effects of raw (PP) and fermented pomegranate pomace (FP) on performance, antioxidant activity, caecal microbiota and ileal morphology in broiler chickens. A total of 175 male broiler chicks were allocated to five treatment groups with five replicates and seven birds per replicate in a completely randomised design. Dietary treatments included a soy-corn based diet (control), diets supplemented with PP at 5 (5PP) and 10 g/kg (10PP), and diets supplemented with FP at 5 (5FP) and 10 g/kg (10FP). Dietary PP and FP did not change the body weight and feed conversion ratio. Moreover, dietary PP and FP did not alter the serum glutathione peroxidase, superoxide dismutase, and catalase levels but decreased malondialdehyde ($p < 0.05$) in breast meat. Caecal *Clostridium perfringens* count was decreased in broiler chickens of groups 10PP, 5FP and 10FP ($p < 0.05$). However, PP and FP had detrimental effects on the ileum morphology of broiler chicks. The villus height was decreased in the 10PP, 5FP and 10FP groups compared with the control group ($p < 0.01$). Crypt depth was higher in the 5PP and 10FP groups than control and 10PP groups ($p < 0.01$). The villus height to crypt depth ratio was also decreased in 5PP, 5FP, and 10FP groups ($p < 0.01$). These results suggest that PP and FP have the potential to be used in broiler diets as antioxidant and antimicrobial agents. However, detailed studies should be conducted to investigate the underlying reasons for the detrimental effects on ileal morphology.

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1. Introduction

Herbal products have been used for thousands of years to support animal growth, prevent diseases, and control pathogenic microorganisms. Use of herbal feed additives is gaining importance with growing concern among consumers about health-related issues associated with the use of antibiotics and synthetic antioxidants (Sarica and Urkmez 2016; Saleh et al. 2018). Billions of tonnes of agricultural residues with high potential for use as feed additives in poultry nutrition are discarded every year worldwide (Xie et al. 2016). Therefore, researchers have focused on natural agricultural residues with strong antimicrobial and antioxidant properties. Previous studies showed that

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agricultural by-products improved the growth performance, antioxidant capacity and supported the intestinal health by enhancing intestinal bacterial population and morphology in broiler chickens (Viveros et al. 2011; Gungor and Erener 2020a).

Pomegranate (*Punica granatum L.*) belongs to the Punicaceae family, and is one of the ancient fruits cultivated in the Mediterranean and South Asian region. Turkey is one of the important pomegranate producers in the world with an annual production of 600,000 tonnes (TUIK 2020). Pomegranate is consumed fresh and also used in the production of juice, jam, syrup and sauce. Pomegranate pomace (PP) is an inedible by-product produced at high quantities consisting of peel and seeds (Saleh et al. 2018). Pomegranate pomace quickly deteriorates due to its high water content and causes environmental pollution when not disposed of properly (Ahmed and Yang 2017).

Pomegranate pomace contains high amounts of phenolic compounds such as ellagic acid, gallic acid, punicalin and punicagalin (Hosseini-Vashan and Raei-Moghadam 2019). It has positive effects on growth performance (Ahmed and Yang 2017), antioxidant capacity (Al-Shammari et al. 2019), intestinal microbiota (Ahmed and Yang 2017), immune status (Sharifian et al. 2019) and lipid metabolism (Hosseini-Vashan and Raei-Moghadam 2019) in broiler chickens. Besides the beneficial effects, PP contains some antinutritional compounds such as hydrolysable and condensed tannins, which limit use in broiler diets (Bostami et al. 2015).

Fermentation has been a unique method for utilising agricultural by-products in recent years. Solid-state fermentation refers to microbial growth in moistened solid media (Gungor and Erener 2020b). *Aspergillus niger* is used as a probiotic in broiler diets (Harimurti and Hadisaputro 2015), and can improve the antioxidant (Gumienna et al. 2016) and antimicrobial activity (Dipnaik et al. 2014) of PP by increasing its phenolic compounds such as ellagic acid and gallic acid. It can also produce digestive enzymes such as protease, cellulase, lipase and tannase (Wu et al. 2015), and can eliminate the hydrolysable and condensed tannins of PP by breaking down their structures with the tannase enzyme (Aguilar et al. 2008). The effects of raw and fermented pomegranate pomace (FP) on growth performance, some carcass traits, antioxidant activity, intestinal microbiota and morphology in broiler chickens were investigated in this study.

2. Materials and methods

2.1. Preparation of fermented pomegranate pomace

Pomegranate pomace, obtained from a juice factory in Turkey, was used in this study. *Aspergillus niger* (ATCC 9142) was provided by American Type Culture Collection (ATCC, Manassas, VA, USA). It was cultured in potato dextrose agar at 30°C for 7 d. The spores of *A. niger* were harvested by turning the plate upside down and tapping the top of the plate. Spore counts were determined with a Thoma counting chamber.

Pomegranate pomace was quickly dried at 65°C to avoid any spoilage, and milled to a size of 2 mm. It was divided into unfermented and fermented groups after sterilisation at 121°C for 15 min. The fermentation medium was prepared by adding 100 ml nutritive solution (40 g glucose, 20 g urea, 60 g $(NH_4)_2SO_4$, 10 g peptone, 40 g KH_2PO_4, and 1 g $MgSO_4$ in 1 l distilled water) to each 100 g of PP. Then 10^4 spores of *A. niger* were

inoculated on each 100 g of PP. Pomegranate pomace was incubated at 30°C for 7 d with gentle mixing once a day. After incubation, the fermented sample was dried on a polyethylene sheet at room temperature to reach 90% dry matter and ground to pass through a 1 mm sieve. Pomegranate pomace and FP were analysed for crude protein (method 976.06), ether extract (method 920.29), ash (method 942.05), and crude fibre (method 973.18) according to AOAC (2000). The neutral detergent fibre, acid detergent fibre and lignin were determined following the procedures of Van Soest et al. (1991). Total phenols were determined using Folin-Ciocalteu reagent with gallic acid as a reference standard as described by Negi and Jayaprakasha (2003). Different concentrations of gallic acid or extract (1 g PP and 10 ml 99% methanol) was added to 1 ml Folin-ciocalteu reagent (1:10 diluted with distilled water) and 0.8 ml 7.5% sodium carbonate solution then mixed thoroughly. Total phenol content was determined spectrophotometrically at 765 nm with a Genesys 10S UV–Vis Genes spectrophotometer (Thermo Scientific, Waltham, MA, USA) and presented as mg gallic acid equivalents per gramme of sample in dry weight. Condensed tannin was analysed by the method described by Makkar et al. (1995). In brief, ground samples (0.01 g) were treated with 6 ml acid butanol reagent (5 ml 37% HCl and 95 ml n-butanol) at 95°C for 60 min and then rapidly cooled down in iced water. Absorbance was measured at 550 nm by a spectrometer (Genesys 10S UV-Vis, Thermo Scientific) and results were given as mg/g sample in dry weight. The radical scavenging activity was determined by the DPPH method (Brand-Williams et al. 1995). Changes in the nutritional composition and radical scavenging activity of PP are shown in Table 1.

