Effect of Fermented Medicinal Plants (*Gynura Procumbens*, *Rehmannia Glutinosa*, *Scutellaria Baicalensis*) as Alternative Performance Enhancers in Broilers

Jin-Suk Jeong and In-Ho Kim

Department of Animal Resource and Science, Dankook University,
Cheonan, Choongnam, 330-714, Republic of Korea

In the present study, the effect of dietary supplementation of mixed fermented medicinal plants (FMP) on growth performance in broilers and whether FMP have potential to serve as antibiotic growth promoter (AGP) alternatives was investigated. A total of 765 1-d-old male broiler chicks (ROSS strain) were used in a 5 wk feeding experiment and randomly divided into 5 groups with 9 replicates of 17 birds each. The treatments were NC (negative control, basal diet), PC (positive control, basal diet with 5 ppm enramycin), FMP 0.05 (basal diet with FMP 0.05%), FMP 0.1 (basal diet with FMP 0.1%), and FMP 0.2 (basal diet with FMP 0.2%). Both body weight gain and feed conversion ratios were improved, as compared with NC or in response to FMP supplementation, respectively. Additionally, dry matter and nitrogen retention, metabolizable energy, and excreta noxious gas emission of ammonia, hydrogen sulfide, and total mercaptans were improved as compared with NC or in response to FMP supplementation, demonstrating a positive correlation. The amount of intestinal microflora was improved (increased *Lactobacillus* spp counts and reduced *E.coli* counts) as compared with NC, PC, and a dose response increase of FMP supplementation, in both the small and large intestines. Taken all together, our results suggest and support the viable possibility that FMPs supplementation can be used as an effective alternative to AGPs for improving the performance of broilers.

Key words: broiler, excreta noxious gas, fermented medicinal plants, growth performance, intestinal microflora

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Introduction

Plants in the context of animal production are thought of as the principal nutrient source, and sometimes as an unwellcome source of anti-nutritional factors (ANFs) that interfere with an animal's utilization of ingested nutrients, since many of the ANFs are either toxic, unpalatable or undigestible. Otherwise, mainstream attitudes are changing, and over the last 15 years there has been growing interest in the use of plants and their extracts as alternative performance enhancers for animals (Greathead, 2003; Diarra *et al.*, 2011). As a result of banning antibiotic supplementation to animal feed, additionally, natural alternatives, such as medicinal plants and herbs, have attracted widespread attention due to their wide range of potential beneficial effects and increasing industry demands for maximizing net returns and carcass quality (Jenkins and Atwal, 1995; Manesh *et al.*, 2012).

Gynura procumbens is a well-known tropical asian medicinal herbal plant, containing flavonoid sterol and steroid, as well as some proteins (Hew and Gam, 2010). *Rehmannia glutinosa* has been extensively used as a tonic for the blood in traditional Chinese medicine to protect to liver and show promise in relieving hepatitis (Zhang *et al.*, 2008). Lastly, the genus *Lamiaceae* comprises over 360 species, many of which are medically important. Some *Scutellaria baicalensis* is used to treat neurological disorders, cancer, inflammatory diseases and viral and bacterial infections (Shang *et al.*, 2010).

Patterson and Burkholder (2003) have proposed the use of probiotic micro-organisms as an alternative approach to sub-therapeutic antibiotics in livestock. Probiotics can beneficially affect the animal by improving the properties of stimulating micro-organisms capable of modifying the gastrointestinal environment to favor health status and improve feed efficiency (Dierick, 1989; Edens, 2003). There is a need to find and determine more efficient alternatives or combinations of different alternatives for maintaining health and improving performance of broilers. To the best of our knowledge, the effects of dietary supplementa-

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Correspondence: Prof. IH Kim, Department of Animal Resource and Science, Dankook University, Cheonan, Choongnam, 330-714, Republic of Korea. (E-mail: inhokim@dankook.ac.kr)

tion of mixed fermented medicinal plants (FMP) obtained from exudates of *Lactobacillus plantarum*-fermented *G. procumbens*, *Saccharomyces cerevisiae*-fermented *R. glutinosa*, and *Bacillus licheniformis*-fermented *S. baicalensis* as an alternative to antibiotic growth promoters (AGP) for broilers has yet to be investigated.

