OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2025.37.45

Research Article
Effects of Probiotic Treated Pomegranate Residue on Growth Performance, Immunity and Microbiome in the Intestines of Broilers

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Abstract

Objectives: This research was undertaken to assess the effect of probiotic treated pomegranate residue (PPR) on growth performance, serum immunoglobulins, intestinal and excreta microbiome, and excreta harmful gas emission in broilers. **Materials and Methods:** A total of 128 day-old Ross-308 chicks were randomly assigned to 4 treatment groups, each consisting of 4 replicates of 8 birds. The experimental diets were formulated to supply 0, 0.5, 1.0 and 2.0% of PPR and were fed for 35 day. **Results:** Dietary inclusion of PPR linearly increased the weight gain of broiler, while reduced FCR by almost 0.10 points without affecting the feed intake ($p<0.05$). Following the addition of PPR, a linear rise in serum IgA concentration was perceived ($p<0.05$). Dietary PPR increased the ileal and cecal *Lactobacillus*, cecal *Bacillus* (linear, $p<0.05$) and ileal yeast and mold (linear, $p = 0.0007$; quadratic, $p = 0.007$) population. In contrary, the *E. coli* population has been decreased in the ileal (linear, $p = 0.004$, quadratic, $p<0.04$) and cecal digesta (linear, $p = 0.004$) and *Salmonella* only in the cecal digesta (linear, $p = 0.0004$) in consequence of dietary PPR. As the dose level increased PPR linearly reduced the ileal and cecal pH ($p<0.05$). Dietary PPR increased the CFU of excreta *Lactobacillus* (linear, $p = 0.002$) and *Bacillus* (quadratic, $p<0.05$), whereas, decreased the *E. coli* population (linear, $p = 0.008$). In relation to dietary PPR supplementation, excreta pH was linearly lowered ($p<0.05$). Inclusion of 1% and 2% PPR reduced the NH_3 and H_2S emission from broiler excreta. **Conclusion:** Therefore, it can be concluded that PPR supplemented up to 2% level may improve the growth performance, immunity and microbiome in the intestines of broilers.

Key words: Broiler, immunity, intestinal microbiology, pomegranate residue, probiotics

Citation: Ahmed, S.T., H.-S. Mun and C.J. Yang, 2025. Effects of probiotic treated pomegranate residue on growth performance, immunity and microbiome in the intestines of broilers. Int. J. Poult. Sci., 24: 37-45.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The gut flora has an impact on the integration of immunity and tolerance, digestion, fermentation, nutritional absorption, and energy metabolism. Therefore, manipulating gut microbiota of domestic animals is of crucial importance for optimal production efficiency, overall health and welfare of animals[1]. Antibiotic Growth Promoters (AGPs) had been tremendously successful as dietary supplement to enhance animal performance by alteration of intestinal microbial ecology. However, ending the use of AGPs due to development of bacterial resistance poses challenges for livestock sector to find alternate products with antimicrobial properties. Due to their supportive antioxidant and antibacterial characteristics, absence of side effects, and ability to modify intestinal microbiota for improving animal health and production, probiotics and medicinal plants have attracted a lot of attention as antibiotic alternatives.

The pomegranate (*Punica granatum* L.) a historically significant, miraculous, and distinctive fruit that is a member of the Punicaceae family, has long been employed for therapeutic purposes in a variety of civilizations. Currently, pomegranates are cultivated and consumed widely around the world, either as fresh arils or juice. It is utilized in the food industry to make jellies, concentrates, flavorings, and colorings due to its potential to be nutritious and health-promoting. Thus, a significant amount of residues are created, including peel, rind, and seeds[2], which contain considerable amounts of phenolic and flavonoid chemicals[3] and have the potential to act as an antioxidant, an antimicrobial, and an immunomodulator[4,5]. The majority of these are disposed of through landfills, incinerators, or composting, which eventually has negative environmental effects. But it is well known for its possible health advantages and can be added to livestock feed as a supplement feed[2].