2.2. Animals, diets and management

In total, 175 one-day-old Ross 308 male broiler chicks (38.85 ± 0.07 g) were obtained from a local commercial hatchery (Ross Breeders Anadolu, Ankara, Turkey). Birds were weighed and divided into five treatment groups with five replicates of seven birds each in a completely randomised design. The birds were housed in floor pens (100 × 115 × 70 cm) placed in an environmentally controlled room at an environmental temperature of 32°C for the first 3 d and reducing temperatures by 0.5°C every day to reach a final temperature of 20°C. The lighting programme was set to continuous

Table 1. Nutritional composition and radical scavenging activity of raw and fermented pomegranate pomace (on dry matter basis).

	PP*	FP#	Difference (FP - PP)
Crude protein [g/kg]	51.7	176.0	124.3
Ether extract [g/kg]	11.8	22.0	10.2
Ash [g/kg]	44.2	76.3	32.1
Nitrogen-free extractives [g/kg]	691.5	543.5	−148
Crude fibre [g/kg]	200.7	182.3	−18.4
Neutral detergent fibre [g/kg]	268.6	232.1	−36.5
Acid detergent fibre [g/kg]	202.4	166.9	−35.5
Lignin [g/kg]	64.0	51.6	−12.4
Condensed tannin [mg/g]	16.1	18.7	2.6
Total polyphenols [mg GAE/g]	41.1	39.3	−1.8
Radical scavenging activity	94.2	93.1	−1.1

*PP, raw pomegranate pomace; #FP, fermented pomegranate pomace; †GAE, gallic acid equivalents.

illumination for 3 d and switched to a 23 h light: 1 h dark schedule. Chicks were vaccinated against infectious bursal disease at 20 d of age, and against Newcastle disease at 26 d of age. All diets in mash form and fresh drinking water were provided *ad libitum* throughout the experimental period (42 d). Body weight (BW) and feed intake (FI) were measured on a pen basis at the end of each week and feed conversion ratio (FCR) was calculated. Mortality was recorded daily to adjust FCR. All animal care and experimental procedures were conducted following the guidelines of Ondokuz Mayis University Local Ethics Committee for Animal Experiments.

The diets were formulated to meet the nutritional requirements of male broilers in different growth periods according to the recommendations of the Ross breeders. Birds were fed a basal diet (control) or the basal diet supplemented with PP at 5 and 10 g/kg (5PP and 10PP) or FP at 5 and 10 g/kg (5FP and 10FP). The ingredients and nutritive value of the basal diets are given in Table 2.

2.3. Sample collection and analysis

At the end of the trial, one bird from each pen with BW closest to the average BW of the replicate was selected. Blood samples were collected from the wing vein in tubes with a clot activator and serum gel separator. After the samples were centrifuged at 3000 *g* for 10 min at 4°C, serum was separated and stored at −20°C for further analysis of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT). The serum biological analysis was conducted using commercial kits (GPx; CK-bio-20413, SOD; CK-bio-19400 and CAT; CK-bio-18096, Shanghai Coon Koon Biotech, Shanghai, China) with an ELISA Plate Reader (RT-2100 C, Rayto, Shenzhen, China) according to the instructions of the manufacturer.

After blood sampling, selected birds were slaughtered and eviscerated for determination of the carcass characteristics. Eviscerated carcass weights were recorded to obtain the dressing percentage. Visceral organs (heart, liver, gizzard, spleen), abdominal fat and gastrointestinal tract were weighed individually and expressed as a percentage of the live weight. Breast meat and liver were removed from each carcass and placed into individually labelled plastic bags and analysed for pH and colour values at 1, 5 and 11 d of storage at 4°C. Colour measurements of skinless breast meat and liver were performed at three different locations on the sample surface using a Chroma Metre (CR300, Konica Minolta, Osaka, Japan). The pH values were recorded in triplicate with a digital pH metre (Testo 205, Testo AG, Lenzkirch, Germany). The breast meat samples were also used to determine malondialdehyde (MDA) levels. The MDA concentration in the breast meat was measured at 45 min by the thiobarbituric acid method described by Tarladgis et al. (1960). Briefly, 10 g of breast meat were homogenised with 50 ml distilled water for 2 min using a homogeniser (Ultra-Turrax T-25, Janke & Kunkel, IKA-Labortechnik, Staufen, Germany). Homogenised meat was added to the distillation tubes and mixed with 47.5 distilled water and 2.5 ml 4 N HCl. The mixtures were then distilled until a 50 ml sample was obtained. Subsequently, 5 ml of distillate and TBA reagent (0.02 M 2-thiobarbituric acid in 90% glacial acetic acid) were heated in a water bath at 100°C for 35 min. After cooling, the absorbance was measured at 538 nm with a spectrophotometer (Genesys 10S UV-Vis, Thermo Scientific). The amount of MDA in breast meat was expressed as mg of MDA per kg of meat.

Table 2. Ingredients and nutritive value of the basal diets.

	Starter (Day 1–11)	Grower 1 (Day 12–21)	Grower 2 (Day 22–35)	Finisher (Day 36–42)
Ingredients [g/kg]				
Corn	197.0	244.0	217.0	257.5
Maize germ meal (9% CP[#])	230.0	230.0	230.0	230.0
Soybean meal (45% CP)	339.0	149.0	143.0	103.0
Full-fat soybean (35% CP)	100.0	125.0	80.0	80.0
Wheat red dog (16% CP)	90.0	90.0	90.0	90.0
Maize germ (16% CP)	-	-	75.0	75.0
Sunflower meal (36% CP)	-	60.0	65.0	65.0
Chicken viscera (55% CP)	-	50.0	50.0	50.0
Meat and bone meal (35% CP)	-	25.0	25.0	25.0
Monocalcium phosphate (22.7% Ca)	12.4	3.9	1.5	1.5
Marble dust (36% Ca)	14.9	6.4	6.3	6.3
Sodium chloride	2.6	1.9	2.4	2.4
Liquid-methionine (88% methionine)	4.0	3.0	2.8	2.8
L-Lysine sulphate (55% lysine)	4.6	6.8	7.0	7.0
L-Threonine (98% threonine)	1.2	1.0	1.0	1.0
Vitamin and mineral premix[*]	2.5	2.5	2.5	2.5
Sodium sulphate	1.2	1.0	1.0	1.0
Anticoccidial	0.6	0.5	0.5	-
Analysed composition [g/kg as fed]				
Crude protein	243.0	228.0	213.7	190.7
Ether extract	56.9	59.2	72.9	70.2
Crude fibre	62.5	61.9	73.3	68.3
Ash	33.3	32.9	48.1	41.2
Calculated composition [g/kg as fed]				
Metabolisable energy [MJ/kg]	12.6	12.9	13.1	13.3
Lysine	15.8	14.8	14.3	13.3
Methionine	6.7	5.9	5.8	5.6
Methionine and cystine	11.0	10.3	10.3	9.9
Threonine	10.2	9.2	9.2	8.6
Tryptophan	3.0	2.6	2.5	2.3
Ca	9.6	11.2	10.8	10.6
Total P	7.4	8.3	8.0	7.9
Available P	4.9	5.7	5.1	5.1
Na	2.5	2.7	3.0	3.0