As such, the objective of this study was to investigate whether FMP could match or even surpass an AGP as a viable alternative. We determined the effects of dietary mixed FMP on the growth performance, nutrient retention, intestinal microflora, and excreta noxious gas emission of broilers as compared to basal diet and the AGP, enramycin, alone.

Materials and Methods

The experimental protocols used in this study were approved by the Animal Care and Use Committee of Dankook University (Anseodong, Cheonan, Choongnam, Korea).

Preparation of Fermented Medicinal Plants

The solid culture of the FMP product used in this study contained a mixture of lactic acid bacteria at 3.0×10^8 CFU/g (*Lactobacillus plantarum* species), yeasts at 7.5×10^7 CFU/g (*Saccharomyces cerevisiae* species), and fermenting bacteria at 8.0×10^8 CFU/g (*Bacillus licheniformis* species). The following three medicinal plants were purchased from a traditional Chinese drug market in Korea. The FMP was prepared as follows: the upper leaves of the *G. procumbens* plant were taken and thoroughly washed, and then cut to approximately 1×1 cm pieces. Processed leaves were mixed with *L. plantarum* NLR11201 inoculants which were fermented under liquid condition with a similar amount of soybean meal (1:1, wt/wt) in advance. The optimal condition for *L. plantarum* production was 40% moisture content for 2 d at 30°C, under solid-state fermentation (SSF). To make the fermented *R. glutinosa*, these roots were thoroughly washed and then cut to approximately 1×1 cm pieces. Processed roots were inoculated with *S. cerevisiae* 5% (vol/wt) and then fermented for 2 months at low temperature. The optimal conditions for *R. glutinosa* production were 40% moisture content for 5 d at 25°C under SSF. The *S. baicalensis* roots were thoroughly washed and then cut to approximately 1×1 cm pieces. Processed roots were inoculated with *B. licheniformis* DK42 5% (vol/wt) and then fermented for 2 mon at low temperature. The optimal conditions for *S. baicalensis* production were 40% moisture content for 5 d at 30°C under SSF. Fresh fermented samples were dried at 50°C for 3 d and then granulating, which equal amounts of all three FMPs were mixed together as feeding additive.

Experimental Design, Animals, Housing, and Diets

A total of 765 1-d-old male broiler chicks (ROSS strain; BW 36.77 ± 0.33 g) were used in an experimental feeding trial, lasting for up to 5 wk, and were allotted into 1 of 5 treatment groups in a completely random block design. Each dietary treatment consisted of 9 replicate cages, with 17 broilers per replicate, serving as a block. Dietary treatments

included: 1) negative control (NC, basal diet), 2) positive control (PC, NC with 5 ppm enramycin), 3) FMP 0.05 (NC with FMP 0.05%), 4) FMP 0.1 (NC with FMP 0.1%), and 5) FMP 0.2 (NC with FMP 0.2%). FMP was administrated by replacing the same amount of corn in the basal diet. The feeding program consisted of a starter diet until 3 wks and a finisher diet until 5 wks, with the ingredients and calculated nutrient composition of the basal diet shown in Table 1. Diets were formulated to meet requirements by the NRC (1994) for broilers, and supplied in mash form. Water was provided *ad libitum* throughout the experimental period. All the chicks were kept in a battery brooder with three levels of stainless steel cages (124 cm width $\times$ 64 cm length $\times$ 40 cm height), with 8 adjacent cages per level. Each cage was equipped with 2 drinker nipples and 2 open trough feeders. The temperature of the battery brooder was maintained at $33 \pm 1^\circ\text{C}$ during the 1st week and subsequently decreased by 3°C per week until reaching a final temperature of 24°C . Artificial light was provided 24 h/d by the use of fluorescent lights.

