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RESEARCH ARTICLE

Growth Performance, Fecal Noxious Gas Emission and Economic Efficacy in Broilers Fed Fermented Pomegranate Byproducts as Residue of Fruit Industry

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Abstract

Byproducts of fruit industry are promising source of nutrients and bioactive compounds. The objective of the present experiment was to evaluate the effect of fermented pomegranate byproducts (FPB) on growth performance, fecal pH and noxious gas emissions; and economic efficacy in broilers. A feeding trial was carried out with 160 day old Ross broilers assigned to four dietary treatments having five replications (8 birds per replicate). The dietary treatments were control (T0), corn-soybean meal based basal diet; and test diets (T1 to T3), T1 = Basal diet + 0.5% FPB; T2 = Basal diet + 1.0% FPB; T3 = Basal diet + 2.0% FPB. The result revealed that, supplementation of FPB (T2 and T3) increased average daily weight gain of broilers during finisher and overall experimental period compared to control (T0) ($P < 0.05$). However, daily feed intake and feed conversion ratio remain unaffected ($P > 0.05$) among the dietary treatments. Fecal pH tended to reduce ($P < 0.05$) in response to FPB supplemented group T1 and T3 compared to T0. In addition, fecal ammonia emission was lower ($P < 0.05$) in all FPB supplemented groups (T1, T2 and T3), where hydrogen sulfide emission was reduced in T1 and T2 during incubation ($P < 0.05$). Overall economic analysis indicated that, feed cost per unit of weight gain was lower ($P < 0.05$) in the FPB supplemented T2 and T3 groups. Therefore, it could be concluded that FPB supplementation can significantly improve growth performance, reduce fecal pH and gas emission from excreta; and economical to utilize in broiler diet.

INTRODUCTION

Demand of processed food is increasing due to increasing population, dynamicity, lifestyle and consumption perception; where disposal of food wastage represents a growing problem since the material is usually prone to microbial spoilage. Costs of drying, storage and shipment of byproducts are also economically limiting factors and in some cases required legislation. In addition, climate change results competition over food between animal and human which consequently impact on the availability and cost of cereals. In that case, byproducts of agroindustry and food process industry utilization have received attention as alternative source in animal nutrition (Afsharhamidi and Razeghi, 2010).

Byproducts of plant food processing represent promising sources of compounds which might use because of their favorable technological or nutritional properties (Schieber et al., 2001). Pomegranate (*Punica granatum*) byproducts are such types of residue show interesting nutritional and health-promoting features due to presence of bioactive compounds (Viuda-Martos et al., 2010). Pomegranate is one of the oldest edible fruits and is widely grown in many tropical and subtropical countries such as United States, Iran and South East Asian countries (Stover and Mercue, 2007). In Korea, it is very popular because of purported health benefits and every year a large amount (13,596 metric ton in 2013) is imported from United States to fulfill the demands beyond the home production (Source: Global Trade Atlas, 2013). Pomegranate fruits are either consumed as fresh or used commercially in the juice, jam and wine industries (Gil et al., 2000). Edible parts of fruit comprise 78% juice and 22% byproducts; while byproducts constitute approximately 52% of the total weight of fruit (Kullkarni and Aradhya, 2005); which is prone to wastage and environmental pollution. But inedible part such as seed, husk, peel etc. has many bioactive components such as polyphenols, ellagitannins, vitamins, minerals and polyunsaturated fatty acids (Gil et al., 2000; Seeram et al., 2005). In particular, byproducts of pomegranate have antioxidant, anti-carcinogenic and antimicrobial properties (Seeram et al., 2005; Reddy et al., 2007). Besides the beneficial properties, there are some limitations on the use of pomegranate byproducts in animal nutrition. Relatively high level of tannin contents in pomegranate byproducts acts as antinutritional factor in animal nutrition (Serrano et al., 2009). However, antinutritional factors like tannins could be minimized through processing such as fermentation (Dei et al., 2008).

Fermentation is a unique biotechnological process with great potential for recycling some feed process industrial or agroindustrial byproducts into useful animal feeds in developing countries. The process does not require the use of chemicals and is easy to manage in on-farm conditions or on an industrial scale. It can reduce the anti-nutritional factors (Zhang et al., 2006), enrich the quality of protein (Oduguwa et al., 2007); and improve nutrient digestibility (Kim et al., 2007). Ahmed et al. (2014) reported improvement of growth performance and immunity in broilers fed citrus junos byproducts fermented with *Bacillus, Lactobacillus, Enterococcus* and *Saccharomyces* in the same laboratory. Furthermore, fermented feed nutrients can suppress the fecal ammonia concentration (Awati et al., 2005). While Cho et al. (2006) reported that plant secondary metabolites and probiotic bacteria can reduce the fecal noxious gas emission. Moreover, fermentation of feed with probiotic can modulate the pH through altering the of lactic acid concentration, which can modulate the intestinal microflora (Canibe et al., 2001, 2007). Several studies have reported that, probiotic produced by solid substrate fermentation using agroindustrial byproducts as growth substrates are both cost-effective and environmental-friendly (Shim et al., 2010).