[#]CP, crude protein; [*]Premix provided the following nutrients per kilogram of diet: 12,000 IU retinol; 2,400 IU cholecalciferol; 40 mg α-tocopherol; 4 mg menadione; 3 mg thiamine; 6 mg riboflavin; 25 mg nicotinic acid; 10 mg pantothenic acid; 5 mg pyridoxine; 0.03 mg cyanocobalamin; 0.05 mg biotin; 1 mg folic acid; 80 mg Mn; 60 mg Zn; 60 mg Fe; 5 mg Cu; 0.2 mg Co; 1 mg I; 0.15 mg Se, 200 mg choline chloride.

After weighing the gastrointestinal tract, ileum samples for histological analysis and caecum samples for microbiological analysis were collected. Ileum samples were obtained by cutting into pieces between Meckel's diverticulum and the ilea-caecal junction. The ileum samples were fixed in 10% formalin and immersed into paraffin wax and sectioned at 5 µm by a microtome (Leica, Nussloch, Germany). After processing for staining with routine haematoxylin and eosin methods, the sections were investigated by using an optical microscope (Primo Star, Zeiss, Jena, Germany) and image processing and analysis software (ZEN 2012 SP2, Zeiss) to measure the villus height (VH), crypt depth (CD) and muscularis mucosa thickness (MMT).

Collected caecal samples were stored at −20°C until microbiological analysis. One gramme of each caecal sample was transferred to a sterile test tube and diluted with 9 ml sterile Ringer solution. The serial dilutions (1:10) were prepared and each dilution was plated onto the appropriate agar plate for bacterial counting. The culture media was MRS (de Man, Rogosa and Sharpe) Agar (Merck 110660) for *Lactobacillus* spp., Slanetz and

Bartley Agar (Merck 105262) for *Enterococcus* spp., EMB (Eosin Methylene Blue) agar (Merck 101347) for *E. coli*, Campylobacter Selective Agar for *Campylobacter jejuni*, Baird-Parker agar (Merck 105406) for *Staphylococcus aureus* and TSC (Tryptose Sulphite Cycloserine) agar (Merck 111972) for *Clostridium perfringens*. The microbial colonies were counted based on the morphology of the colonies with a digital colony counter (Colony Star, Funke Gerber, Berlin, Germany) and expressed as $\log_{10}$ colony-forming units (CFU) per gramme of wet caecal content.

2.4. Statistical procedures

Data were subjected to one-way analysis of variance (ANOVA) using the SPSS statistical software (SPSS 21.0 for Windows; SPSS Inc., Chicago, IL, USA). Treatment means were separated using Duncan's multiple range test at $p < 0.05$. The results are presented as mean and pooled SEM values. The cage represented the experimental unit for performance parameters while the bird was the experimental unit for other parameters. A chi-square test was performed to determine the association between dietary treatments and mortality rates. Microbiological values were subjected to logarithmic transformation before analysis.

3. Results

3.1. Growth performance, carcass traits and meat quality

The average mortality rate of broiler chickens was 2.29% and there were no significant differences among the treatment groups. The growth performance of broilers fed diets containing PP and FP is shown in Table 3. Dietary PP and FP did not affect the growth performance of broiler chickens.

The carcass characteristics of broilers fed diets containing PP and FP are shown in Table 4. Carcass characteristics were not changed by dietary PP and FP in broilers. The b* value of breast meat at day 5 in birds in the 5FP group was higher than chicks in the control group ($p < 0.05$, Table 5). The chicks in the 10PP group had lower L* value ($p < 0.05$) in the liver compared with the birds in the 10FP group at day 5, while L* value

Table 3. Effect of dietary raw (PP) and fermented pomegranate pomace (FP) on the growth performance in broiler chickens.

	Day	Control*	5PP†	10PP♦	5FP◊	10FP♦	SEM#	*p*-Value
Body weight [g]	0	38.7	38.8	39.0	38.8	39.0	0,07	0.817
	21	963	1005	991	990	1000	8.0	0.563
	42	3195	3159	3155	3178	3196	17.9	0.933
Feed intake [g]	1–21	1198	1237	1233	1213	1217	7.8	0.558
	21–42	3748	3703	3726	3768	3807	20.7	0.590
	1–42	4947	4940	4959	4982	5024	25.9	0.872
Feed conversion ratio [g:g]	1–21	1.30	1.28	1.30	1.28	1.27	0.010	0.839
	21–42	1.68	1.72	1.72	1.72	1.73	0.007	0.138
	1–42	1.57	1.58	1.59	1.59	1.59	0.004	0.303

*Control, unsupplemented basal diet; †5PP, basal diet supplemented with 5 g PP per kg; ‡10PP, basal diet supplemented with 10 g PP per kg; ◊5FP, basal diet supplemented with 5 g FP per kg; ♦10FP, basal diet supplemented with 10 g FP per kg; #SEM, standard error of the mean.

Each value represents the mean of five replicate values (seven chickens per replicate).

Table 4. Effect of dietary raw (PP) and fermented pomegranate pomace (FP) on carcass characteristics in broiler chickens.

Characteristic [g/100 g BW[$]]	Control*	5PP[†]	10PP[‡]	5FP[◊]	10FP[♦]	SEM[#]	*p*-Value
Dressing percentage	77.9	78.7	79.1	78.0	78.1	0.24	0.520
Heart	0.49	0.45	0.55	0.43	0.51	0.020	0.350
Liver	1.66	1.61	1.66	1.66	1.64	0.032	0.982
Gizzard	1.36	1.32	1.38	1.33	1.29	0.047	0.980
Gastrointestinal weight	4.78	4.81	5.04	4.96	4.64	0.083	0.595
Abdominal fat	0.34	0.34	0.39	0.43	0.49	0.043	0.814
Spleen	0.09	0.12	0.13	0.11	0.10	0.005	0.253
Edible internal organs	3.51	3.38	3.59	3.41	3.43	0.060	0.847

[$]BW, body weight; *Control, unsupplemented basal diet; [†]5PP, basal diet supplemented with 5 g PP per kg; [‡]10PP, basal diet supplemented with 10 g PP per kg; [◊]5FP, basal diet supplemented with 5 g FP per kg; [♦]10FP, basal diet supplemented with 10 g FP per kg; [#]SEM, standard error of the mean.
Each value represents the mean of five replicate values (one chicken per replicate).