Sampling and Measurements

Body weight gain (BWG) and feed intake (FI) were recorded on d 0, 2 wks, 4 wks, and at the end of the 5 wk feeding trial, which was then used to calculate feed conversion ratio (FCR) after correcting for mortality. On d 35, 9 broilers were randomly selected from each treatment (1 bird per cage) and blood samples were collected from the brachial vein into a sterile syringe. After collection, blood samples were aliquoted into both a vacuum (clot activator with gel) and K_3EDTA vacuum tube separately (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) and stored at 4°C . Samples for serum analysis were then centrifuged at $3,000 \times g$ for 15 min, and then separated and stored at 4°C . White blood cells (WBC), red blood cells (RBC), and lymphocyte percentages were analyzed using an automatic blood analyser (ADVIA 120, Bayer, Leverkusen, Germany). After blood collection, the same broilers were individually weighed and euthanized by cervical dislocation and exsanguinated. The breast muscle (*pectoralis major*), bursa of fabricius, liver, spleen, gizzard, and abdominal fat were then removed by trained personnel and weighed. Organ weights were expressed as a relative percentage to the whole body weight. The pH of the breast muscle sample was measured by a calibrated, glass-electrode pH meter (WTW pH 340-A, WTH Measurement Systems Inc., Ft. Myers, FL, USA). The breast muscle Hunter lightness (L^*), redness (a^*), and yellowness (b^*) values were determined using a Minolta CR410 chromameter (Konica Minolta Sensing Inc., Osaka, Japan). The water-holding capacity (WHC) was measured in accordance with the methods described by Kauffman *et al.* (1986). Briefly, 0.3 g sample was pressed at $3,000 \times g$ for 3 min at 26°C on a 125 mm diameter piece of filter paper. The areas of the pressed sample and the expressed moisture were delineated and then determined using a digitizing area line sensor (MT-10S, M.T. Precision Co. Ltd., Tokyo, Japan). The ratio of water:meat area was then calculated, giving a measure of WHC (a smaller ratio in-

dicates increased WHC). Drip loss was measured using approximately 2 g of heated sample according to the plastic bag method described by Honikel (1998). All samples were ground to pass through a 1mm screen, after which they were determined for dry matter (DM, Method 930.15) and nitrogen (N, Method 984.13) retention, in accordance with the methods established by AOAC (1995). Broilers were fed diets mixed with chromic oxide (0.2%) as an indigestible marker at d 5 before excreta collection at wk 5. Excreta samples were collected for 3 d, and then dried at 60°C for 72 hr and finely ground to pass through a 1 mm screen. DM and N retention was calculated from chromic oxide concentrations in the diets and excreta. Chromic oxide content were analyzed with UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan), as described by Fenton and Fenton (1979). N was determined using a Kjeltec 2300 analyzer (Foss Tecator AB, Hoeganaes, Sweden). Gross energy (GE) was measured by the heat of combustion in diet and fecal samples, respectively using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline, IL, USA). Metabolizable energy (ME) was calculated as gross energy intake - gross energy content of feces, and energy metabolizability (%) was estimated to be $(ME/GE \times 100)$ (Göranzan *et al.*, 1983).

Small and large intestinal contents were collected into Qorpak glass containers (118 mL) under CO₂, sealed, and placed on ice until transported to the laboratory for enumeration of microbial populations. These samples were assessed for populations of *Lactobacilli* and *E. coli*. One gram of composite excreta sample from each cage was diluted with 9 mL of 1% peptone broth (Becton Dickinson Vacutainer Systems) and then homogenized. Viable counts of bacteria in the fecal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto lactobacilli medium III agar plates (Medium 638; DSMZ, Braunschweig, Germany) and MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) supplemented with glacial acetic acid (1 mL/L) and mupirocin (100 mg/L) extracted from antimicrobial discs (Oxoid; Nepean, Ontario, Canada) to isolate the *Lactobacilli* and *E. coli*, respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 37°C under anaerobic conditions, whereas MacConkey agar plates were incubated for 24 h at 37°C. The microflora colonies were counted immediately after removal from the incubator, and concentration of microflora was finally expressed as log₁₀ colony-forming units per gram of intestinal content.

Fresh excreta samples were collected from each cage on the last 2 d of the experiment and then mixed well for each respective cage. Analysis was then conducted using excreta samples (300 g) obtained from each cage that were stored in 2.6 L sealed plastic boxes. At 72 h post collection, the excreta samples were shaken manually for approximately 30 s in order to disrupt any crust formation on the surface of the sample and to homogenize the samples prior to measurement. The adhesive plaster was then punctured and 100 mL of headspace air was sampled approximately 2 cm above the

surface. The boxes used for measuring the gas emission had a small hole in the middle of one side wall that ferment for a period of 24 h at room temperature (25°C), after which the gas was sampled using a Gastec (model GV-100) gas sampling pump (Gastec Corp., Japan; Gastec detector tube No. 3M and 3La for ammonia; No. 4LL and 4LK for hydrogen sulfide; No. 70 and 70L for mercaptan).