Utilizing probiotics for feed fermentation, such as liquid fermenting feed or silage for ruminants and pigs with beneficial effects are in practice (Boguhn et al., 2006). However, limited information and research is available on the use of fermented feeds in case of chicken especially on broilers. The use of antibiotics in broilers has banned in many countries which triggered the use of potential alternatives like probiotics, prebiotics and medical plants or their combination. In addition, unconventional feed resource such as byproducts; and cost of feed for poultry production is major concern. To the best of our knowledge, fermentation of pomegranate byproducts with multispecies probiotics has not been considered before as an alternative biotechnology for broiler nutrition. We hypothesized that, the pomegranate byproducts with multispecies probiotic (*Bacillus subtilis, Lactobacillus acidophilus* and *Saccharomyces cerevisiae*) fermentation will complement each other's effect to show synergistic probiotic properties; reduce the feed cost and environmental pollution through biological conversion. Consequently, it might be an effective approach for improvement of growth performance; reduction of fecal noxious gas emission and feed cost of broilers. Therefore, present study was undertaken to investigate the beneficial effects of a fermented pomegranate byproduct with multispecies probiotics on the growth performance, fecal pH and fecal noxious gas emission; and economic analysis in broilers.

MATERIALS AND METHODS

The use and care of birds were carried out in accordance with the guidelines for the care and use of animals in research (Korean Ministry for Food Agriculture Forestry and fisheries, 2008). All experimental procedures in this study were approved by the Animal Care and Use Committee of Sunchon National University, Republic of Korea.

Preparation of fermented pomegranate byproduct (FPB)

A total of 9 strains, including *Lactobacillus acidophilus* (KCTC 3111, KCTC 3146, KCTC 3150), *Bacillus subtilis* (KCTC 1022, KCTC 1103, KCTC 3239) and *Saccharomyces cerevisiae* (KCTC 7107, KCTC 7915, KCTC 7928) were used as candidate probiotic strains. These microbes were purchased from the Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea. Among these *Lactobacillus acidophilus* KCTC 3111, *Bacillus subtilis* KCTC 3239 *and Saccharomyces cerevisiae* KCTC 7928 were selected as starter cultures based on their acid, bile and heat tolerance levels according to Hossain et al. (2012).

Strains selected according to their acid, bile, and heat tolerance levels were used for the preparation of FPB by solid substrate fermentation (SSF). The byproduct of pomegranate obtained from a juice manufacturing company (includes the peel, rind and seeds) was used as solid media for fermentation. At first, the pomegranate byproducts were dried in a force air dry oven (Doori TEC, Doori TEC, FA, Co., Ltd) at $80^{o}C$ for 2 days and ground using a milling machine to pass through a 100-mesh sieve followed by sterilization for 30 min at 85°C. A two-step fermentation method was accomplished using a commercial fermenter (W-1000; Wonbalhyo Industry Co., Icheon, South Korea). At first 0.5% *Lactobacillus acidophilus* KCTC 3111 were added to the solid substrate media and fermented at 40°C repeating 5 h of anaerobic and 3 h of aerobic conditions for 48 h. A second fermentation was performed with 0.5% *Saccharomyces cerevisiae* and 0.5% *Bacillus subtilis*, for 72 h at 40°C under aerobic condition. After completion the fermented products were dried to less than 15% moisture at 80°C for 48 h using a drying oven. To determine the number of cells, 1g of FPB was dilutedwith sterilized distilled water (10 ml) at room temperature. After approximately 10 min, 1 ml of the dilution was serially diluted 10-fold in NaCl (0.85%) solution and cultured in agar media. The culture plate was then incubated at 37°C for 24 to 48 h and the number of colonies counted. The FPB were then analyzed in triplicate for moisture content by oven-drying method (930.15), total ash content using a muffle furnace (942.05), crude protein content by the Kjeldahl method (990.03), crude fiber content by sequential extraction with diluted acid and alkali (978.10) and crude fat content by the ether extraction technique (991.36) as described by AOAC International, 2000 (Official Methods of Analysis. 17th ed. AOAC Int., Washington, DC). The chemical composition and concentration of microorganismsin FPB are shown in Table 1.

Experimental design, diets and bird's husbandry

A total of 160 day old Ross broiler chicks obtained from a commercial hatchery were weighed and randomly allocated into four dietary treatment groups with five replicate pens of 8 birds for a 5 weeks feeding trial based on completely randomized design. The average initial body weight (43.8 ± 0.17 g) did not differ among all groups. The dietary treatments consisted of control (T0), corn-soybean meal based basal diet; and test diets (T1 to T3), T1 = Basal diet + 0.5% FPB; T2 = Basal diet + 1.0% FPB; T3 = Basal diet + 2.0% FPB. Commercially available broiler diets were used as basal diet prepared with the same batch of ingredients for starter (0 to 3 weeks) and finisher (4 to 5 weeks) periods. The ingredients, chemical composition, vitamin and mineral content of the experimental basal diets are shown in Table 2.

Broilers were kept in a closed, ventilated, wire-floor caged broiler house (100 cm long × 80 cm wide × 40 cm high/cage). The cages had a linear feeder in the front and a nipple drinker in the back to provide feed and water *ad libitum* throughout the experiment. The diets were provided in mash form in two phases during the experiment, a starter phase from 0 to 3 weeks and a finisher phase from 4 to 5 weeks. Temperature was maintained at 33°C from day 0 to 7, after which it was gradually reduced to 24°C at a rate of 3°C per week and then maintained at this temperature until the end of the experiment. The relative humidity was maintained at around 50% and continuous lighting was provided throughout the experimental period.

Measurement of growth performance

Feed intake (FI) and body weight (BW) were recorded by replicate at 1st, 2nd, 3rd, 4th and 5th weeks of age. From these data, average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) were calculated per cage by period and for the entire experimental period. Mortality was recorded daily.