Table 5. Effect of dietary raw (PP) and fermented pomegranate pomace (FP) on pH and colour values of breast meat and liver in broiler chickens.

	Control[#]	5PP[$]	10PP[¶]	5FP[◊]	10FP[♦]	SEM[#]	*p*-Value
Day 1							
Breast meat							
pH	6.01	6.08	6.13	6.03	6.01	0.017	0.131
L*[†]	63.42	62.32	63.40	62.33	62.55	0.445	0.888
a*[‡]	2.63	3.47	2.47	3.06	2.44	0.192	0.381
b*[§]	8.83	9.07	8.99	10.39	9.88	0.331	0.539
Liver							
pH	6.25	6.33	6.37	6.30	6.36	0.024	0.494
L*	33.96	33.35	31.97	33.28	33.60	0.293	0.268
a*	17.00	16.30	16.21	17.02	17.18	0.216	0.507
b*	8.56	8.92	8.23	8.71	9.95	0.223	0.133
Day 5							
Breast meat							
pH	6.09	6.16	6.16	6.12	6.04	0.019	0.260
L*	62.89	61.87	61.64	61.77	60.69	0.372	0.501
a*	2.14	2.93	2.57	3.23	2.31	0.184	0.365
b*	8.94[b]	8.65[b]	8.79[b]	11.02[a]	9.62[ab]	0.292	0.044
Liver							
pH	6.26	6.23	6.35	6.27	6.21	0.022	0.353
L*	34.24[ab]	34.20[ab]	32.29[b]	32.75[ab]	34.62[a]	0.315	0.048
a*	16.60	15.39	15.56	17.04	15.94	0.240	0.141
b*	9.56	9.34	8.18	9.27	10.19	0.256	0.140
Day 11							
Breast meat							
pH	6.11	6.13	6.18	6.16	6.17	0.016	0.598
L*	62.75	60.59	60.84	60.74	60.63	0.462	0.559
a*	2.35	3.50	2.72	3.05	2.52	0.187	0.314
b*	8.24	8.81	7.91	9.42	8.70	0.273	0.499
Liver							
pH	6.15	6.13	6.33	6.26	6.18	0.033	0.294
L*	35.27[a]	35.35[a]	33.07[b]	34.88[a]	35.87[a]	0.316	0.036
a*	15.26	15.23	15.22	16.16	15.57	0.214	0.622
b*	8.60	8.70	8.32	9.11	10.16	0.235	0.097

Control, unsupplemented basal diet; [$]5PP, basal diet supplemented with 5 g PP per kg; [¶]10PP, basal diet supplemented with 10 g PP per kg; [◊]5FP, basal diet supplemented with 5 g FP per kg; [♦]10FP, basal diet supplemented with 10 g FP per kg; [†]L, lightness; [‡]a*, redness; [§]b*, yellowness; [#]SEM, standard error of the mean.
Each value represents the mean of five replicate values (one chicken per replicate, three measurements per sample). [a,b]Values within a row not sharing the same superscript differ significantly at $p < 0.05$.

of liver was lowest in chicks in the 10PP group compared with the other groups at day 11 ($p < 0.05$).

3.2. Antioxidant activity

The serum GPx, SOD, and CAT levels and MDA level of breast meat in broiler chickens fed diets containing PP and FP are shown in Table 6. There was no significant effect on the serum GPx, SOD, and CAT level by dietary inclusion of PP and FP. The MDA level of breast meat was lower in all dietary treatment groups compared with the control group ($p < 0.05$).

3.3. Caecal microbiota

The composition of caecal microbiota of broiler chickens fed diets containing PP and FP is shown in Table 7. Chicks fed 10PP, 5FP and 10FP had lower caecal *C. perfringens* ($p < 0.05$) than birds in the control group. Dietary PP and FP had no effect on the caecal *Lactobacillus* spp., *Enterococcus* spp., *Escherichia coli, C. jejuni*, and *S. aureus* counts in broiler chicks.

Table 6. Effect of dietary raw (PP) and fermented pomegranate pomace (FP) on serum glutathione peroxidase, superoxide dismutase, and catalase and malondialdehyde level of breast meat in broiler chickens.

	Control*	5PP[$]	10PP[!]	5FP[◊]	10FP[♦]	SEM[#]	*p*-Value
GPx[†] [U/ml]	281	285	288	294	273	11.0	0.988
SOD[‡] [U/ml]	314	360	352	318	323	12.9	0.749
CAT[§] [U/ml]	103	110	102	114	106	3.6	0.839
MDA[¶] [mg/kg]	0.104[a]	0.031[b]	0.057[b]	0.058[b]	0.054[b]	0.0080	0.018

*Control, unsupplemented basal diet; [$]5PP, basal diet supplemented with 5 g PP per kg; [!]10PP, basal diet supplemented with 10 g PP per kg; [◊]5FP, basal diet supplemented with 5 g FP per kg; [♦]10FP, basal diet supplemented with 10 g FP per kg; [†]GPx, glutathione peroxidase; [‡]SOD, superoxide dismutase; [§]CAT, catalase; [¶]MDA, malondialdehyde; [#]SEM, standard error of mean.

Each value represents the mean of five replicate values (one chicken per replicate); [a,b]Values within a row with different superscripts differ significantly at $p < 0.05$.

Table 7. Effect of dietary raw (PP) and fermented pomegranate pomace (FP) on caecal microbiota in broiler chickens.

Microorganism [$\log_{10}$ CFU/g]	Control*	5PP[$]	10PP[!]	5FP[◊]	10FP[♦]	SEM[#]	*p*-Value
Lactobacillus spp.	8.62	9.02	8.70	8.56	8.88	0.120	0.740
Enterococcus spp.	8.00	8.19	8.01	8.05	8.26	0.098	0.903
Escherichia coli	8.86	8.98	8.68	8.51	9.28	0.115	0.272
Campylobacter jejuni	6.30	6.17	6.17	5.78	5.92	0.120	0.680
Staphylococcus aureus	6.01	6.38	5.50	5.78	6.37	0.157	0.442
Clostridium perfringens	6.99[a]	6.34[ab]	5.15[c]	5.36[bc]	5.60[bc]	0.205	0.016

*Control, unsupplemented basal diet; [$]5PP, basal diet supplemented with 5 g PP per kg; [!]10PP, basal diet supplemented with 10 g PP per kg; [◊]5FP, basal diet supplemented with 5 g FP per kg; [♦]10FP, basal diet supplemented with 10 g FP per kg; [#]SEM, standard error of the mean.