A special technique called fermentation has a lot of potential for turning some agricultural waste items into beneficial animal feeds in underdeveloped nations. Oboh *et al*.[6] claim that fermentation can increase a product's nutraceutical value by reducing some harmful substances and triggering efficient microbial conversion. Fermented foods are more suited for birds because they are more pleasant, more nutritious, and have greater antibacterial potential[7,8]. Probiotic strains like *Lactobacillus spp., Enterococcus faecium*, *Bacillus spp*., and *Saccharomyces cerevisiae* have been demonstrated in the past to improve the nutrient content and antibacterial capacity of several medicinal plants and agro-industrial byproducts through fermentation[9-11]. The present research sought to determine how the pomegranate fermentation process with *Lactobacillus plantarum* and *Saccharomyces cerevisiae* impacted broiler chicken growth, immunity, intestinal and excreta microbiology, and pH. Moreover, the harmful gas emissions from broiler excrement were also assessed.

MATERIALS AND METHODS

Probiotic treated pomegranate residues (PPR) production: According to Hossain *et al*.[10], probiotic bacteria *Lactobacillus plantarum* KCTC 3099 and *Saccharomyces cerevisiae* KCTC 7928 were chosen as starter cultures considering their levels of acid, bile, and heat tolerance. These bacteria were bought from the Korea Research Institute of Bioscience and Biotechnology in Daejeon, Korea. The peel, skin, and seeds of pomegranate, which were a byproduct of pomegranate juice production, were utilized as solid media for fermentation. The fermentation procedure was carried out as stated by Ahmed *et al*.[11]. After fermentation, fermented goods were dried at 80° C for 48 hours to less than 15% moisture. Then, for measuring the cell number, 1g of PPR was added to 10 mL of sterile, purified water. One milliliter of the solution was diluted successively 10 times in NaCl (0.85%) buffer, and then it was used to cultivate bacteria on an agar substrate. The inoculated plate was allowed to incubate for 24-48 hrs at 37°C, after which the colonies were counted. The microbial contents of PPR were *Lactobacillus plantarum* KCTC 3099 5.2×10^9CFU/g and *Saccharomyces cerevisiae* KCTC 7928 2.9×10^8 CFU/g. Following this, the PPR were subjected to a triple analysis for moisture, ash, Crude Protein (CP), crude fiber (CF), and crude fat (EE) content using the AOAC[12] technique. Table 1 displays the chemical make-up of the PPR as well as the quantity of bacteria present.

Table 1: Microbial content, chemical composition, and pH (Mean±SD) of pomegranate residue (PR) and probiotic treated pomegranate residues (PPR)

Chemical composition	PR	PPR
Moisture (% dry matter)	11.02±0.21	12.67±0.23
Crude Protein (% dry matter)	7.55±0.08	9.11±0.22
Crude Fat (% dry matter)	3.73±0.35	3.17±0.06
Crude Fiber (% dry matter)	23.53±0.21	24.92±0.29
Crude Ash (% dry matter)	4.28±0.04	4.35±0.07
Calcium (mg/100g)	55.25±1.07	59.71±1.01
Iron (mg/100g)	22.49±0.43	28.06±0.56
Magnesium (mg/100g)	6.25±1.13	8.37±1.05
Sodium (mg/100g)	5.40±0.31	6.52±0.55
pH	4.28±0.03	4.04±0.01
Total polyphenol (mg/g)	143.54±3.12	149.24±2.25
Hydrolysable Tannins (mg/g)	15.24±2.13	13.74±1.95

Each value is the average obtained from three replicates

Table 2: Feed ingredients and chemical compositions of the broiler diets

	Starter diet (0 to 21 day)				Finisher diet (22 to 35 day)			
Item	PPR 0%	PPR 0.5%	PPR 1.0%	PPR 2.0%	PPR 0%	PPR 0.5%	PPR 1.0%	PPR 2.0%
Ingredients (Fed basis%)								
Corn grain	57.37	56.87	56.37	55.37	60.78	60.28	59.78	58.78
Soybean meal	26.55	26.55	26.55	26.55	24.75	24.65	24.65	24.65
Corn gluten	4.50	4.50	4.50	4.50	4.00	4.00	4.00	4.00
Soybean oil	2.50	2.50	2.50	2.50	2.80	2.80	2.80	2.80
Animal fats	4.00	4.00	4.00	4.00	3.00	3.00	3.00	3.00
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Dicalcium phosphate	2.20	2.20	2.20	2.20	2.00	2.00	2.00	2.00
Limestone	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Vitamin-mineral premix[a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Choline	0.08	0.08	0.08	0.08	0.07	0.07	0.07	0.07
L-lysine HCl (78%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
DL-Methionine	0.20	0.20	0.20	0.20	0.10	0.10	0.10	0.10
Pomegranate residues	0.00	0.50	1.00	2.00	0.00	0.50	1.00	2.00
Calculated composition (DM%)								
ME (kcal/kg)	3150.00	3126.00	3123.00	3116.00	3210.00	3205.00	3188.00	3175.00
Moisture	12.07	11.39	11.34	11.68	13.08	13.16	13.28	13.25
Crude protein	20.89	20.73	21.97	20.12	19.12	18.72	18.74	18.26
Ether extract	4.65	4.90	5.57	5.93	2.43	2.40	3.71	3.42
Crude fiber	4.42	4.61	4.51	4.00	3.71	3.35	3.49	3.46
Crude ash	5.63	5.37	5.76	5.54	5.61	4.68	5.47	5.25
Calcium	1.00	1.00	1.00	1.00	0.83	0.83	0.83	0.83
Available phosphorus	0.50	0.50	0.50	0.50	0.45	0.45	0.45	0.45
Lysine	1.40	1.40	1.40	1.40	1.10	1.10	1.10	1.10
Methionine	0.50	0.50	0.50	0.50	0.42	0.42	0.42	0.42