Statistical Analysis

All experimental data were analyzed as a randomized complete block design using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). Before conducting statistical analysis of the microbial counts, we performed a logarithmic conversion of the data. The cage was used as the experimental unit. Orthogonal contrasts were used to test the overall effect of growth promoting materials supplementation (NC vs. FMPs) and the effect of difference between of PC and FMPs (PC vs. FMPs), additionally, orthogonal comparison were conducted using polynomial regression to measure the linear and quadratic effects of increasing con-

Table 1. Ingredient and composition of the basal diet (as-fed basis)¹

Ingredient (%)	Starter	Grower/Finisher
Corn	55.42	62.98
Soybean meal (CP 48%)	28.25	24.61
Corn gluten meal (CP 60%)	6.50	3.50
Soybean oil	5.50	4.89
Dicalcium phosphate	2.46	2.29
Limestone	0.89	0.75
Salt	0.20	0.20
DL-Methionine (98%)	0.17	0.17
L-Lysine-HCl (78%)	0.21	0.21
Vitamin premix ²	0.20	0.20
Trace mineral premix ³	0.20	0.20
Total	100.00	100.00
Chemical composition		
ME, kcal/kg	3,140	3,200
CP, %	22.00	20.09
Lysine, %	1.10	1.05
Met, %	0.54	0.41
Met + Cys, %	0.93	0.93
Ca, %	1.00	0.87
Total P, %	0.80	0.75
Crude Fat, %	4.32	5.87
Crude Fiber, %	4.71	6.21

¹ The experimental diets were formulated by replacing soybean meal with 0 (negative control), 0.05%, 0.1%, and 0.2% fermented medicinal plants extract.

² Provided per kg of complete diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D₃, 37.5 mg of vitamin E, 2.25 mg of vitamin K₃, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B₆, 24 µg of vitamin B₁₂, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin and 13.5 mg of Ca-Pantothenate.

³ Provided per kg of complete diet: 37.5 mg Zn (as ZnSO₄); 37.5 mg Mn (as MnO₂); 37.5 mg Fe (as FeSO₄·7H₂O); 3.75 mg Cu (as CuSO₄·5H₂O); 0.83 mg I (as KI); and 0.23 mg Se (as Na₂SeO₃·5H₂O).

Table 2. Effect of fermented medicinal plants (FMP) supplementation on growth performance in broilers¹

Treatment ³							P-value			
	NC	PC	FMP 0.05	FMP 0.1	FMP 0.2	SEM ⁴	NC vs. FMPs	PC vs. FMPs	Linear	Quadric
Starter (0–2 wk)										
BWG (g)	413	412	419	420	419	13.91	0.3045	0.3754	0.3385	0.9188
FI (g)	561	569	566	565	569	14.19	0.3047	0.7306	0.2268	0.5880
FCR	1.359	1.375	1.353	1.347	1.359	0.049	0.7475	0.2747	0.9271	0.8352
Grower (3–4 wk)										
BWG (g)	876	869	897	909	940	74.58	0.2171	0.1734	0.0869	0.9007
FI (g)	1697	1708	1693	1693	1701	19.02	0.9101	0.1866	0.6085	0.9795
FCR	1.945	1.972	1.903	1.872	1.817	0.147	0.1991	0.1132	0.0763	0.9864
Finisher (4–5 wk)										
BWG (g)	397	393	362	392	416	74.58	0.8479	0.9410	0.4843	0.5695
FI (g)	480	484	468	471	486	24.64	0.6363	0.3614	0.6383	0.9700
FCR	1.285	1.528	1.364	1.253	1.184	0.607	0.9431	0.3015	0.4424	0.6649
Overall										
BWG (g)	1681	1733	1663	1730	1779	56.63	0.0849	0.7216	0.0016	0.3114
FI (g)	2732	2754	2720	2731	2757	38.08	0.7871	0.3069	0.1481	0.9202
FCR	1.628	1.590	1.636	1.580	1.552	0.057	0.1141	0.9860	0.0087	0.3585

¹ Based on 765 birds with initial body weight of 36.8 ± 0.33 . Each mean represented by 9 replications (17 birds/replication).

² BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

³ NC, negative control (basal diet); PC, positive control (basal diet + 5 ppm enramycin); FMP 0.05, basal diet with 0.05% FMP extract; FMP 0.1, basal diet with 0.1% FMP extract; FMP 0.2, basal diet with 0.2% FMP extract.