Each value represents the mean of five replicate values (one chicken per replicate).

[a,b,c]Values within a row not sharing the same superscript differ significantly at $p < 0.05$.

Table 8. Effect of dietary raw (PP) and fermented pomegranate pomace (FP) on ileal morphology in broiler chickens.

	Control*	5PP[$]	10PP[I]	5FP[◊]	10FP[♦]	SEM[#]	*p*-Value
VH[†] [µm]	939.5[a]	926.1[ab]	832.6[c]	813.6[c]	856.7[bc]	13.04	0.002
CD[‡] [µm]	77.2[b]	94.3[a]	75.4[b]	85.5[ab]	88.7[a]	1.87	0.003
VH:CD [µm:µm]	12.5[a]	10.0[b]	11.1[ab]	9.6[b]	9.8[b]	0.29	0.005
MMT[§] [µm]	132.8	149.6	129.1	128.6	138.0	4.05	0.458

*Control, unsupplemented basal diet; [$]5PP, basal diet supplemented with 5 g PP per kg; [I]10PP, basal diet supplemented with 10 g PP per kg; [◊]5FP, basal diet supplemented with 5 g FP per kg; [♦]10FP, basal diet supplemented with 10 g FP per kg; [#]SEM, standard error of the mean; [†]VH, villus height; [‡]CD, crypt depth; [§]MMT, muscularis mucosa thickness.
Each value represents the mean of five replicate values (one chicken per replicate).
[a,b,c]Values within a row not sharing the same superscript differ significantly at $p < 0.05$.

3.4. Intestinal histology

The VH of the birds from the 10PP, 5FP, and 10FP groups was lower than that of control chicks ($p < 0.01$, Table 8). The 5PP and 10FP groups had higher CD ($p < 0.01$) compared with the control group. Similarly, the VH:CD ratio of chicks in the 5PP, 5FP, and 10FP groups was lower than birds in the control group ($p < 0.01$). However, the MMT of the broiler chicks was not affected by dietary treatments.

4. Discussion

Solid-state fermentation has been a useful method for the utilisation of agricultural residues, where the nutritional composition of agricultural residues can be improved (Altop et al. 2018). Similar to the findings of the present study, *A. niger* increased the crude protein content and ash of pomegranate peels after solid-state fermentation (Aguilar et al. 2008). Similarly, Ahmed et al. (2017) reported that crude protein and ash contents of PP were increased after fermentation with *Lactobacillus plantarum* and *Saccharomyces cerevisiae*. The increase in crude protein may be attributed to produced enzymes and/or mycelia by *A. niger* (Raimbault 1998). Gungor et al. (2020) noted that *A. niger* increased the ash content of pomegranate seed due to the decline in the other nutrients. Similarly, the increase in ash content of PP may be due to the decrease in the nitrogen-free extractives, crude fibre, neutral and acid detergent fibre and lignin content.

Microorganisms like *A. niger* can produce microbial lipids and can therefore increase the ether extract content of the substrates (Hui et al. 2010). Furthermore, *A. niger* can produce cellulase to break down the structural carbohydrates in the substrates (Xie et al. 2016). The decrease in structural carbohydrates (crude fibre, neutral and acid detergent fibre, and lignin) in FP can be attributed to the production of cellulase enzyme during fermentation. Papagianni (2007) reported that *A. niger* prefers soluble carbohydrates to other nutrients for use as a carbon source. Similar to the result of the present study, Gungor et al. (2020) reported a reduced contents of nitrogen-free extractives in pomegranate seeds through solid-state fermentation with *A. niger*.

Aguilar et al. (2008) showed that *A. niger* decreased the condensed tannins from 12 g/kg to 6 g/kg and reduced hydrolysable tannins from 61 g/kg to 9 g/kg in pomegranate peels. Similarly, reduced hydrolysable tannin (from 14.9 g/kg to 13.9 g/kg) was reported in PP after solid-state fermentation with *L. plantarum* and *S. cerevisiae* by Ahmed et al. (2017).

However, condensed tannin content was slightly increased after fermentation of PP in this study. Similarly, Akiyama et al. (2001) reported that condensed tannins are harder to be degraded than hydrolysable tannins due to the complicated structures. The inoculation level of *A. niger* may not be sufficient to break down the condensed tannins of PP in the present study. The discrepancies between the results of the present study may also be attributed to different *A. niger* strains and/or different pomegranate by-products having different fractions of condensed tannins (Ambigaipalan et al. 2016).

Gumienna et al. (2016) showed that fermentation increases the radical scavenging activity by increasing the amount and/or effectiveness of polyphenols in the fermentation medium. However, a slight decrease was observed in the total phenols and radical scavenging activity after solid-state fermentation in this study. Similarly, *A. niger* decreased the total phenols of pomegranate peels from 81 mg/g to 19 mg/g (Aguilar et al. 2008). However, Ahmed et al. (2017) reported a slight increase in total phenols after solid-state fermentation of PP from 145 mg/g to 147 mg/g. These differences may be due to differences in *A. niger* strains, by-product types, pomegranate cultivars, and/or fermentation conditions among the studies.

