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Egg production, egg quality, and cecal microbial populations of layers fed diets supplemented with fermented phytogenic feed additive

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Abstract: This study was undertaken to examine the effect of fermented phytogenic feed additive (FPFA) containing *Gynura procumbens* and *Rehmannia glutinosa* on egg production, egg quality, and cecal microflora population in layers. In total, 288 Hy-Line brown layers were randomly divided into 4 dietary treatment groups with 6 replicate pens per treatment group for 5 weeks. The dietary treatments were the control group (CON; basal diet without FPFA), FPFA1 (CON + 0.5% FPFA), FPFA2 (CON + 1% FPFA), and FPFA3 (CON + 2% FPFA). Egg production and egg weight during the experiment (35–39 weeks) improved linearly with the supplementation of 1% and 2% FPFA. Eggshell strength, albumen height, and Haugh unit were significantly higher in birds fed 1% or 2% FPFA compared with the control group. The cecal microflora populations of *Lactobacillus* showed a linear increase, whereas cecal microflora populations of *E. coli* showed a linear decrease related to the level of FPFA addition in the diet. The results of the experiment indicate that adding 1% and 2% FPFA (but not 0.5% FPFA) to the diet of layers can improve their laying performance, egg weight, eggshell strength, albumen height, Haugh unit, and cecal microflora; therefore, diets containing FPFA may enhance the physiologic condition of layers during the laying period.

Key words: Egg production, egg quality, cecal microflora, fermented phytogenic feed additive, layer

1. Introduction

Antibiotic growth promoters have been under thorough management for many years because of concerns and worries about antibiotic resistance and residues, and they have been removed from the livestock sector in many countries. The use of antibiotics in animal feed as a growth promoter has been prohibited in EU countries since 2006, and a complete ban on the use of antibiotics in animal feed has been enforced to ensure the safety of livestock products for consumers in Korea since 2011. Thus, there is a need to find safe and effective alternatives to antibiotics as prophylactic antibacterial agents and growth promoters.

In poultry, plant-derived feed additives have various physiological effects including antioxidative effects, promotion of digestion and appetite, and improvement of gut function and growth (1,2). *Gynura procumbens* is an herb of the family Compositae and is widely found in Africa and Southeast Asia; it is used traditionally in folk medicine as a remedy for eruptive fevers, rash, kidney disease, migraine, hypertension, and diabetes mellitus (3). *Rehmannia glutinosa* is a perennial plant that grows in Korea, China, Japan, and Vietnam (4). *R. glutinosa* is used for hemostasis, contusion, inflammation, and alleviating fever (5). *R. glutinosa* has been also shown to nourish the blood, has tonic and cardiotonic activities, positively affects diabetes and hypotension, and increases body fluids (6).

Although some evidence for *G. procumbens* and *R. glutinosa* as dietary supplements has been reported in humans and mice, limited data are available, in particular with regard to these fermented products in layers. Thus, the objective of this study was to assess the effects of the dietary addition of a fermented plant mixture with *G. procumbens* and *R. glutinosa* on laying performance, the cecal microbial population of layers, and their influences on egg quality properties in layers.

2. Materials and methods

2.1. Preparation of fermented plants

G. procumbens and *R. glutinosa*, which are commonly grown in Korea, were purchased in a local market in Korea. The leaf (*G. procumbens*) or root (*R. glutinosa*) was separated, washed, dried at room temperature, and then cut into small pieces (1 × 1 cm). Fermented feed

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was prepared with *G. procumbens* leaves and *R. glutinosa* roots (50/50 w/w) supplemented with *Lactobacillus planetarium* (NLRI1201; 10^4 cfu/g), *Bacillus licheniformis* (DK42; 10^4 cfu/g), and *Saccharomyces cerevisiae* (10^4 cfu/g); it was then appropriately mixed and maintained at the optimal temperature of 25 °C for 5 days to achieve proper fermentation in confined conditions. Finally, these fermented feeds were stored outside for 24 h to achieve normal conditions.