⁴ SEM: standard error of means.

Table 3. Effect of fermented medicinal plants (FMP) supplementation on nutrient retention and energy metabolizability in broilers¹

Treatment ²							P-value			
	NC	PC	FMP 0.05	FMP 0.1	FMP 0.2	SEM ³	NC vs. FMPs	PC vs. FMPs	Linear	Quadric
DM, %	73.26	74.62	74.83	74.66	76.30	1.483	0.0097	0.3717	0.0100	0.2546
N, %	62.71	63.01	63.71	65.06	65.32	1.909	0.0390	0.0764	0.0015	0.7567
Energy Metabolizability (ME/GE), %	72.21	73.35	73.73	74.33	73.01	1.647	0.0714	0.6689	0.3512	0.7374

¹ Each mean represented by 9 replications.

² NC, negative control (basal diet); PC, positive control (basal diet + 5 ppm enramycin); FMP 0.05, basal diet with 0.05% FMP extract; FMP 0.1, basal diet with 0.1% FMP extract; FMP 0.2, basal diet with 0.2% FMP extract.

³ SEM: standard error of means.

⁴ DM, dry matter; N, nitrogen; ME, metabolizable energy; GE, gross energy.

centrations of FMPs. Variability in the data was expressed as the pooled MSE, and $P < 0.05$ was considered statistically significant, whereas $P < 0.10$ was considered a tendency.

Results

Growth Performance

BWG improved in the overall period ($P = 0.0849$) as compared with NC. In addition, increased FMP supplementation demonstrated a linear improvement in the grower (linear, $P = 0.0869$) and overall (linear, $P = 0.0016$) periods, respectively (Table 2). However, there was no effect in the starter and finisher periods ($P > 0.10$). Furthermore, no significant effects of FMPs supplementation were observed

on FI as compared with NC and PC treatments. However, FCR tended to decrease in the grower period (linear, $P = 0.0763$) and significantly decreased during the overall period (linear, $P = 0.0087$) in accordance with increased FMPs supplementation.

With regards to nutrient retention, FMP supplementation significantly improved DM ($P = 0.0097$ and linear, $P = 0.0100$) and N ($P = 0.0390$ and linear, $P = 0.0015$) retention, as compared with NC (Table 3). Furthermore, FMP supplementation also appears to improve ME ($P = 0.0714$) as compared with NC.

Meat Quality and Relative Organ Weight

Broilers fed the FMP supplemental diet did not signifi-

Table 4. Effect of fermented medicinal plants (FMP) supplementation on meat quality and visceral organ weight in broilers¹

Items ²	Treatment ³						P-value			
	NC	PC	FMP 0.05	FMP 0.1	FMP 0.2	SEM ⁴	NC vs. FMPs	PC vs. FMPs	Linear	Quadric
pH value	5.44	5.43	5.48	5.52	5.52	0.155	0.3972	0.3285	0.5064	0.8992
Breast muscle color										
L*	54.87	55.64	56.24	55.15	56.20	2.823	0.4623	0.8721	0.6018	0.4113
a*	13.75	14.32	14.17	13.79	13.15	2.085	0.9655	0.5398	0.5239	0.8727
b*	14.94	15.31	14.81	15.18	15.86	1.761	0.6853	0.9776	0.2799	0.9466
WHC, %	62.05	63.05	63.46	61.54	63.59	3.351	0.4572	0.9050	0.8162	0.1268
Drip loss, %										
1d	2.43	2.34	2.63	2.63	2.66	0.503	0.3748	0.2135	0.4445	0.8012
3d	5.28	5.02	5.27	5.05	5.30	0.646	0.8232	0.5434	0.9066	0.5668
5d	8.22	8.01	7.92	8.23	8.33	0.944	0.8861	0.7425	0.7428	0.6651
7d	10.84	10.39	10.32	10.76	10.48	0.854	0.4386	0.7521	0.6737	0.2681
Visceral organ weight, g/kg live weight										
Liver	19.91	20.99	20.07	21.09	20.99	0.355	0.4283	0.7859	0.2498	0.5877
Spleen	1.18	1.07	1.12	1.02	1.05	0.031	0.1350	0.8951	0.1124	0.5665
Bursa of fabricius	1.18	1.32	1.21	1.22	1.19	0.050	0.8575	0.4069	0.9873	0.9305
Breast muscle	91.79	89.53	92.03	91.18	92.60	0.939	0.9523	0.2289	0.9433	0.7951
Abdominal fat	12.07	11.65	12.40	12.72	12.56	0.285	0.5019	0.2061	0.5650	0.8399
Gizzard	12.03	11.95	11.83	12.18	13.08	0.194	0.5318	0.4359	0.0383	0.9723