Pomegranate pomace contains antioxidant polyphenolic compounds such as hydrolysable tannin, ellagic acid, gallic acid and punicalin (Hosseini-Vashan and Raei-Moghadam 2019). Phenolic compounds in PP react with free radicals to convert them to more stable products by donating electrons and thus breaking the free radical chain reactions (Negi and Jayaprakasha 2003). Antioxidant enzymes such as GPx, SOD, and CAT act as a first barrier in the antioxidant defence system, and therefore higher levels of these enzymes in serum or tissues indicate strong antioxidant capacity (Surai 2016). Malondialdehyde is an important indicator of oxidative stability as a secondary product of lipid oxidation occurring during the conversion of muscle to meat after slaughtering (Jiang and Xiong 2016). Lower levels of MDA indicate a reduction in oxidative deterioration (Zhang et al. 2008). Al-Shammari et al. (2019) stated that 10 g dietary pomegranate peel per kg (total polyphenols 170 mg/g) increased GPx, SOD and CAT, and also decreased MDA in plasma of broilers. Similarly, erythrocyte GPx and blood total antioxidant capacity were raised in broilers fed diets supplemented with 70 and 100 g/kg pomegranate pulp containing 36.8 mg total polyphenols per g (Hosseini-Vashan and Raei-Moghadam 2019). Besides, dietary addition of 1 g PP/kg (total polyphenols 190 mg/g) and 100 mg PP extract/kg (total polyphenols 810 mg/g) increased the radical scavenging activity of the breast and thigh meat and reduced the MDA level of thigh meat at day 0 of storage (Saleh et al. 2017, 2018). Similarly, dietary PP and FP (total polyphenols 41.1 and 39.3 mg/g, respectively) showed an antioxidant effect by decreasing the MDA level of breast meat although no change in the GPx, SOD and CAT level was observed in the present study. Akuru et al. (2020) demonstrated that dietary supplementation of pomegranate peel at 6 g/kg (total polyphenols 144 mg/g) did not change the CAT level and also decreased radical scavenging activity of breast meat at 16 d of storage. However, dietary pomegranate peel at 8 g/kg was reported to increase the CAT level but decreased the SOD level of breast meat at 16 d of storage. Besides, no change in MDA at 1, 3 and 5 d of storage, and a decline in MDA on the 7th storage day were shown in breast meat of broiler chickens receiving a diet including 5 and 10 g PP/kg (Ahmed et al. 2015) and FP containing 147 mg/g total polyphenols (Ahmed et al. 2017). The differences between the results may be due to differences in the by-product types, dietary inclusion level, and total polyphenol content

and composition of phenolic compounds of PP between the studies. Indeed, PP and FP used in this study had relatively lower amounts of total polyphenols compared with the previous studies. Ambigaipalan et al. (2016) showed that different pomegranate by-products (pomace, peel, seed etc.) have different phenolic compounds. Besides, phenolic compounds vary depending on the cultivar of pomegranates (Mousavinejad et al. 2009).

Oxidative stress suppresses the growth performance of broiler chickens by constant detrimental effects throughout the chicks' lives (Surai 2016). Dietary supplementation of pomegranate pulp at 100 g/kg raised the total antioxidant capacity in plasma and increased the body weight gain (BWG) of broiler chicks (Hosseini-Vashan and Raei-Moghadam 2019). Similarly, 10 and 20 g of dietary FP per kg diet diminished the MDA in the breast and thigh meat and also improved BWG and FCR in broilers (Ahmed et al. 2017). In the present study, FP did not affect the growth performance of broiler chicks although an antioxidant effect was observed by decreasing the MDA level of breast meat. It is thought that total phenol contents and/or the inclusion levels of PP and FP were not sufficient to observe antioxidant activity strong enough to improve the growth performance. Similar to the results of the present study, supplementation of 15 g pomegranate peel per kg did not alter the BW, BWG, and FCR in broiler chicks, notwithstanding the decrease in the MDA level of breast and thigh meat (Rajani et al. 2011). Similarly, no changes in BWG and FCR were observed in broiler chickens with dietary addition of pomegranate peel at 0.25 and 0.5 g/kg, in spite of a decrease in lipid peroxidation and increase in GPx level in the liver (Rama Rao et al. 2019). Similar results were obtained from the broilers fed diets supplemented with 8 g pomegranate peel per kg (Akuru et al. 2021).

Dietary FP using *Lactobacillus* spp. and *Saccharomyces cerevisiae* was reported to enhance the BW and FCR at 10 and 20 g/kg but did not change the growth performance at 5 g/kg in broiler chickens (Ahmed et al. 2017). Bostami et al. (2015) also reported an improvement in BWG but no change in FCR of boiler chickens with dietary supplementation of 5, 10, and 20 g FP/kg using *Lactobacillus acidophilus, Bacillus subtilis* and *S. cerevisiae*. In the present study, dietary FP at 5 and 10 g/kg had no effect on the growth performance of broilers. The difference in results may be attributed to the difference in microorganisms and/or fermentation conditions in the studies.

Colour is one of the important quality traits influencing consumer preferences. Trampel et al. (2005) reported a linkage between L* value and ether extract content in the liver of broilers. They observed a decrease in L* value with a decline in the ether extract content. In this study, the L* value in the liver of chicks fed diets supplemented with 10 g PP/kg was lower than the chicks fed 10 g FP/kg after 5 d of storage and was the lowest among all treatment groups after 11 d of storage. This may be due to a possible decline in the ether extract content in the liver by a regulating effect of PP on the lipid metabolism of broiler chicks. Indeed, Ahmed et al. (2015) demonstrated that 10 g dietary PP per kg decreased the ether extract content of breast and thigh meat of broiler chickens.

Pomegranate peel has antibacterial effects on various bacteria such as *E. coli, S. aureus*, and *Salmonella* spp. thanks to its phenolic compounds (Gullon et al. 2016). Ahmed and Yang (2017) indicated that PP can improve the intestinal microbiota of broiler chickens due to high amounts of polyphenols, flavonoids and hydrolysable tannins, and by acidifying the intestines by its low pH level (pH 3.56). Sarica and Urkmez (2016) demonstrated that dietary supplementation of pomegranate peel extract at 0.1 and

0.2 g/kg reduced total coliform and *E. coli* counts in the ileum and escalated the ileal *Lactobacillus* spp. count in broiler chicks. Similarly, caecal *Lactobacillus* spp. count was raised by inclusion of 20 g pomegranate seed oil per kg diet in broilers (Rezvani et al. 2018). Similar to the results on caecal *C. perfringens* count in the present study, Ahmed and Yang (2017) reported that 10 g PP/kg diet diminished the *E. coli* counts in caecum and ileum although no effect was observed on the *E. coli* counts at the inclusion of 5 g/kg in broiler chickens. However, Rezvani et al. (2018) reported no changes in ileal *E. coli* and *Lactobacillus* spp. counts of ileum and caecum by addition of 20 g pomegranate seed oil per kg diet. Similarly, supplementation of 5 and 10 g PP/kg in broiler diets did not alter the *Lactobacillus* spp. count in the ileum (Ahmed and Yang 2017).

Zhao et al. (2013) reported that *A. niger*-fermented *Ginkgo biloba* leaves reduced caecal *E. coli* count in broiler chicks. Similarly, fermented cottonseed meal (Jazi et al. 2017) and fermented rapeseed meal (Ashayerizadeh et al. 2018) diminished total coliform bacteria in the ileum of broiler chickens. Besides, Bostami et al. (2015) noted that FP with *L. acidophilus, B. subtilis*, and *S. cerevisiae* decreased the faecal pH in broiler chicks. Gogol et al. (2005) showed that the inclusion of *A. niger* to broiler diets as a probiotic declined the *C. perfringens* count in the ileum of chicks. In the present study, the decline in caecal *C. perfringens* count with dietary FP may be due to the antibacterial effects of PP and the probiotic effect of *A. niger*.