2.2. Microbial analysis of the fermented plant

Lactobacillus, *Bacillus*, and yeast in the fermented phytogenic feed additive (FPFA) were measured on days 1, 3, and 5 of fermentation. Tenfold serial dilutions of samples were made and appropriate dilutions were spread on plates to count viable cells of *Lactobacillus*, *Bacillus*, and yeast. For *Lactobacillus*, *Bacillus*, and yeast, Rogosa agar (Difco Laboratories, Detroit, MI, USA), mannitol egg yolk polymyxin (MYP) agar (Oxoid Ltd., Basingstoke, UK), and yeast morphology agar (Difco Laboratories) were used, respectively, and colony numbers were counted after 25 °C for 5 days. Results of viable cell counts are presented as average values of colony-forming units (cfu)/g of FPFA.

2.3. Feeding trial

In total, 288 Hy-Line brown layers, 35 weeks old, were divided randomly into four treatment groups (6 replicates with 12 birds each per group) from 35 to 39 weeks of age. Dietary treatments in this study included an unsupplemented control diet (CON; no FPFA) and three FPFA treatment groups. The FPFA treatment groups consisted of 0.5% (FPFA1), 1% (FPFA2), and 2% FPFA (FPFA3)-fed groups. The experimental diets were formulated to meet the nutrient requirements of the National Research Council (7) and were formulated consisting of a starter diet (ME: 2900 kcal/kg, CP: 18%, lysine: 0.85%, Met + Cys: 0.65%, Ca: 3.87%, total P: 0.61%) (Table). The experimental diets and water were provided ad libitum. An environmental temperature of 18 ± 3 °C and a photoperiod of 16/8-h light/dark cycle were maintained throughout the experimental period. All animal care procedures were approved by Institutional Animal Care and Use Committee at Dankook University.

2.4. Laying performance

Egg production was recorded daily in replicates and the mean egg weight was determined by the daily average weight of eggs, excluding abnormal eggs (soft-shell eggs).

2.5. Eggshell quality

Eggshell strength was measured for 30 eggs collected randomly from six replicates of each treatment. The eggs were weighed individually and then exposed to a breaking force using an eggshell strength tester (FHK, Fugihira Ltd., Tokyo, Japan). The eggshell strength was measured as the maximum force required to fracture each egg. On breaking, the egg contents were poured into a glass plate to measure the albumen height. Haugh unit values, along with albumen height and egg weight, were determined using a QCM+ Tester (QCM+, Technical Services and Supplies Ltd., York, UK).

2.6. Cecal microbial analysis

Twelve birds per treatment were slaughtered to take cecal samples at the end of the experiment. For the measurement of cecal *Lactobacillus* and *E. coli*, samples of about 1 g were diluted 10fold (1:9, w/v) by blending them with sterile

Table. Formula and chemical composition of basal diet (as-fed basis).

	(%)
Ingredients	
Corn	51.47
Soybean meal (CP 46%)	22.01
Corn gluten meal	6.00
Wheat bran	5.24
Animal fat	4.40
Limestone	9.45
Dicalcium phosphate (P 18%)	0.91
Salt	0.30
$_{DL}$-Met (98%)	0.03
Vitamin premix[1]	0.10
Trace mineral premix[2]	0.10
Total	100
Calculated values	
ME (kcal/kg)	2900
CP (%)	18.00
Lys (%)	0.85
Met + Cys (%)	0.65
Ca (%)	3.87
P (%)	0.61

[1]Provided per kilogram of premix: 125,000 IU vitamin A; 2500 IU vitamin D_3; 10 mg vitamin E; 2 mg vitamin K_3; 1 mg vitamin B_1; 5 mg vitamin B_2; 1 mg vitamin B_6; 15 mg vitamin B_{12}; 500 mg folic acid; 35,000 mg niacin; 10,000 mg Ca-pantothenate; and 50 mg biotin.