Collection of fecal sample and noxious gas measurements

After finishing the growth trial, fecal samples (mixtures of feces and urine) were collected. Where excreta sample was collected from the bottom tray of each wire-floor cage after proper homogenization and put in plastic zipper bags. About 500g of fecal sample from each replicate cage was placed in 2-liter plastic box in triplicate to measure the emission of ammonia (NH_3), hydrogen sulfide (H_2S), sulfur dioxide (SO_2) from broiler excreta. The boxes used for measuring the gas emission were equipped with a cover containing two holes. One hole was used to insert a tube with cap to facilitate gas measurement. The other hole was sealed with an Advantec® membrane filter (pore size 1.0µm, Toyo Roshi Kaisha Ltd., Otowa, Tokyo, Japan) to facilitate insertion of fresh air to fill up the negative pressure create during drawing of headspace air with pump. Following one sampling at 0 h, the samples were allowed to ferment at room temperature (average 27°C) and subsequent sampling was taken at 3 h, 6 h, 12 h, 24 h and 48 h. Gastec (model AP-20) gas sampling pump (Gastec Corp., Kitagawa, Japan) and Gastec detector tube (3 LA, 3M for NH_3; 4 LB, 4LK for H_2S; 5 LA for SO_2) was used to measure the gas. During measurement, the tube was open and 100 mL of headspace air was sampled approximately 2.0 cm above the sample surface. The concentration of lethal gases was expressed as ppm/100 mL.

Measurement of fecal pH

To measure fecal pH, 4 g of fecal sample was weighed and diluted in 36 mL of distilled water (DW) to a 1:9 (weight : volume) ratio and the pH was measured directly using a Uni pH testa (Trans Instruments (S) PTE Ltd, 5 Jalan Kilang Barat, Petro Centre, Singapore) at 25°C.

Economic analysis

For economic analysis, the total feed intake, weight gain and feed cost was measured and calculated. Finally, per kg feed cost for per unit of weight gain was measured for calculating the economy of supplementation of probiotic fermented pomegranate byproducts in broilers.

Statistical analyses

The experiment was carried out as a completely randomized design with four treatments. Data were subjected to ANOVA using the PROC GLM function of the Statistical Analysis System (SAS, 2003) [SAS User's Guide. Version 9.1, Cary, NC, SAS Institute Incorporation]. Pens were used as the experimental unit for growth performance parameters (ADG, ADFI and FCR), whereas an individual bird served as the experimental unit for gas measurements and pH measurements. Variability in the data was expressed as the standard error (SE) and a probability level of $P \leq 0.05$ was considered statistically significant.

RESULTS

Effect of FPB supplementation on growth performance of broilers

The effects of dietary fermented pomegranate byproducts (FPB) supplementation on growth performance of broilers at different phases were shown in Table 3. The overall result of the present study revealed that, dietary supplementation of FPB (T2 and T3) had significant effect ($P < 0.05$) on average daily gain (ADG) and there was no adverse effect on feed intake and feed efficiency in broilers. During starter phase dietary FPB supplementation had positive effect on ADG but there were no significant differences among the dietary treatments, while during finisher phase FPB supplementation had significant effect in T2 and T3 dietary groups compared to T0 ($P < 0.05$). During overall period, supplementation of FPB had significant effect on ADG among all FPB supplemented (T1, T2 and T3) groups compared to control (T0) ($P < 0.05$). Among the dietary treatments highest ADG value being observed at T3 supplemented group which was 5.5% higher compared to T0 ($P < 0.05$). In addition, average daily feed intake (ADFI) was not significantly changed ($P > 0.05$) during starter, finisher and overall phase of experimental period although during overall period there was found little bit higher numerical value in the T2 and T3 supplemented groups. Furthermore, feed conversion ratio (FCR) value was not significantly differed ($P > 0.05$) among the dietary treatments during starter, finisher and overall phase of experimental period. However, there was found better trend of FCR at T2 and T3 compared to T0 ($P > 0.05$).

Effect of FPB supplementation on fecal pH in broilers

The excreta pH was presented at figure 1. It was found tendency of reduction of the fecal pH in response to dietary supplementation of FPB both before and after fermentation. The fecal pH before fermentation was lower in all FPB treated groups but it was significant only in the T1 group compared to control (T0) ($P < 0.05$). While the fecal pH after fermentation was reduced in T1 and T3 but there were no significant ($P > 0.05$)differences among the dietary treatments.

Effect of FPB supplementation on fecal noxious gas emission from broilers

The fecal noxious gas (NH_3, H_2S and SO_2) emission from broilers excreta was presented in the figure 2, figure 3 and figure 4. The result of the present experiment on fecal gas emission revealed that FPB supplementation had significant impact ($P < 0.05$) on reduction of NH_3 and H_2S but there was no significant impact on reduction of SO_2 ($P > 0.05$).

The effects of FPB supplementation on the emission of noxious NH_3 from broiler excreta was found positive on the aspect of reduction in all FPB supplemented (T1, T2 and T3) groups (Figure2). Among the dietary treatments, T3 supplemented group showed lowest value of NH_3 gas emission during 0, 3, 6, 12, 24 and 48 hours of incubation followed by other dietary treatments but it was nonsignificant. During 24 hours of incubation NH_3 gas was significantly lower ($P < 0.05$) in all FPB supplemented (T1, T2 and T3) groups. It was found reduction of NH_3 gas on an average in all FPB groups but optimal reduction (37%) was found at T3 (190.25 ppm) compared to T0 (119.25ppm).

As shown in figure 3, supplementation of FPB in broiler diets had negative effects on emission of H_2S from the excreta. It was found significant reduction of H_2S in T1 group during 6, 12, 24 and 48 hours of incubation ($P < 0.05$), while it was also observed significant reduction of H_2S in T2 during 48 hours of incubation ($P < 0.05$). The optimum efficacy was observed in response to supplementation of FPB in T1 where on an average 86% reduced compared to T0 ($P < 0.05$).