[a]Vitamin-mineral mixture provided the following nutrients per kilogram of diet: vitamin A, 15,000 IU; vitamin D3, 1,500 IU; vitamin E, 20.0 mg; vitamin K3, 0.70 mg; vitamin B12, 0.02 mg; niacin, 22.5 mg; thiamine, 5.0 mg; folic acid, 0.70 mg; pyridoxine, 1.3 mg; riboflavin, 5 mg; pantothenic acid, 25 mg; choline chloride, 175 mg; Mn, 60 mg; Zn, 45 mg; I, 1.25 mg; Se, 0.4 mg; Cu, 10.0 mg; Fe, 72 mg; Co, 2.5 mg (Bayer Korea Ltd., Dongjak-Ku, Seoul, Korea).

Dietary regimens and birds management: A completely randomized design was used to allocate 128 Ross 308 male broiler chicks (one-day-old) to 1 of 4 feeding regimens. There were 4 replicates of each regimen, each containing 8 chicks. The dietary regimens included a control diet consisting solely of a basal diet (PPR 0%) and a basal diet added with 0.5% (PPR 0.5%), 1.0% (PPR 1.0%) and 2.0% (PPR 2.0%) probiotic treated pomegranate residues. The starter period (day 0 to 21) and the finisher period (day 22 to 35) of the experimental diets were administered where PPR were added by replacing corn gain in equal amounts. The nutrients all fulfilled or surpassed the NRC[13] recommended nutritional requirements. Table 2 lists the components and chemical make-up of the experimental diets. Broilers were kept in a climate controlled wire-floor caged house giving a floor space of 664 cm^2/bird (85 cm long×62.5 cm wide×40 cm high/cage). The indoor temperature was initially kept at around 34°C and then dropped to between 23°C and 24°C with a 65% humidity level. By taking daily observations other environmental factors were controlled. Through the duration of the experiment, there was constant lighting. The birds had unrestrained entree to food and water during the whole raising process.

Growth measurement: On day 1, 21, and 35, body weight and feed intake were recorded, the Average Daily Gain (ADG), Average Daily Feed Intake (ADFI), and feed/gain were subsequently determined.

Immunoglobulin analysis: At day 35, 3 broilers were indiscriminately chosen from each replication and blood specimens were drawn (10 mL) from the wing veins and placed into a 10 mL anticoagulant-free vacutainer tube (Greiner Bio-One GmbH, Kremsmunster, Austria). During the collection time, the samples were kept on ice. Once collected, they were immediately centrifuged for 15 minutes at 1,610×g at 4°C to separate the serum. After that, the specimens of serum were securely shifted to plastic vials and kept there at -20°C until immunoglobulin analysis was carried out in accordance with the technique explained by Ahmed and Yang[14]. The concentrations of immunoglobulins were articulated as mg/mL of serum.

Analysis of intestinal and fecal microbiology and pH: Following blood collection, the experimental birds were slaughtered by severing their jugular vein and the GIT was excised from the bodies. Subsequently, 10-cm lengths from

the same portion of both ceca as well as the ileum (Meckel's diverticulum to the joint of ceca) were separated. A 15 mL safe-lock falcon tube (SPL Lifesciences Co., Ltd, Pocheon, Gyenggi-do, Korea) was used to place approximately 1 g of ileal, cecal and fecal sample. The analysis of intestinal and fecal microbiology and pH were carried out in accordance with the methodology described by Ahmed and Yang[14].