[2]Provided per kilogram of diet: 8 mg Mn (as MnO_2); 60 mg Zn (as ZnSO4); 5 mg Cu (as $CuSO_4 \cdot 5H_2O$); 40 mg Fe (as $FeSO_4 \cdot 7H_2O$); 0.3 mg Co (as $CoSO_4 \cdot 5H_2O$); 1.5 mg I (as KI); and 0.15 mg Se (as $Na_2SeO_3 \cdot 5H_2O$).

phosphate-buffered saline (PBS, 0.1 M, pH 7.0) and were then homogenized. A 0.1-mL sample was then serially diluted, 10^3 to 10^6, and spread onto *Lactobacillus* agar (MRS agar; Difco Laboratories) and *E. coli* agar (MacConkey; Difco Laboratories) for culture. The *Lactobacillus* and *E. coli* medium agar plates were then incubated for 24 h at 37 °C under anaerobic and aerobic conditions, respectively. The colonies of each were counted using a colony counter and are reported as cfu $\log_{10}$ per gram.

2.7. Statistical analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA), using the GLM procedure of the SAS program. Tukey's test was performed to detect the significance of differences among groups. Statements of statistical significance are based on $P < 0.05$, and results are expressed as means ± SE.

3. Results

3.1. Growth pattern of microorganisms in FPFA

The total viable bacteria of *Lactobacillus*, *Bacillus*, and yeast in FPFA were counted on Rogosa agar, mannitol egg yolk polymyxin agar, and yeast morphology agar, respectively. In the FPFA fermented with *Lactobacillus planetarium*, *Bacillus licheniformis*, and *Saccharomyces cerevisiae*, viable cell counts of *Lactobacillus*, *Bacillus*, and yeast were increased as fermentation periods increased from day 1 to day 5. At day 5 of fermentation, the total counts of *Lactobacillus*, *Bacillus*, and yeast were 4.5×10^8 cfu/g, 1.3×10^8 cfu/g, and 1.5×10^8 cfu/g, respectively (Figures 1–3). The FPFA at this time (day 5) was acidic (pH range of 4–5; data not shown).

3.2. Egg production, egg weight, and mortality

The inclusion of FPFA in the layers' diet did not affect feed intake and feed conversion ratio among treatments (data not shown). In addition, there was no mortality caused by the supplementation of FPFA among treatments. However, egg production (CON = 93.5% vs. FPFA2 = 96.2%, FPFA3 = 96.8%) and egg weight (CON = 64.7 vs. FPFA2 = 69.5, FPFA3 = 69.4 g) were increased in the FPFA2- and FPFA3-fed groups ($P < 0.05$; Figures 4 and 5).

3.3. Egg quality

Supplemental FPFA increased eggshell strength (CON = 4.24 vs. FPFA2 = 4.56, FPFA3 = 4.65 kg/cm^2), albumen height (CON = 8.33 vs. FPFA3 = 8.85 mm), and Haugh unit (CON = 86.5 vs. FPFA2 = 91.2, FPFA3 = 91.8) ($P < 0.05$; Figures 6–8).

3.4. Cecal microflora

The inclusion of FPFA led to an increase in cecal *Lactobacillus* counts (CON = 6.75 vs. FPFA2 = 7.54, FPFA3 = 7.77 cfu/g) and a decrease in *E. coli* counts (CON = 6.48 vs. FPFA2 = 6.01, FPFA3 = 5.84 cfu/g) ($P < 0.05$; Figures 9 and 10).

4. Discussion

The primary objective of the current study was to investigate the effect of FPFA (a mixture of *G. procumbens* and *R. glutinosa*) as a feed ingredient for layers. First, the presence of *Lactobacillus*, *Bacillus*, and yeast in FPFA was identified as being 4.5×10^8 cfu/g, 1.3×10^8 cfu/g, and 1.5×10^8 cfu/g, respectively. Considering the role of *Lactobacillus*, *Bacillus licheniformis*, and yeast strains in the processing and fermentation of the plants, the presence of these strains in FPFA could be an essential feed factor as functional ingredients in feed.