¹ Each mean represented by 9 replications.² L*, lightness; a*, redness, b*, yellowness, WHC, water holding capacity.³ NC, negative control (basal diet); PC, positive control (basal diet + 5 ppm enramycin); FMP 0.05, basal diet with 0.05% FMP extract; FMP 0.1, basal diet with 0.1% FMP extract; FMP 0.2, basal diet with 0.2% FMP extract.⁴ SEM: standard error of means.**Table 5. Effect of fermented medicinal plants (FMP) supplementation on blood profiles in broilers¹**

Items ²	Treatment ³						P-value			
	NC	PC	FMP 0.05	FMP 0.1	FMP 0.2	SEM ⁴	NC vs. FMPs	PC vs. FMPs	Linear	Quadric
WBC, 10 ³ /μl	30.2	30.8	30.9	30.8	30.7	11.43	0.3265	0.9473	0.5995	0.7402
RBC, 10 ⁶ /μl	1.95	2.04	2.03	2.06	2.03	0.144	0.1924	0.9807	0.2565	0.9945
LYM, %	81.7	79.2	84.5	78.7	79.2	4.98	0.7090	0.5004	0.1971	0.1489

¹ Each mean represented by 9 replications.² WBC, white blood cell; RBC, red blood cell; LYM, lymphocyte.³ NC, negative control (basal diet); PC, positive control (basal diet + 5 ppm enramycin); FMP 0.05, basal diet with 0.05% FMP extract; FMP 0.1, basal diet with 0.1% FMP extract; FMP 0.2, basal diet with 0.2% FMP extract.⁴ SEM: standard error of means.

cantly differ in breast muscle color (L*, a*, and b*) or pH value as compared with those fed NC and PC treatments ($P > 0.10$) (Table 4). Additionally, no significant differences were observed in WHC, drip loss, and visceral organ weights between treatments, except for the gizzard, which demonstrated a linear improvement in weight gain (linear, $P = 0.0383$) with increasing FMP amount supplementation.

Blood Profiles

FMPs supplementation did not affect RBC, WBC, and lymphocyte percentages in the current study ($P > 0.10$) (Table 5).

Intestinal Microflora and Excreta Noxious Gas Emission

FMP supplementation increased *Lactobacilli* counts in the small ($P = 0.0243$, $P = 0.0296$, and linear, $P = 0.0014$) and large intestines ($P = 0.0030$, $P = 0.0032$, and linear, $P = 0.0034$) as compared with NC or PC and in accordance with increasing amount of FMP, respectively (Table 6). Furthermore, *E. coli* counts were reduced in the small ($P = 0.0151$) and large intestines ($P < 0.0001$) as compared with NC. However, no differences were observed in *E. coli* counts when compared with PC (small intestine, $P = 0.2821$; large intestine, $P = 0.2995$). Nonetheless, FMP supplementation was significant in both small ($P = 0.0350$) and large

Table 6. Effect of fermented medicinal plants (FMP) supplementation on small and large intestinal microflora in broilers¹

Items	Treatment ²						P-value			
	NC	PC	FMP 0.05	FMP 0.1	FMP 0.2	SEM ³	NC vs. FMPs	PC vs. FMPs	Linear	Quadric
Small intestine (log ₁₀ CFU/g)										
<i>Lactobacillus</i>	7.56	7.57	7.59	7.67	7.73	0.089	0.0243	0.0296	0.0014	0.7009
<i>E. coli</i>	6.45	6.33	6.37	6.39	6.34	0.069	0.0151	0.2821	0.0350	0.2460
Large intestine (log ₁₀ CFU/g)										
<i>Lactobacillus</i>	8.01	8.01	8.04	8.05	8.08	0.029	0.0030	0.0032	0.0034	0.3589
<i>E. coli</i>	7.02	6.90	6.91	6.85	6.83	0.065	<.0001	0.2995	<.0001	0.9908