Measuring malodourous gas emission from broiler excreta: After being properly homogenized, an excreta sample was taken from the underneath tray of each replicate cage and being stored in zipper bags. For measuring the release of ammonia (NH_3), hydrogen sulfide (H_2S), sulfur dioxide (SO_2), and mercaptan from broiler excreta, approximately 600 g of feces/replication was put in a 2-liter plastic box in triplets. The covers of the plastic boxes have two holes in them. One hole was used to draw gas through a tube with a cap and another was vacuum-packed with an Advantec® membrane filter (pore size 1.0µm, Toyo Roshi Kaisha Ltd., Otowa, Tokyo, Japan) which made it simpler to provide fresh air to balance the negative pressure that was produced as the pump drew headspace air. The samples were first collected at 0 hours, then allow to decompose at ambient temperature (on average 28°C), and then samples were obtained at 3, 6, 12, 24 and 48 hrs. Gastec (model AP-20) gas sampling pump (Gastec Corp., Kitagawa, Japan) and Gastec detector tube (3 LA, 3M for NH3; 4 LB, 4LK for H2S) were used to draw 100 mL of headspace air from 2.0 cm above the sample surface. The ppm/100 ml unit used to represent the concentration of deadly gases.

Statistical analysis: The data collected for the current study were statistically analyzed using the General Linear Model (GLM) Procedure created by the Statistics Analysis Systems Institute (SAS[15]). For growth performance, excreta pH, and harmful gas emission, each replicate cage served as the experimental unit, whereas data on immunity, intestinal pH, and microbial population were based on groups of three broilers. To ascertain the linear and quadratic effects of PPR in diets on each assessment, an orthogonal polynomial contrast test was used. Treatment means were calculated using the SAS program's LSMEANS option, and Statistical significance was determined using a probability level of $p \leq 0.05$.

RESULTS

Growth performance and immunity: Table 3 shows the impact of PPR on the growth performance indicators of broilers at various stages of rearing. There was a linear increase in the BW of broiler in response to supplemental at day 21 ($p<0.01$) and 35 ($p = 0.001$). Over the course of the trial, broiler weight gain increased linearly as PPR levels increased ($p<0.05$). The incorporation of PPR in broiler feed had no discernible impact on ADFI, however significantly lowered the feed/gain ratio of broiler during 0 to 21 day (linear, $p<0.05$; quadratic, $p<0.02$) and 0 to 35 day (linear and quadratic, $p<0.02$) experimental period. Dietary PPR improved the humoral immunity of broiler by linearly increasing ($p<0.05$) the level of serum IgA (Table 4).

Table 3: Effects of different level of probiotic treated pomegranate residue (PPR) on the growth performance of broilers

	Fermented pomegranate residues (PPR)					p-value	
Performance parameter	0%	0.5%	1.0%	2.0%	SEM	Linear	Quadratic
Weight gain (g/bird/day)							
0 to 21 day	41.81	42.46	46.66	45.03	1.00	0.01	0.30
21 to 35 day	74.34	75.27	77.19	76.41	0.62	0.02	0.21
0 to 35 day	54.82	55.59	58.87	57.58	0.56	0.001	0.12
Feed intake (g/bird/day)							
0 to 21 day	61.31	58.29	62.26	62.63	1.13	0.19	0.21
21 to 35 day	142.77	138.7	139.4	140.7	2.79	0.67	0.37
0 to 35 day	93.89	90.46	93.11	93.86	1.40	0.69	0.16
Feed conversion ratio (FCR)							
0 to 21 day	1.47	1.38	1.34	1.39	0.05	0.05	0.02
21 to 35 day	1.92	1.84	1.81	1.84	0.04	0.14	0.16
0 to 35 day	1.71	1.63	1.58	1.63	0.02	0.02	0.02

Each number corresponds to the average over 4 replications with 8 birds each replication. SEM: Standard error of the means.

Table 4: Effects of probiotic treated pomegranate residue (PPR) on the serum immunoglobulins of broilers

	Probiotic treated pomegranate residues (PPR)					p-value	
Immunoglobulins	0%	0.5%	1.0%	2.0%	SEM	Linear	Quadratic
IgA	0.792	0.929	0.803	0.996	0.05	0.05	0.57
IgG	1.718	1.773	1.745	1.744	0.03	0.74	0.46

Each number corresponds to the average over 4 replications with 3 birds each replication, SEM: Standard error of the means.