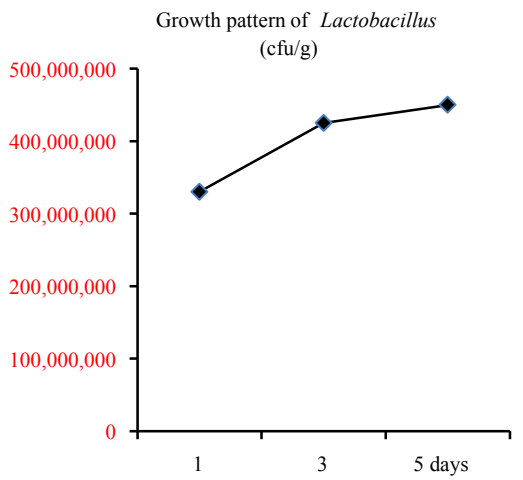

Figure 1. Growth pattern of *Lactobacillus* in the plants fermented with *Lactobacillus planetarium*, *Bacillus licheniformis*, and *Saccharomyces cerevisiae* strains for 5 days at 25 °C.

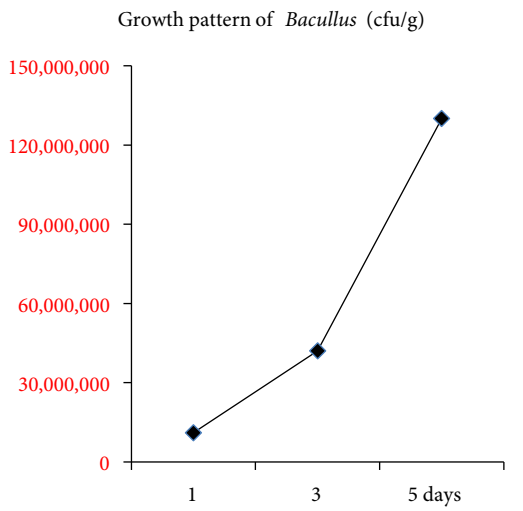

Figure 2. Growth pattern of *Bacillus* in the plants fermented with *Lactobacillus planetarium*, *Bacillus licheniformis*, and *Saccharomyces cerevisiae* strains for 5 days at 25 °C.

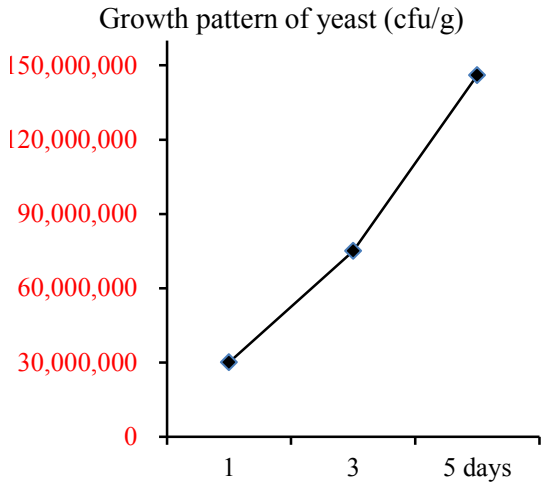

Figure 3. Growth pattern of yeast in the plants fermented with *Lactobacillus planetarium*, *Bacillus licheniformis*, and *Saccharomyces cerevisiae* strains for 5 days at 25 °C.

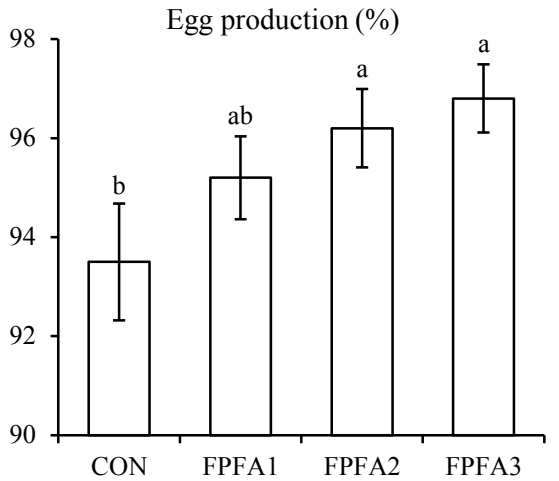

Figure 4. The effects of fermented phytogenic feed additive (FPFA) on egg production in layers. Dietary treatments: CON = basal control diet with no additive; FPFA1 = CON + 0.5% FPFA; FPFA2 = CON + 1% FPFA; and FPFA3 = CON + 2% FPFA. [a–b]Different superscripts mean a significant difference at $P < 0.05$. Each value represents the mean ± SE of 6 observations.