Villus height, CD, VH:CD, and MMT are important indicators of intestinal development, absorption capacity and health status (Gungor and Erener 2020b). Shorter villus, deeper crypt and thinner muscularis mucosa may indicate the low absorption capacity and also the presence of toxins (Xu et al. 2003). Ahmed et al. (2017) showed that the lower levels of polyphenols, hydrolysable tannin and condensed tannin in PP have positive effects on broilers, but higher levels exert a toxic effect on broiler chickens. Some phenolic compounds at higher levels may cause a reduction in digestibility of nutrients by binding to proteins in the intestinal lumen (Chamorro et al. 2013) and suppressing the villus development (Viveros et al. 2011) in broiler chickens. The worsened intestinal morphology may because of the detrimental effects of the condensed tannin and/or other possible antinutritional compounds of PP. Indeed, the condensed tannin contents of PP (16.1 mg/kg PP) and FP (18.7 mg/kg FP) used in this study were higher than in previous studies by Saleh et al. (2018) (6.72 mg/kg PP) and Hosseini-Vashan and Raei-Moghadam (2019) (1.3 mg/kg pomegranate pulp). Nyamambi et al. (2007) demonstrated that high dietary condensed tannin caused detrimental effects on intestinal morphology with reducing VH of broiler chickens. Similar to the result of the present study, Sharifian et al. (2019) observed a tendency to impair the intestinal morphology by raising CD of jejunum in broiler chicks with addition of pomegranate peel extract at 0.25–0.65 g/kg diet containing 53.8 mg condensed tannins per g.

5. Conclusions

This study demonstrated that PP and FP decreased the MDA level of breast meat with no detrimental effects on the growth performance of broiler chickens. Additionally, dietary inclusion of PP at 10 g/kg and FP at 5 and 10 g/kg improved the intestinal microbiota by reducing the caecal *C. perfringens* count of broilers. However, some adverse effects of

dietary PP and FP supplementation were observed on the intestinal morphology. Further studies investigating the possible reasons for the harmful effects on the intestinal morphology of PP and FP are needed.

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References

Aguilar CN, Aguilera-Carbo A, Robledo A, Ventura J, Belmares R, Martinez D, Rodríguez-Herrera R, Contreras J. 2008. Production of antioxidant nutraceuticals by solid-state cultures of pomegranate (*Punica granatum*) peel and creosote bush (*Larrea tridentata*) leaves. Food Technol Biotechnol. 46:218–222.

Ahmed S, Ko S, Yang C. 2017. Improving the nutritional quality and shelf life of broiler meat by feeding diets supplemented with fermented pomegranate (*Punica granatum* L.) by-products. Br Poult Sci. 58:694–703.

Ahmed ST, Islam MM, Bostami AR, Mun H-S, Kim YJ, Yang CJ. 2015. Meat composition, fatty acid profile and oxidative stability of meat from broilers supplemented with pomegranate (*Punica granatum* L.) by-products. Food Chem. 188:481–488.

Ahmed ST, Yang CJ. 2017. Effects of dietary *Punica granatum* L. by-products on performance, immunity, intestinal and fecal microbiology, and odorous gas emissions from excreta in broilers. J Poult Sci. 54:157–166.

Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. 2001. Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother. 48:487–491.

Akuru EA, Mpendulo CT, Oyeagu CE, Nantapo CWT. 2021. Pomegranate (*Punica granatum* L.) peel powder meal supplementation in broilers: effect on growth performance, digestibility, carcase and organ weights, serum and some meat antioxidant enzyme biomarkers. Ital J Anim Sci. 20:119–131.

Akuru EA, Oyeagu CE, Mpendulo TC, Rautenbach F, Oguntibeju OO. 2020. Effect of pomegranate (*Punica granatum* L) peel powder meal dietary supplementation on antioxidant status and quality of breast meat in broilers. Heliyon. 6:e05709.

Al-Shammari KIA, Batkowska J, Zamil SJ. 2019. Role of pomegranate peels and black pepper powder and their mixture in alleviating the oxidative stress in broiler chickens. Int J Poult Sci. 18:122–128.

Altop A, Coskun I, Filik G, Kucukgul A, Bekiroglu YG, Cayan H, Gungor E, Sahin A, Erener G. 2018. Amino acid, mineral, condensed tannin, and other chemical contents of olive leaves (*Olea*

europaea L.) processed via solid-state fermentation using selected *Aspergillus niger* strains. Int J Agric Nat Res. 45:220–230.
Ambigaipalan P, De Camargo AC, Shahidi F. 2016. Phenolic compounds of pomegranate byproducts (outer skin, mesocarp, divider membrane) and their antioxidant activities. J Agric Food Chem. 64:6584–6604.
AOAC. 2000. Official methods of analysis of AOAC international. USA:AOAC International.
Ashayerizadeh A, Dastar B, Shargh MS, Mahoonak AS, Zerehdaran S. 2018. Effects of feeding fermented rapeseed meal on growth performance, gastrointestinal microflora population, blood metabolites, meat quality, and lipid metabolism in broiler chickens. Livest Sci. 216:183–190.
Bostami A, Ahmed S, Islam M, Mun H, Ko S, Kim S, Yang C. 2015. Growth performance, fecal noxious gas emission and economic efficacy in broilers fed fermented pomegranate byproducts as residue of fruit industry. Int J Adv Res. 3:102–114.
Brand-Williams W, Cuvelier M-E, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 28:25–30.
Chamorro S, Viveros A, Centeno C, Romero C, Arija I, Brenes A. 2013. Effects of dietary grape seed extract on growth performance, amino acid digestibility and plasma lipids and mineral content in broiler chicks. Animal. 7:555–561.
Dipnaik HS, Shrivastava P, Jacob SM. 2014. *Aspergillus niger* mediated solid state fermentation (SSF) of pomegranate peels, yields bioactive product having antibacterial, antioxidant, radical scavenging and anti-UV properties. World J Pharm Res. 3:1377–1394.
Gogol D, Koreleski J, Żyła K. 2005. Effects of an *Aspergillus niger* mycelium, antibiotic and probiotic on growth performance, P and Ca retention and *Clostridium perfringens* ileal counts in broiler chickens. J Anim Feed Sci. 14:151–160.
Gullon B, Pintado ME, Pérez-Álvarez JA, Viuda-Martos M. 2016. Assessment of polyphenolic profile and antibacterial activity of pomegranate peel (*Punica granatum*) flour obtained from co-product of juice extraction. Food Control. 59:94–98.
Gumienna M, Szwengiel A, Górna B. 2016. Bioactive components of pomegranate fruit and their transformation by fermentation processes. Eur Food Res Technol. 242:631–640. doi:10.1007/s00217-015-2582-z.
Gungor E, Altop A, Erener G. 2020. Improvement in the nutritional composition and nutrient digestibility of pomegranate (*Punica granatum* L.) seed by *Bacillus subtilis* and *Aspergillus niger* solid-state fermentation. Anadolu J Agric Sci. 35:268–273.
Gungor E, Erener G. 2020a. Effect of dietary raw and fermented sour cherry kernel (*Prunus cerasus* L.) on growth performance, carcass traits, and meat quality in broiler chickens. Poult Sci. 99:301–309.
Gungor E, Erener G. 2020b. Effect of dietary raw and fermented sour cherry kernel (*Prunus cerasus* L.) on digestibility, intestinal morphology and caecal microflora in broiler chickens. Poult Sci. 99:471–478.
Harimurti S, Hadisaputro W. 2015. Probiotics in poultry. In: Liong MT, editor. Beneficial microorganisms in agriculture, aquaculture and other areas. Switzerland: Springer International Publishing; p. 1–19.
Hosseini-Vashan SJ, Raei-Moghadam MS. 2019. Antioxidant and immune system status, plasma lipid, abdominal fat, and growth performance of broilers exposed to heat stress and fed diets supplemented with pomegranate pulp (*Punica granatum* L.). J Appl Anim Res. 47:521–531.
Hui L, Wan C, Hai-Tao D, Xue-Jiao C, Qi-Fa Z, Yu-Hua Z. 2010. Direct microbial conversion of wheat straw into lipid by a cellulolytic fungus of *Aspergillus oryzae* A-4 in solid-state fermentation. Bioresour Technol. 101:7556–7562.
Jazi V, Boldaji F, Dastar B, Hashemi S, Ashayerizadeh A. 2017. Effects of fermented cottonseed meal on the growth performance, gastrointestinal microflora population and small intestinal morphology in broiler chickens. Br Poult Sci. 58:402–408.
Jiang J, Xiong YL. 2016. Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: a review. Meat Sci. 120:107–117.