¹ Each mean represented by 9 replications.² NC, negative control (basal diet); PC, positive control (basal diet + 5 ppm enramycin); FMP 0.05, basal diet with 0.05% FMP extract; FMP 0.1, basal diet with 0.1% FMP extract; FMP 0.2, basal diet with 0.2% FMP extract.³ SEM: standard error of means.**Table 7. Effect of fermented medicinal plants (FMP) supplementation on fecal noxious gas emission in broilers¹**

Items, ppm	Treatment ²						P-value			
	NC	PC	FMP 0.05	FMP 0.1	FMP 0.2	MSE ³	NC vs. FMPs	PC vs. FMPs	Linear	Quadric
Ammonia	83.1	79.9	79.6	75.3	74.8	5.46	0.0199	0.2148	0.0059	0.6276
Hydrogen sulfide	33.3	33.0	32.6	31.9	30.5	2.09	0.1188	0.1892	0.0313	0.9017
Total mercaptans	12.4	12.1	12.2	11.9	11.2	0.92	0.1747	0.4370	0.0467	0.9858

¹ Each mean represented by 9 replications.² NC, negative control (basal diet); PC, positive control (basal diet + 5 ppm enramycin); FMP 0.05, basal diet with 0.05% FMP extract; FMP 0.1, basal diet with 0.1% FMP extract; FMP 0.2, basal diet with 0.2% FMP extract.³ SEM: standard error of means.

intestines ($P = < 0.0001$) in accordance with increasing amount of FMP.

The effect of FMP supplementation on the emission of ammonia, hydrogen sulfide, and total mercaptans is shown in Table 7. FMPs supplementation significantly reduced ammonia emission ($P = 0.0199$) as compared with NC. Additionally, ammonia (linear, $P = 0.0059$), hydrogen sulfide (linear, $P = 0.0313$), and total mercaptans (linear, $P = 0.0467$) was significantly reduced in accordance with increasing FMP supplementation.

Discussion

In the current study, significant improvements to BWG and FCR were observed during the overall period as a result of dietary supplementation with FMP. Unfortunately, there are currently no available studies, focusing on the influence of feeding diets supplemented with mixed FMP to broilers to compare against. However, there are other studies reporting on the use of other fermented plant extracts, similar to the FMP used in this study, as food supplementation in broilers. Lokaewmanee *et al.* (2012) reported improved BWG and FCR in broilers with an increase in fermented fruit and vegetable products consumption as compared to NC treatment at 49 d of age. In addition, Kassie (2009) observed improvement in BWG and FCR, after feeding chicks with

100 ppm essential oils, derived from thyme and cinnamon, as compared to NC treatment. Meanwhile, Ao *et al.* (2011a, 2011b) reported no significant effect on BWG and FCR throughout the overall period, in broilers fed with up to 4 g/kg fermented garlic powder and red ginseng extract as compared with NC treatment. Khonyoung *et al.* (2012) also concluded that 1% dried fermented ginger treatment did not affect BWG and FCR, throughout the overall period, as compared with NC treatment. These contradictory results observed, amongst many studies, may be due to differences in the quality, as well as the quantity of active chemicals derived from plant extracts, which will determine and influence broiler response.

Our results showed that FMP derived from *G. procumbens*, *R. glutinosa*, and *S. baicalensis* in broiler diets significantly improved BWG and FCR, which may be due to bioactive chemicals (peroxidase, osmotin-like protein, thaumatin-like protein (Hew and Gam, 2010); catalpol, mannitol, mannitol, stachyose (Zhang *et al.*, 2008); baicalin, wogonin, wogonoside (Shang *et al.*, 2010), found in medicinal plants. Furthermore, the fermentation process used in the current study improved 1) the extraction quantity of active materials derived from medicinal plants from 40–50% to 90%, due to leaves processing rendering smaller particles for microbial digestion (Kim *et al.*, 2012), and 2)

nutrient utilization and retention resulting from favourable manipulations to the digestive and absorptive processes of the intestine in poultry (Yazdankish *et al.*, 2010). Consequently, these effects originate from the interaction of complex active chemicals derived from FMP. However, the exact growth promoting mechanisms of medicinal plants in broilers is still poorly understood. Perhaps, in future studies which will examine individual medicinal plants, we might be able to elucidate the specific contribution and effect of each plant component of the mixed FMP used in our current study.