FPB supplementation to broiler diet was found no significant ($P > 0.05$) impact on reduction of fecal noxious gas SO_2 emission (Figure 4). However, it was observed lower value of SO_2 in T1 during 12, 24 and 48 hours; and in T3 group during 0, 24 and 48 hours of incubation period.

Economic efficacy of FPB supplementation in broilers

The economic analysis of dietary supplementation of FPB for overall period of experiment was shown at figure 5. Economic analysis of the current study indicated that, overall it was significantly lower ($P < 0.05$) feed cost per kg weight gain in the FPB supplemented groups (T2 and T3) compared to control (T0).

Table 1. The number of microflora population and chemical composition of multispecies fermented pomegranate byproducts (cfu/g)

Microbial strain	Microbial concentration (cfu/g)
Lactobacillus acidophilus KCTC 3111	5.2×10^9
Bacillus subtilis KCTC 3239	2.5×10^8
Saccharomyces cerevisiae KCTC 7928	2.9×10^8
Chemical composition (% dry matter)	
Moisture	32.6
Crude protein	9.11
Ether extract	2.47
Crude fiber	23.11
Crude ash	5.45

Table 2. Ingredients and nutrient composition (% DM) of broiler starter and finisher diets

Ingredients	Starter	Finisher
Corn grain	42.91	45.64
Wheat bran	19.35	20.00
Soybean meal	21.80	18.68
Corn gluten meal	5.00	4.65
Whole soybean	5.00	5.00
Animal fats	2.63	3.00
Sodium chloride	0.38	0.38
Dicalcium phosphate	1.88	1.78
Limestone	0.70	0.65
Vit-min. mix[1]	0.30	0.22
Yeast agent	0.05	0.00
Chemical composition[2]		
ME (kcal/kg)	3200.00	3200.00
Crude protein (%)	20.02	23.02
Lysine (%)	0.72	0.77
Methionine (%)	0.24	0.18
Calcium (%)	0.99	1.24
Available P (%)	0.35	0.45
Na	0.17	0.21

[1]Vit-min.mix provided the following nutrients per kg of diet: Vitamin A 9,000,000 IU; Vitamin D3, 2,100,000 IU; Vitamin E, 15,000 IU; Vitamin K, 2,000 mg; Vitamin B1, 1,500 mg; Vitamin B2, 4,000 mg; Vitamin B6, 3,000 mg; Vitamin B12, 15 mg; Pan-acid Ca, 8,500 mg; Niacin, 20,000 mg; Biotin, 110 mg; Folic acid, 600 mg; Fe, 40,000 mg; Co, 300 mg; Cu, 3500 mg; Mn, 55,000 mg; Zn, 40,000 mg; I, 600 mg; Se, 130 mg.
[2] Calculated values.
DM, dry matter; ME, metabolizable energy

Table 3. Effects of fermented pomegranate byproducts (FPB) on growth performance of broilers

Parameters	Period	Dietary Treatments				SEM	P-value
		T0	T1	T2	T3		
IBW (g)		43.5	43.7	44.0	44.0	0.17	0.19
ADG (g/d)	Starter	44.7	45.0	46.2	46.6	1.32	0.25
	Finisher	64.7^{b}	65.1^{b}	69.2^{a}	69.1^{a}	0.97	0.02
	Overall	52.7^{c}	53.1^{b}	55.4^{a}	55.6^{a}	0.56	<0.01
ADFI (g/d)	Starter	73.6	73.5	73.5	73.0	1.32	0.81
	Finisher	104.3	104.6	107.9	119.6	2.03	0.30
	Overall	85.9	85.9	87.3	87.7	1.50	0.79
FCR (F:G)	Starter	1.65	1.62	1.59	1.57	0.04	0.30
	Finisher	1.61	1.61	1.56	1.59	0.04	0.73
	Overall	1.63	1.62	1.58	1.58	0.03	0.51

ADG = Average daily gain (g/d); ADFI = Average daily feed intake (g/d); FCR = Feed conversion ratio (feed:gain)
^{a,b,c} Means with different superscripts within the same row are significantly different at $P < 0.05$.
SEM: Standard error of mean.
The dietary treatments consisted of control (T0), corn-soybean meal based basal diet; and test diets (T1 to T3), T1 = Basal diet + 0.5% FPB; T2 = Basal diet + 1.0% FPB; T3 = Basal diet + 2.0% FPB.