Table 5: Effects of probiotic treated pomegranate residue (PPR) on ileal, cecal and excreta microbiology in broilers

Microorganisms	Probiotic treated pomegranate residue (PPR)					p-value	
(log_{10} CFU/g)	0%	0.5%	1.0%	2.0%	SEM	Linear	Quadratic
Ileum							
Lactobacillus spp.	5.06	5.46	5.48	6.03	0.19	0.005	0.710
Bacillus spp.	4.81	4.57	4.36	4.73	0.56	0.87	0.630
Yeast and mold	3.73	5.57	5.92	5.71	0. 31	0.0007	0.007
Escherichia coli	5.34	4.71	3.87	4.34	0.23	0.004	0.040
Salmonella spp.	0.96	0.58	0.77	0.43	0.62	0.80	0.800
pH	6.03	6.05	5.86	5.76	0.24	0.04	0.240
Cecum							
Lactobacillus spp.	6.62	7.05	7.13	7.35	0.16	0.009	0.520
Bacillus spp.	5.88	6.28	6.23	6.74	0.24	0.05	0.820
Yeast and mold	5.69	5.61	6.01	5.54	0.24	0.97	0.390
Escherichia coli	6.84	6.84	6.37	5.46	0.28	0.004	0.140
Salmonella spp.	2.99	2.14	1.55	0.54	0.30	0.0004	0.290
pH	6.76	6.60	6.29	6.28	0.17	0.02	0.640
Excreta							
Lactobacillus spp.	6.60	7.22	7.21	7.68	0.18	0.002	0.710
Bacillus spp.	6.05	6.70	6.72	7.03	0.21	0.12	0.050
Yeast and mold	5.86	6.17	6.13	6.10	0.23	0.54	0.510
Escherichia coli	5.63	4.90	4.64	4.41	0.22	0.008	0.390
Salmonella spp.	4.84	5.17	5.34	4.55	0.39	0.72	0.210
pH	7.10	6.78	6.72	6.70	0.09	0.01	0.130

Each number corresponds to the average over 4 replications with 3 birds each replication, SEM: Standard error of the means.

Intestinal and excreta microbiology and pH: As shown in Table 5, inclusion of PPR at a rate of 0.5, 1.0, and 2.0% of diet resulted in linearly increased CFU number of *Lactobacillus* bacteria both in ileal ($p = 0.005$) and cecal ($p = 0.009$) digesta. Higher CFU number of yeast and mold in the ileal content (linear, $p = 0.0007$; quadratic, $p=0.007$) and *Bacillus* spp. in the cecal digesta (linear, $p<0.05$) were also documented in broiler supplemented with PPR with their diet. In contrast, dietary PPR condensed the CFU number of *E. coli* both in ileal (linear, $p = 0.004$; quadratic, $p<0.05$) and cecal (linear, $p = 0.004$) digesta. In addition, broiler diet supplemented with PPR linearly reduced the CFU number of *Salmonella* ($p = 0.0004$) in the cecal digesta. Inclusion of PPR in broiler diet resulted in linearly lower pH of both ileal and cecal digesta ($p<0.05$). Dietary inclusion of 0.5 to 2.0% PPR significantly increased the CFU number of excreta *Lactobacillus* (linear, $p=0.002$) and *Bacillus* (quadratic, $p<0.05$) bacteria. On the other hand, as dietary PPR levels increased, the quantity of excreta *E. coli* was linearly decreased ($p = 0.008$). Dietary PPR had no discernible impact on the concentration of *Salmonella* in excreta ($p>0.05$). The pH of the excreta was linearly decreased by dietary addition of PPR ($p<0.05$).

Fecal harmful gas emissions: The effects of dietary PPR on fecal NH_3 and H_2S, emissions are shown in Fig. 1 and 2, respectively. Dietary inclusion of 1.0 or 2.0% PPR considerably decreased the NH_3 release from broiler excreta at 0, 3, 6, 12, and 48 hrs of incubation in comparison to control group ($p<0.05$). In addition, inclusion of 1.0 or 2.0% PPR in broiler diet reduced the emissions of H_2S from broiler feces at 12 and 48 hrs ($p<005$).