Supplementation with FMP lead to increased *Lactobacilli* and reduced *E. coli* counts as compared with NC treatment, and increased *Lactobacilli* counts as compared to PC treatment. These results corroborate with results from Jamroz (2005) who reported that broilers fed a diet supplemented with plant extracts consisting of capsaicin, cinnamaldehyde, and carvacrol demonstrated reduced *E. coli* and increased *Lactobacilli* counts as compared to control diet. Annuk (2003) and Tucker (2002) have reported that medicinal plant extracts may be able to affect the cell surface hydrophobicity of microbes, which in turn may affect their adhesion/binding capacity to host tissue, which may have resulted in the observed reduction in *E. coli* counts. Alterations to the bacterial cell surface may be mediated via two possible mechanisms: 1) medicinal plant extracts may modulate the permeability of the cytoplasmic membrane, resulting in loss of chemiosmotic control of the affected cell, leading to cell death (Cox *et al.*, 2000), and 2) monoterpenes, derived from plant extracts, can cross the lipid bilayer, penetrating the cell and interacting at specific sites, exerting their antimicrobial activity intracellularly (Brenes and Roura, 2010). Based on the results of the present study, it may be considered that the peculiar bioactive chemicals derived from medicinal plant and microflora grown under a fermented plants matrix may modulate synergistically by suppressing intestinal harmful microflora while concomitantly favoring beneficial microflora, thereby ultimately enhancing overall gut health (Fuller, 1989; Kim *et al.*, 2007).

FMP supplementation leads to increased and improved DM and N retention, as compared with NC treatment, in addition to increased DM retention as compared to PC treatment. Our study indicates that FMP supplementation can be more effective than PC (enramycin) treatment at modulating the gastrointestinal environment and subsequent nutrient utilization in broilers. Hernandez *et al.* (2004) demonstrated that supplementation of plant extracts consisting of carvacrol, cinnamaldehyde, and capsaicin improved apparent fecal digestibility of DM and CP of the broiler diet. Garcia *et al.* (2007) also observed that finisher broiler diets supplemented with a mixture of sage, thyme, and rosemary or with a commercial product containing capsaicin, cinnamaldehyde, and carvacrol resulted in similar ileal DM and N digestibilities as the diet supplemented with antibiotic, and was significantly higher overall than the control diet. It may be considered that plant extracts may stimulate the production of saliva, and favor pancreatic and intestinal origin enzyme secretion, improving nutrient digestibility (Platel and

Srinivasan, 2004). In addition, the plant matrix may contain different molecules that have intrinsic bioactivities on animal physiology and metabolism, similar to prebiotics (Dorman and Deans, 2000).

The shift of excreta fecal microbial composition by FMP 0.2% supplementation was followed by a reduction in excreta ammonia, hydrogen sulfide, and mercaptans gas emission as compared with NC treatment and a reduction in excreta hydrogen sulfide gas emission as compared with PC treatment. Excreta noxious gas emission has been suggested to be related to nutrient utilization and the intestinal microbial ecosystem (Ferket *et al.*, 2002). As such, alterations to the intestinal microbial population may have relevant and important implications on reducing excreta noxious gas emissions. Our results indicate that FMP supplementation in broilers reduced excreta ammonia and hydrogen sulfide gas emission, which is consistent with the findings of Sung (2013) and Ra *et al.* (2004) who reported that fermented phytonics, prepared by fermented functional herbs with probiotics, have the ability for reducing excreta noxious gas emission. Additionally, Chiand and Hsieh (1995) and Zhang and Kim (2013) reported that probiotics can improve dietary protein digestibility, thereby indirectly reducing excreta N content.

In conclusion, the results of our study indicate that FMPs can be used as a possible viable alternative to AGP in feeding broilers, while obtaining most of the same benefits without any of the negative associated with an AGP. In addition, the source of FMP, which included 3 different microorganism (*L. plantarum*, *S. cerevisiae*, and *B. licheniformis*) and 3 different types of medicinal plants (*G. procumbens*, *R. glutinosa*, and *S. baicalensis*), can be a major factor in explaining any variation observed when producing batches of FMPs. In further studies, we plan to test and compare individual FMPs and combinations thereof, used in this study, to determine the effect of each type of plant alone.

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