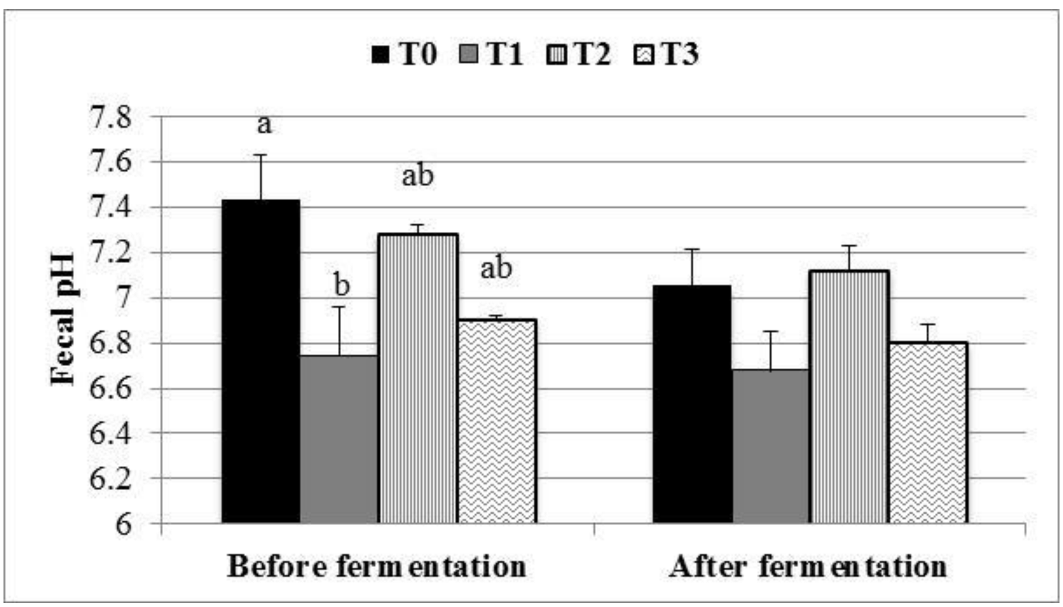

Figure 1. Effect of fermented pomegranate byproducts (FPB) on fecal pH
^{a,b} with different error bars and different superscript letters are significantly different at $P < 0.05$.
The dietary treatments consisted of control (T0), corn-soybean meal based basal diet; and test diets (T1 to T3), T1 = Basal diet + 0.5% FPB; T2 = Basal diet + 1.0% FPB; T3 = Basal diet + 2.0% FPB.

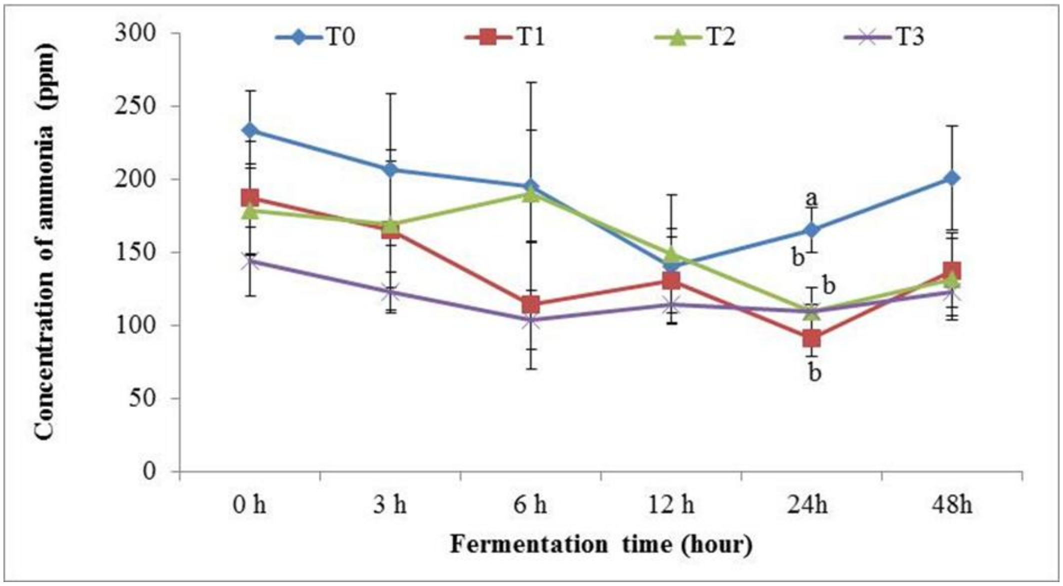

Figure 2. Effect of fermented pomegranate byproduct (FPB) on ammonia (NH_3) emission from broiler excreta
[a, b] with different error bars and different superscript letters are significantly different at P <0.05.
The dietary treatments consisted of control (T0), corn-soybean meal based basal diet; and test diets (T1 to T3), T1 = Basal diet + 0.5% FPB; T2 = Basal diet + 1.0% FPB; T3 = Basal diet + 2.0% FPB.

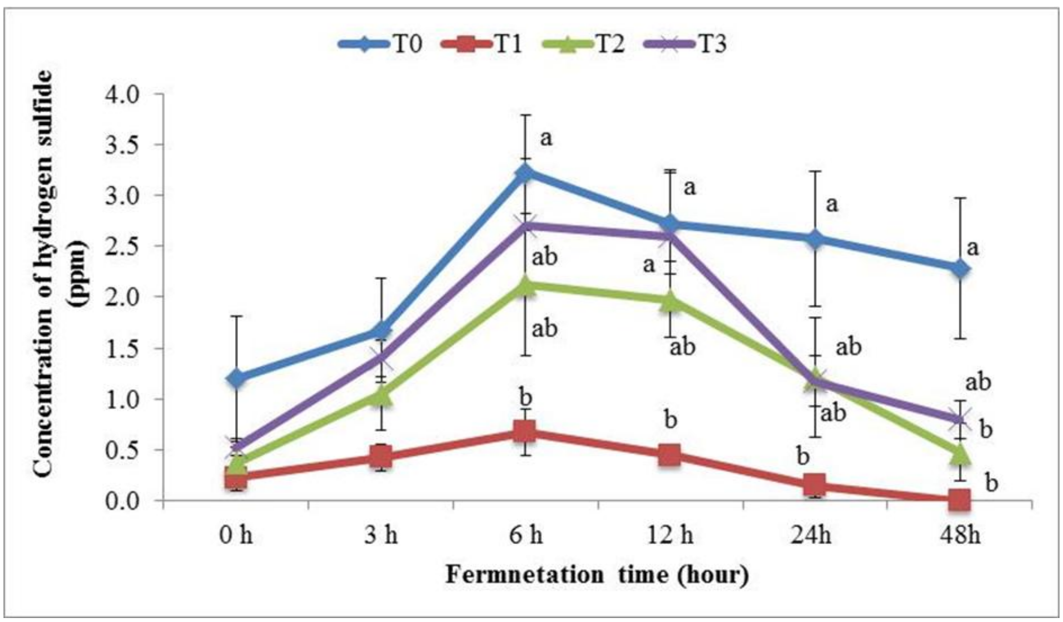

Figure 3. Effect of fermented pomegranate byproduct (FPB) on hydrogen sulfide (H_2S) emission from broiler excreta
[a, b] with different error bars and different superscript letters are significantly different at P <0.05.
The dietary treatments consisted of control (T0), corn-soybean meal based basal diet; and test diets (T1 to T3), T1 = Basal diet + 0.5% FPB; T2 = Basal diet + 1.0% FPB; T3 = Basal diet + 2.0% FPB.