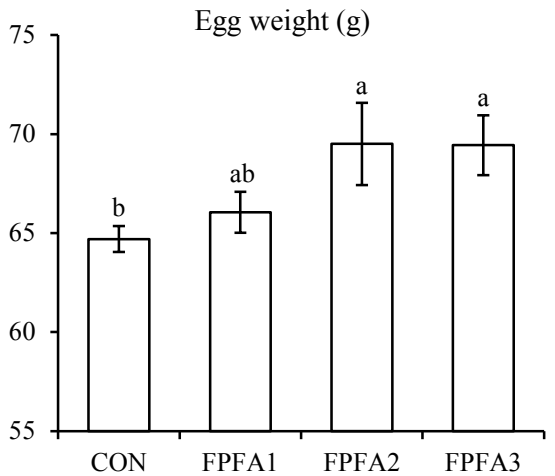

Figure 5. The effects of fermented phytogenic feed additive (FPFA) on egg weight in layers. Dietary treatments: CON = basal control diet with no additive; FPFA1 = CON + 0.5% FPFA; FPFA2 = CON + 1% FPFA; and FPFA3 = CON + 2% FPFA. [a–b]Different superscripts mean a significant difference at $P < 0.05$. Each value represents the mean ± SE of 6 observations.

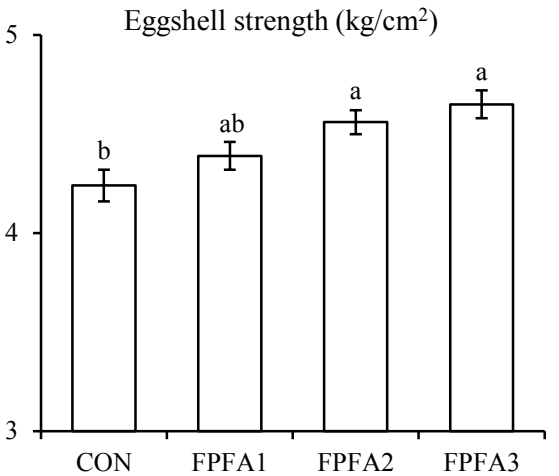

Figure 6. The effects of fermented phytogenic feed additive (FPFA) on eggshell strength in layers. Dietary treatments: CON = basal control diet with no additive; FPFA1 = CON + 0.5% FPFA; FPFA2 = CON + 1% FPFA; and FPFA3 = CON + 2% FPFA. [a–b]Different superscripts mean a significant difference at $P < 0.05$. Each value represents the mean ± SE of 30 observations.

In our in vivo study, increasing concentrations of FPFA showed linear improvements in egg production and egg weight of layers compared with the control treatment during weeks 35–39. These results are consistent with results reported by Lee and Paik (8) and Hong et al. (9), who observed increased egg production and egg weight in layers fed diets supplemented with a mixture of medicinal plants. An increase in eggshell breaking strength was observed with FPFA treatment, and the results of this study were also similar to the reports of Aydin et al. (10) and Lokaewmanee et al. (11), which showed significant effects on the eggshell breaking strength between control and phytogenic feed additive treatments. Albumen height and Haugh units are important factors used to evaluate

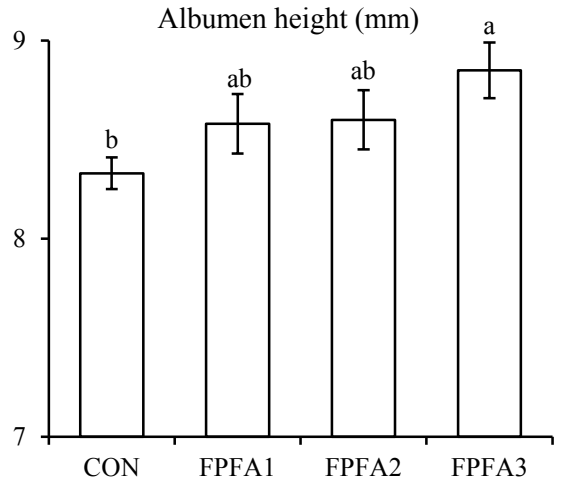

Figure 7. The effects of fermented phytogenic feed additive (FPFA) on albumen height in layers. Dietary treatments: CON = basal control diet with no additive; FPFA1 = CON + 0.5% FPFA; FPFA2 = CON + 1% FPFA; and FPFA3 = CON + 2% FPFA. [a–b]Different superscripts mean a significant difference at $P < 0.05$. Each value represents the mean ± SE of 30 observations.