Makkar H, Blümmel M, Becker K. 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in in vitro techniques. Br J Nutr. 73:897–913.
Mousavinejad G, Emam-Djomeh Z, Rezaei K, Khodaparast MHH. 2009. Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. Food Chem. 115:1274–1278.
Negi P, Jayaprakasha G. 2003. Antioxidant and antibacterial activities of *Punica granatum* peel extracts. J Food Sci. 68:1473–1477.
Nyamambi B, Ndlovu LR, Naik YS, Kock N. 2007. Intestinal growth and function of broiler chicks fed sorghum based diets differing in condensed tannin levels. S Afr J Anim Sci. 37:202–214.
Papagianni M. 2007. Advances in citric acid fermentation by *Aspergillus niger*: biochemical aspects, membrane transport and modeling. Biotechnol Adv. 25:244–263. doi:10.1016/j.biotechadv.2007.01.002.
Raimbault M. 1998. General and microbiological aspects of solid substrate fermentation. Electron J Biotechnol. 1:26–27.
Rajani J, Torshizi MK, Rahimi S. 2011. Control of ascites mortality and improved performance and meat shelf-life in broilers using feed adjuncts with presumed antioxidant activity. Anim Feed Sci Technol. 170:239–245.
Rama Rao S, Raju M, Prakash B, Rajkumar U, Reddy E. 2019. Effect of supplementing moringa (*Moringa oleifera*) leaf meal and pomegranate (*Punica granatum*) peel meal on performance, carcass attributes, immune and antioxidant responses in broiler chickens. Anim Prod Sci. 59:288–294.
Rezvani M, Sayadpour N, Saemi F. 2018. The effect of adding pomegranate seed extract to fat-containing diets on nutrients digestibility, intestinal microflora and growth performance of broilers. Iran J Vet Res. 19:310–317.
Saleh H, Golian A, Kermanshahi H, Mirakzehi M. 2018. Antioxidant status and thigh meat quality of broiler chickens fed diet supplemented with α-tocopherolacetate, pomegranate pomace and pomegranate pomace extract. Ital J Anim Sci. 17:386–395.
Saleh H, Golian A, Kermanshahi H, Mirakzehi MT. 2017. Effects of dietary α-tocopherol acetate, pomegranate peel, and pomegranate peel extract on phenolic content, fatty acid composition, and meat quality of broiler chickens. J Appl Anim Res. 45:629–636.
Sarica S, Urkmez D. 2016. The use of grape seed-, olive leaf-and pomegranate peel-extracts as alternative natural antimicrobial feed additives in broiler diets. Eur Poult Sci. 80:1–13.
Sharifian M, Hosseini-Vashan S, Nasri MF, Perai A. 2019. Pomegranate peel extract for broiler chickens under heat stress: its influence on growth performance, carcass traits, blood metabolites, immunity, jejunal morphology, and meat quality. Livest Sci. 227:22–28.
Surai P. 2016. Antioxidant systems in poultry biology: superoxide dismutase. J Anim Res Nutr. 1:1–17.
Tarladgis BG, Watts BM, Younathan MT, Dugan L. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J Am Oil Chem Soc. 37:44–48.
Trampel D, Sell J, Ahn D, Sebranek J. 2005. Preharvest feed withdrawal affects liver lipid and liver color in broiler chickens. Poult Sci. 84:137–142.
TUIK, 2020. Turkish Statistical Institute. http://www.turkstat.gov.tr/UstMenu.do?metod=temelist Accessed 2021 Jan 31.
Van Soest PV, Robertson J, Lewis B. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 74:3583–3597.
Viveros A, Chamorro S, Pizarro M, Arija I, Centeno C, Brenes A. 2011. Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. Poult Sci. 90:566–578.
Wu Q, Wang Z, Wang G, Li Y, Qi Y. 2015. Effects of feed supplemented with fermented pine needles (*Pinus ponderosa*) on growth performance and antioxidant status in broilers. Poult Sci. 94:1138–1144.
Xie P, Huang L, Zhang C, Zhang YL. 2016. Nutrient assessment of olive leaf residues processed by solid-state fermentation as an innovative feedstuff additive. J Appl Microbiol. 121:28–40.

Xu Z, Hu C, Xia M, Zhan X, Wang M. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult Sci. 82:1030–1036.

Zhang H, Guo Y, Tian Y, Yuan J. 2008. Dietary conjugated linoleic acid improves antioxidant capacity in broiler chicks. Br Poult Sci. 49:213–221.

Zhao L, Zhang X, Cao F, Sun D, Wang T, Wang G. 2013. Effect of dietary supplementation with fermented *Ginkgo*-leaves on performance, egg quality, lipid metabolism and egg-yolk fatty acids composition in laying hens. Livest Sci. 155:77–85.