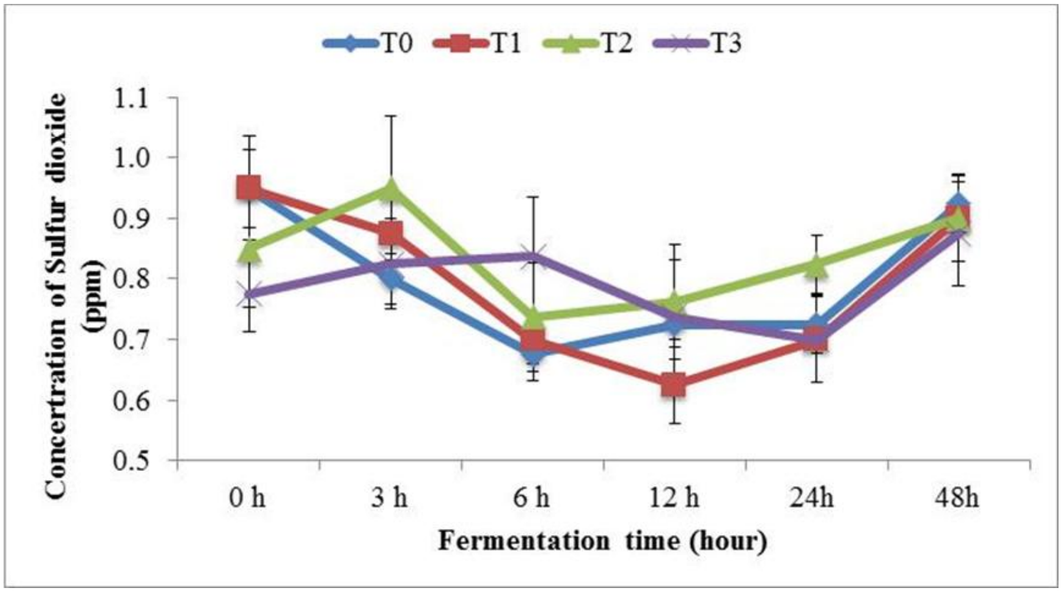

Figure 4. Effect of fermented pomegranate byproduct (FPB) on sulfur dioxide (SO_2) emission from broiler excreta
The dietary treatments consisted of control (T0), corn-soybean meal based basal diet; and test diets (T1 to T3), T1 = Basal diet + 0.5% FPB; T2 = Basal diet + 1.0% FPB; T3 = Basal diet + 2.0% FPB.

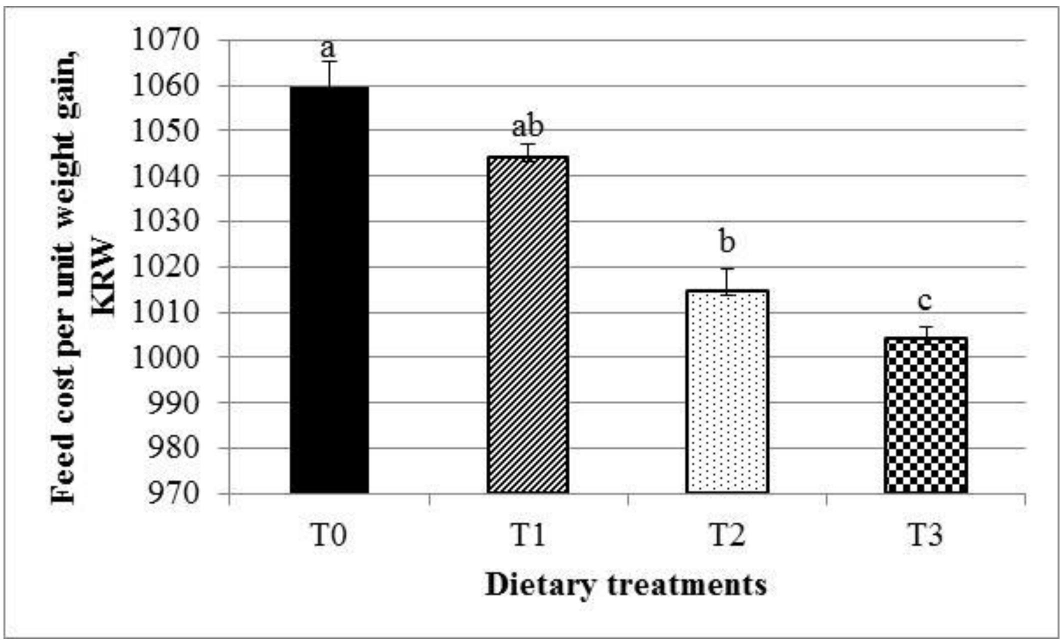

Figure 5. Effect of fermented pomegranate byproducts (FPB) on economic efficacy in broilers
[a, b, c] with different error bars and different superscript letters are significantly different at $P < 0.05$.
The dietary treatments consisted of control (T0), corn-soybean meal based basal diet; and test diets (T1 to T3), T1 = Basal diet + 0.5% FPB; T2 = Basal diet + 1.0% FPB; T3 = Basal diet + 2.0% FPB.