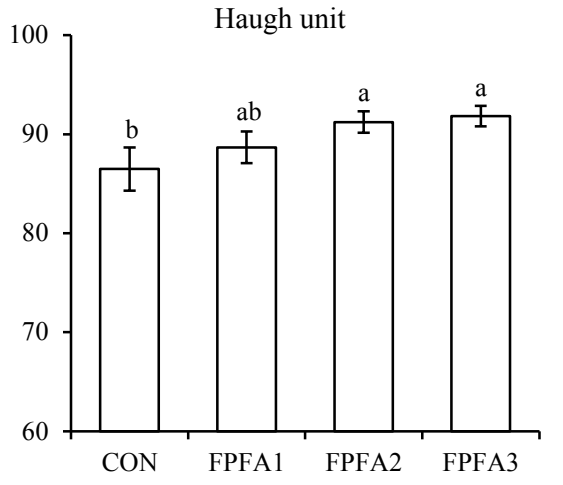

Figure 8. The effects of fermented phytogenic feed additive (FPFA) on Haugh unit in layers. Dietary treatments: CON = basal control diet with no additive; FPFA1 = CON + 0.5% FPFA; FPFA2 = CON + 1% FPFA; and FPFA3 = CON + 2% FPFA. [a–b]Different superscripts mean a significant difference at $P < 0.05$. Each value represents the mean ± SE of 30 observations.

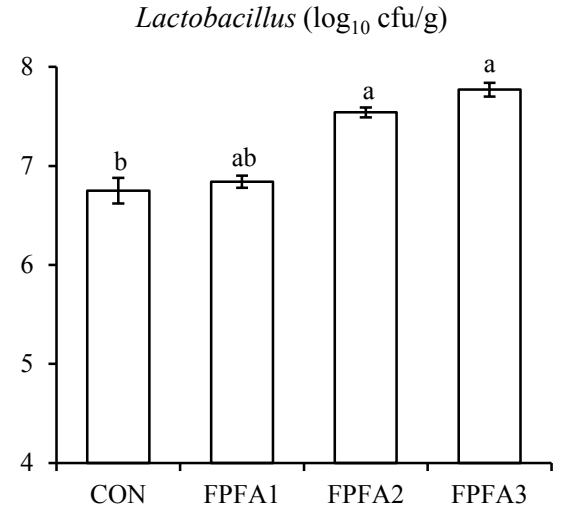

Figure 9. The effects of fermented phytogenic feed additive (FPFA) on cecal *Lactobacillus* in layers. Dietary treatments: CON = basal control diet with no additive; FPFA1 = CON + 0.5% FPFA; FPFA2 = CON + 1% FPFA; and FPFA3 = CON + 2% FPFA. [a–b]Different superscripts mean a significant difference at $P < 0.05$. Each value represents the mean ± SE of 12 observations.

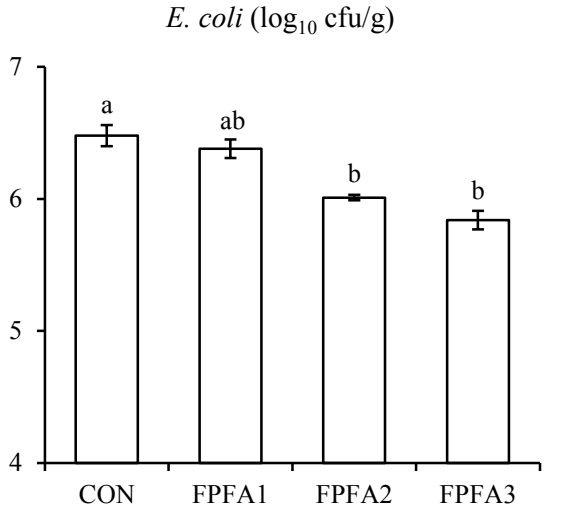

Figure 10. The effects of fermented phytogenic feed additive (FPFA) on cecal *E. coli* in layers. Dietary treatments: CON = basal control diet with no additive; FPFA1 = CON + 0.5% FPFA; FPFA2 = CON + 1% FPFA; and FPFA3 = CON + 2% FPFA. [a–b]Different superscripts mean a significant difference at $P < 0.05$. Each value represents the mean ± SE of 12 observations.