DISCUSSION

The biological significance of tannin-rich feed stuff in chicken nutrition is connected to their features' unfavorable impacts on feed consumption[16] and the absorption of nutrients, notably protein, which has an unfavorable ending on growth performance[17]. However, fermentation of tannin rich feedstuffs can reduce the amount of tannin by microbial breakdown[18,19]. Yeast like *Candida* spp., and *S. cerevisiae*[20] and bacteria like *Bacillus* spp., *Corynebacterium* spp. and *Lactobacillus* spp.[21] are renowned for their tannase synthesis ability, that convert hydrolysable tannins to glucose and gallic acid. The degradation of food elements by microbial enzymes during fermentation with beneficial bacteria has been demonstrated to boost the benefits of fermented feeds in terms of nutrition and nutritional supplements[22,23] and this eventually promotes broiler growth efficiency[24]. The fermentation of the pomegranate residue in our study decreased the tannin content of the PPR (Table 1), which may

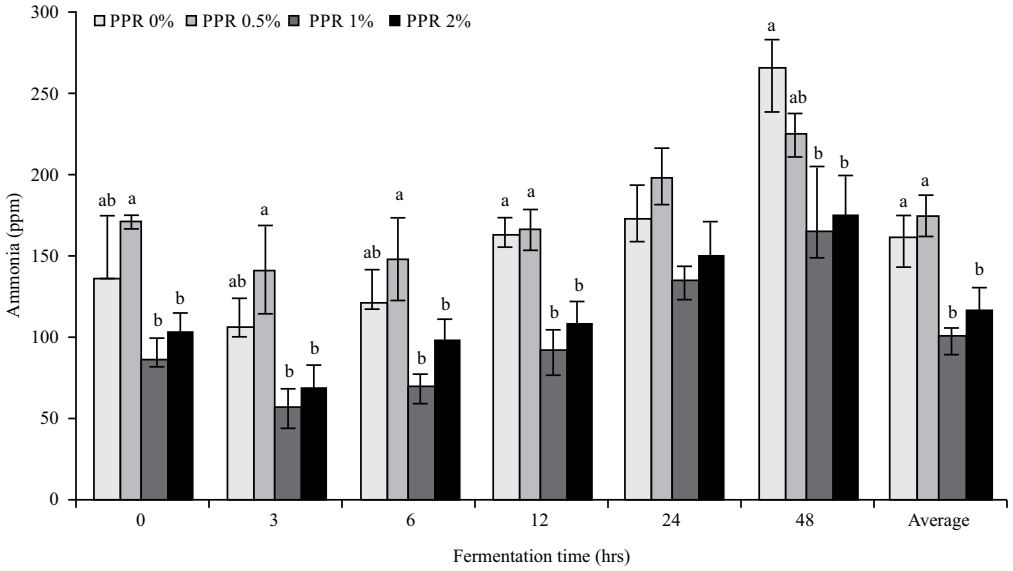

Fig. 1: Effects of different level of fermented pomegranate residue (PPR) on fecal Ammonia (NH_3) emission for 48 hrs. A different letter at a specific time point denotes significant difference ($p<0.05$)