DISCUSSION

Growth performance of broilers

The exploitation of byproducts of fruit and vegetable processing industry as a source of functional compounds is a promising field. Many byproducts have potentiality as animal feedstuffs (Schieber et al., 2001) which indicate replacement of some feed ingredients could be possible. Consequently the competition between human and animal nutrition and environmental pollution problems could be minimized (Mirzaei-Aghsaghali and

Maheri-Sis, 2011). Pomegranate byproducts has interesting nutritional and health-promoting features (Viuda-Martos et al., 2010); mainly attributed to the level of polyphenolic compounds, especially ellagitannins (Gil et al., 2000). Seed of pomegranate is rich in sugars, vitamins, minerals, polysaccharides and polyphenols (Sestili et al., 2007); pomegranate husk is rich in ellagitannins such as punicalagin and its isomers, punicalin, gallagic acid and ellagic acid (Gil et al., 2000; Seeram et al., 2005); and pomegranate peel (fruit pericarp) is rich in tannins (Reddy et al., 2007). Pomegranate has as antioxidative and antimicrobial properties due to the presence of such type of phytochemicals (Gil et al., 2000; Reddy et al., 2007). Some investigations showed that, phenolic compounds particularly ellagic acid and punicalagin of pomegranate are responsible for antibiotic activity (Reddy et al., 2007; Hassan et al., 2012).

Nowadays, due to improvement of nutritional quality and digestibility of the fermented end product people are becoming interested on fermentation (Kim et al., 2007). In addition, fermentation of feedstuffs with desirable microbes breakdown the feed components by microbial enzymes and increase the nutritional and nutraceutical value of fermented feeds(Feng et al., 2007); which might improve the growth performance and influence the microbial modulation into the gastrointestinal tract. (Zhang et al., 2006) reported that, fermentation can reduce the anti-nutritional factors of feed staffs (e.g. trypsin content of soybean meal and gossypol content of cottonseed meal). Improvement of average daily gain (ADG) by supplementing fermented pomegranate byproducts (FPB) in broilers during finisher and overall period of experimental period in the present experiment (Table 3) was supported by Dei et al. (2008). They reported enhancement in growth performance of broiler supplemented with fermented diet with probiotic bacteria (*Bacillus subtilis* and *S. cerevisiae,* and *Aspergillus niger*). Improvement of growth performance, nutritive value and antimicrobial potential with agroindustrial byproducts after fermentation with probiotic strains like *Lactobacillus acidophilus*, *Enterococcus faecium, Bacillus subtilis*, *Saccharomyces cerevisiae* have been tested for green tea byproduct (Yosef et al., 2012), and citrus junos byproduct (Ahmed et al., 2014). It was opined from the present experiment that, pomegranate byproduct fermented with multispecies probiotic might improve its nutritional quality and utilization, as well as reduced the antinutritional contents likes tannin. Consequently it favors improvement of the body weight gain of broilers in the present study.

Consistent to the present study, Shabtay et al. (2008) demonstrated that pomegranate intake has no deleterious effect on feed intake (Table 3).Mathivanan et al. (2006) reported that fermented feed improve the feed conversion ratio (FCR) in broiler which support the tendency of improvement of FCR in the FPB supplemented groups (T2 and T3) in the present experiment (Table 3). Fermented feeds have been shown to be beneficial for improving feed efficiency (Canibe and Jensen, 2012). The tendency of improvement in feed intake and feed efficiency during finisher and overall period in the current study could be attributed to the palatability of the fermented feed with its aroma and acidification. Because, pomegranate contains polyphenolic compounds, which can affect the functional and nutritional values; and can contribute to the sensory and organoleptic properties (color, taste, astringency) of fermented diets (Serra and Ventura, 1997).

Fecal pH

In recent years, fermentation becomes useful means to stimulate processing of various products for producing biological materials with health enhancing efficacy (Dei et al., 2008: Chen et al., 2009). Pomegranate byproducts have plant secondary metabolites and inherent chemical composition (e.g., high content of polyphenolic compounds, tannins, low pH, and high fiber) (Reddy et al., 2007; Hassan et al., 2012). Polyphenols can decrease the pH of the intestinal environment and affect intestinal microbial population (Bialonska et al., 2009). The antimicrobial activity of phenolic extracts has been attributed to the generation of organic acids resulting in lowering the pH (Puupponen-Pimia et al., 2005; Bialonska et al., 2009). While high fiber content can increase the excretion of short chain fatty acids and reduces the pH of excreta (Mroz et al., 2000). In addition, microbial cultures have positive impact on balancing microbial population and nutritional characteristics of fermented feed via changing the pH (Canibe et al., 2001, 2007). Due to fermentation of feed lactic acid production is increased which can affect the pH and produce antimicrobials which are active against pathogenic microorganisms (Olsen et al., 1995). The pH change affect the microbial colonization might indicate the improvement of broiler's performance, which was concurred in the present study in case of FPB supplementation (Table 3).

Fecal noxious gas (NH_3, H_2S and SO_2) emission from broilers

Livestock and poultry are the source of emission of different types of gases which are mainly emitted from their excreta. Among the probable 136 gases emitted from animal house and its surroundings, the noxious ammonia and sulfur containing gases are most important based on environmental pollution and health impact on animals (Hartung and Phillips, 1994). In addition, it is well documented that ammonia nitrogen and hydrogen sulfide are the main components of animal excreta contributing to air pollution (Zahn et al., 1997). However, noxious gas emission related to a number of factors including nutrient utilization, intestinal and fecal microflora; and fecal pH etc. (Ferket et al., 2002; Wang et al., 2011).