interior egg quality characteristics and the freshness of the egg. In our study, the layers fed FPFA diets containing 1% and 2% FPFA had higher values of albumen and Haugh units. In egg production and egg quality in layers, the exact mechanism of action of *G. procumbens* and *R. glutinosa* is not clear yet, but the improved egg production and egg quality in layers fed diets supplemented with FPFA could be associated with improved gut health. In the present study, layers fed diets supplemented with 1% FPFA and 2% FPFA had increased cecal *Lactobacillus* and reduced *E. coli*. Changes in cecal microbial populations most likely reflected the presence of *Lactobacillus*, *Bacillus*, and yeast in

FPFA. These results are consistent with the findings of Guo et al. (12), who reported reduced pathogenic microflora in layers fed phytogenic feed additives, mushrooms, and herb extracts. Phytogenic feed in the diet also may lead to the enhancement of nutrient absorption due to increased villus length and crypt depth (2). Similarly, Hernandez et al. (13) reported that phytogenic feed additives improved nutrient digestibility in broilers. Amad et al. (14) reported that the apparent ileal digestibility of crude ash, calcium, and phosphorus showed a linear increase related to the increase of phytogenic feed additives (essential oils of thyme and anise) in broiler diets. Thus, the beneficial effects on egg production, egg weight, and eggshell breaking strength may be attributable to favorable alterations in the intestinal environment and function, which may have resulted in increased intestinal calcium absorption. Moreover, *G. procumbens* has antioxidant (15), antihyperlipidemic (16), antihypertensive (17), antihyperglycemic (18), antiinflammatory (19), antiulcerogenic (20), and anticarcinogenic (21) activities. It has also been reported that *G. procumbens* contains β-sitosterol, stigmasterol, kaempferol-3-O-rutinoside, quercetin, saponin, tannin, and terpenoids as active compounds (15). The root of *R. glutinosa* also contains some biologically active substances, such as iridoids, monoterpenes, glycosides, saccharides, amino acids, and organic acids (22). Many clinical and experimental studies have shown that *R. glutinosa* possesses various pharmacological properties, such as hemostatic (23), antifatigue (24), immune enhancement (25), antitumor (26), antiinflammatory (6), and antioxidant (27) activities. Thus, in our study, improved production performance and egg quality might also be due to overall improvement of the health of FPFA-supplemented layers.

Other probable reasons for the improved egg production and egg quality found with FPFA treatments may be associated with microbial fermentation. This fermentation process increases the extraction quantity of active materials derived from plants. For example, the microbial fermentation (*Lactobacillus johnsonii*, *Lactobacillus reuteri*, and *Lactobacillus acidophilus*) increased the concentration of total free phenolic acids in cereals (barley and oat) by 20-fold, compared with unfermented samples (28). A similar effect on enhancing the release of phenolic acids and flavanols was reported by Cho et al. (29) after 60 h of fermentation of soybeans with *Bacillus pumilus*, showing 2.8-fold, 7.6-fold, and 4.5fold increases in gallic acid, catechin, and epicatechin, respectively. Increased release of phenolic compounds in fermented foods could be due to the activities of cell wall-degrading enzymes produced during microbial fermentation processes (30). Additionally, microbial fermentation can induce the bioconversion of flavonoids into metabolites by different pathways, including glycosylation, deglycosylation, ring cleavage, methylation, glucuronidation, and sulfate conjugation according to microbial strains and substrates (30). Thus, microbial fermentation could be considered as a potential technology for promoting phenolic compounds from plant-derived foods, as well as for extracting new bioactive compounds. Further investigations are needed to evaluate the efficacy of FPFA in terms of producing new bioactive compounds.