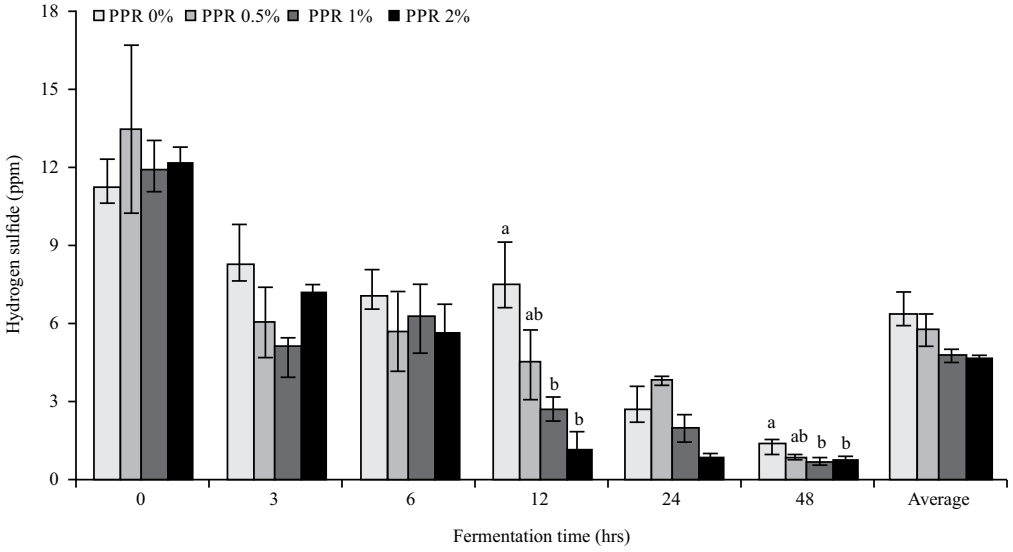

Fig. 2: Effects of different level of fermented pomegranate residue (PPR) on fecal Hydrogen sulfide (H_2S) emission for 48 hrs. A different letter at a specific time point denotes significant difference ($p<0.05$)

have improved the nutrient utilization and improved the growth performance (higher BW and ADG and lower FCR) of broilers. Furthermore, Dei *et al.*[19] found that broiler given diets enriched with fermented Shea nut meal (a high tannin rich feedstuff) resulted in greater weight gain compared to non-fermented meal.

The immune enhancing capacity of pomegranate peel and seed oil was previously reported by Ross *et al.*[4] and Yamasaki *et al.*[25]. Furthermore, fermentation of pomegranate residues with *L. plantarum* and *S. cerevisiae* may increase immunomodulatory capacity via stimulation of the growth of lymphocytes.

The antimicrobial capacity of pomegranate peel, aril and peel extracts against *E. coli* O157: H7, and *S. typhimurium* has been exposed by a number of scientist[26,27]. In addition, Filannino *et al.*[28] revealed that, fermentation with lactic acid bacteria boosted the amount of ellagic acid and the antimicrobial activity of pomegranate juice against human food borne pathogens including *E. coli*. Previous studies have suggested that probiotic bacteria like *Lactobacillus* spp., *Bacillus* subtilis, *S. cerevisiae* may be utilized to enrich and conserve the good bacteria in the intestine[29]. In this study, higher number of *Lactobacillus* and *Bacillus* together with lower number of *E. coli* in the intestinal digesta and excreta can be explained by the formation of organic acids, primarily lactic acid[30] by the probiotic bacteria, which reduced the ileal and cecal pH and thereby create favorable condition for the growth of *Lactobacillus* bacteria.

Harmful gas emission of non-ruminants is influenced by a variety of variables that include food uptake, gut and fecal microbiota, fecal pH, etc.[31]. The primary noxious substance in broiler houses, ammonia, is produced when microbial urease found in feces hydrolyzes uric acid[32]. *E. coli*, *Klebsiella* spp., and other urease-producing bacteria[33] have all been demonstrated to be inhibited by dietary probiotics, either by creating antimicrobials or by lowering pH, which in turn lowers the quantities of ammonia in fowl excreta[34]. The generation of H_2S, SO_2, mercaptans, and organic sulfide in animal dung is mostly caused by degradation of sulfur-containing molecules in feces or sulfate reduction by microbs[35]. *Salmonella*, *E. coli*, *Pseudomonas*, *Citrobacter*, *Aeromonas* and *Desulfovibrio* are the primary sulfur-reducing bacteria in the intestines[36]. Low pH also inhibits the ability of anaerobic fermentative bacteria to metabolize amino acids that contain sulfur[37]. In this experiment, increasing the level of PPR not only increased feed efficiency but also significantly decreased the population of *E. coli* and *Salmonella* and the pH in intestinal content and broiler excreta, which may be the reason for less ammonia and sulfur-containing harmful compounds being released from broiler excreta.

CONCLUSION

In conclusion, broiler diet supplemented with PPR has befitted to promote growth performance, with improved intestinal microbial ecology. Dietary PPR increased the serum IgA level of chickens. In addition, PPR also reduced pathogenic microorganism and lowered the harmful gas emission from broiler excreta. Thus, to enhance performance and the environment inside the poultry house, probiotic treated pomegranate residues have the potential to be added to broiler feed.

ACKNOWLEDGMENTS

Business for Academic-Industrial Cooperative enterprises financed by the Korea Small and Medium Business Administration provided support for this study (Grants No. C0193546). This work was also supported by the Korean Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) and Korea Smart Farm R&D Foundation through the Smart Farm Innovation Technology Development Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) and Ministry of Science and ICT (MSIT) and Rural Development Administration (RDA) (421023-04).

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