Ammonia (NH_3), the major noxious gas from broiler house is generated either by hydrolysis of uric acid by microbial urease present in feces (McCrory and Hobbs, 2001; Wrong, 1981) or through deamination of protein or other nitrogenous substances (Vince et al., 1973). Fecal noxious gas content can be decreased with plant extracts supplementation due to presence of secondary metabolites. Results obtained by Cho et al. (2006) reported that the ammonia concentration in feces can significantly reduce in response to the addition of plant extracts. Polyphenolic compounds, especially ellagitannins of pomegranate has antimicrobial actions (Reddy et al., 2007; Gil et al., 2000). Pomegranate tannins can inhibitthe growth of pathogenic bacteria without adverse effects on beneficial bacteria (Bialonska et al., 2009). Tannins can create stable complexes mainly with proteins and then with enzymes change their structural conformation, thereby inhibiting enzymatic activity into the gastro intestinal tract (Goel etal., 2005; Bialonska et al., 2009). Therefore, tannins can affect the yields of protein extraction and reduce nitrogen excretion which might affect the ammonia emission (Youssef, 1998). In addition, probiotics are also able to suppress the emission of noxious gas from broilers. Ji and Kim (2002) noted that the production of NH_3 in chickens is reduced by 21% in response to the addition of a mixture of probiotic organisms (*Lactobacillus acidophilus*, *Bacillus* spp. and *Aspergillus oryzae*) to their diets. Ferket et al. (2002) suggested that fecal odor and NH_3 emission are related the intestinal microbiota ecosystem. In the herein study, reduction of NH_3 in the fecal material in response to FPB supplementation might be the reflection to balance microflora in the gastrointestinal tract (Wenk, 2003). Furthermore,Aarnink et al. (1998) and Mroz et al. (2000) reported that reducing the pH of excreta is the means of reducing the NH_3 emission. Therefore, the significant decline observed in the pH of broiler excreta obtained from dietary FPB supplemented groups (Figure 1) may also have led to the decrease in NH_3 emission.

Sulfur-containing compounds play an important role in noxious gas emission from chicken house (Mackie et al., 1998). Numerous laboratory and field studies have shown how ammonia and sulfur-containing gas affects birds' health and performance (Wang et al., 2011). While the production of H_2S and SO_2 is related with the decomposition of sulfur containing compounds by microorganisms (Smet and Langenhove, 1998; Kamoun, 2004). Animal diet as the main source of substrate for the metabolism of microbiota therefore any changes through fermentation has effects on the intestinal microbiota and gas production (Bedford and Apajalahti, 2001). The fiber concentration of FPB might be one of the reasons of reduction of hydrogen sulfide gas in the present study because fiber concentration could enhance substantial fermentation and enzymatic action into the gastrointestinal tract (Jensen and Jørgensen, 1994; Awati, 2005). Another reason might be pomegranate byproducts containing polyphenols, which can significantly affect intestinal bacterial population and formation of tannin complexes (Bialonska et al., 2009; Mirzaei-Aghsaghali et al., 2011). Tannin complexes decrease the cell wall permeability and reduce the transport of substrates into the cell, which alter the microbial population (Mirzaei-Aghsaghali et al., 2011). In addition, tannin decrease metal ion availability to bacteria which might result in reduction of pathogenic microbial activity and H_2S gas production (Goel et al., 2005; Bialonska et al., 2009). Furthermore, Chiang and Hsieh, (1995) reported that feeding probiotics containing *Lactobacillus, Streptococcus* and *Bacillus* reduced the concentration of noxious gas in the excreta of broilers. As microbes are responsible for gas production into the gastrointestinal tract (Jensen and Jørgensen., 1994); fermentation of pomegranate byproducts with probiotics might result combined effect on the reduction of fecal noxious gas concentration. Because it results changing in pH, enzyme activity and microbial population into the gastrointestinal tract.

Economic analysis

Byproducts are getting preference in the feed and fiber system because they are available for use as animal feeds at competitive prices relative to other commodities. Byproducts have important aspects regarding wastage and environmental pollution; while utilization by animals can minimize pollution and reduce the cost of animal production (Ferket et al., 2002; Iyayi and Aderolu, 2004). Fermentation is an important biotechnology for bioconversion; and to maintain and improve the nutritional and sensory features. Studies have demonstrated that fermentation not only alters the original bioactivities but also enhances the original treatment efficacy (Miyake et al., 2005; Viuda-Martos et al., 2010). Pomegranate has nutritional and health assiting attributes of antioxidative, antimicrobial and disease prevention actions (Reddy et al., 2007; Shabtay et al., 2008); which facilitate the improvement of growth performance. The probable reason of low feed cost for per unit of weight gain in the FPB supplemented group (T2 and T3) might be due to basal diet replacement by the fruit industry waste material (pomegranate byproducts); better utilization due to fermentation with multispecies probiotics; and improvement of overall growth performance of broilers through reduction of noxious gas emission from the excreta of broilers.

The results of the present study indicated that, supplementation of multispecies fermented pomegranate byproducts (FPB) improve the body weight gain of broilers, reduce fecal pH and noxious gas emission; and economical to use in broiler diet. In conclusion, multispecies fermented pomegranate byproducts with higher level expected to be beneficial for broiler production. Further detail studies required on mechanism, meat composition and preservation quality parameters.

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