In conclusion, the results of this study indicated that FPFA supplementation of layer diets improved egg production and quality. Furthermore, increased cecal *Lactobacillus* and decreased *E. coli* populations suggest that FPFA may promote a more beneficial gut environment in layers.

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References

1. Park JH, Kang SN, Chu GM, Jin SK. Growth performance, blood cell profiles, and meat quality properties of broilers fed with *Saposhnikovia divaricata*, *Lonicera japonica*, and *Chelidonium majus* extracts. Livest Sci 2014; 165: 87-94.
2. Khalaji S, Zaghari M, Hatami KH, Hedari-Dastjerdi S, Lotfi L, Nazarian H. Black cumin seeds, *Artemisia* leaves (*Artemisia sieberi*), and *Camellia* L. plant extract as phytogenic products in broiler diets and their effects on performance, blood constituents, immunity, and cecal microbial population. Poultry Sci 2011; 90: 2500-2510.
3. Perry LM. Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses. Cambridge, MA, USA: MIT Press; 1980.
4. Piątczak E, Królicka A, Wielanek M, Wysokińska H. Hairy root cultures of *Rehmannia glutinosa* and production of iridoid and phenylethanoid glycosides. Acta Physiol Plant 2012; 34: 2215-2224.
5. Huang KC. The Pharmacology of Chinese Herbs. 2nd ed. Boca Raton, FL, USA: CRC Press; 1998.
6. Jin CH, Lee YM, Kang MA, Park YD, Choi DS, Byun MW, Jeong IY. Anti-inflammatory activities of ethylacetate extract of *Rehmannia glutinosa* in LPS-induced RAW 264.7 cells. Food Sci Biotechnol 2009; 18: 923-927.
7. National Research Council. Nutrient Requirements of Poultry. 9th ed. Washington, DC, USA: National Academy Press; 1994.

8. Lee WS, Paik IK. Modification of herbal product (Herb Mix®) to improve the efficacy on the growth and laying performance of chickens. Korean Journal of Poultry Science 2007; 34: 245-251.

9. Hong JW, Kim IH, Kwon OS, Lee SH, Lee JM, Kim YC, Min BJ, Lee WB. Effects of Korean medical herb residue supplementation on the egg quality and serum cholesterol of laying hens under heat stress. Korean Journal of Poultry Science 2001; 3: 259-265.

10. Aydin R, Karaman M, Cicek T, Yardibi H. Black cumin (*Nigella sativa* L.) supplementation into the diet of the laying hen positively influences egg yield parameters, shell quality, and decrease egg cholesterol. Poultry Sci 2008; 87: 2590-2595.

11. Lokaewmanee K, Yamauchi K, Komori T, Saito K. Eggshell quality, eggshell structure and small intestinal histology in laying hens fed dietary Pantoea-6® and plant extracts. Ital J Anim Sci 2014; 13: 332-339.

12. Guo FC, Williams BA, Kwakkel RP, Li HS, Li XP, Luo JY, Li WK, Verstegen MWA. Effect of mushroom and herb polysaccharides, as alternative for antibiotics, on the cecal microbial ecosystem in broiler chickens. Poultry Sci 2004; 83: 175-182.

13. Hernandez F, Madrid J, Garcia V, Orengo J, Megias M. D. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poultry Sci 2004; 83: 169-174.

14. Amad AA, Männer K, Wendler KR, Neumann K, Zentek J. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Poultry Sci 2011; 90: 2811-2816.

15. Yam M, Sadikun A, Asmawi M. Antioxidant potential of *Gynura procumbens*. Pharm Biol 2008; 46: 616-625.

16. Zhang XF, Tan BKH. Effects of an ethanolic extract of *Gynura procumbens* on serum glucose, cholesterol and triglyceride levels in normal and streptozotocin-induced diabetic rats. Singapore Med J 2000; 41: 9-13.

17. Kim MJ, Lee HJ, Wiryowidagdo S, Kim HK. Antihypertensive effects of *Gynura procumbens* extract in spontaneously hypertensive rats. J Med Food 2006; 9: 587-590.

18. Akowuah GA, Sadikun A, Mariam A. Flavonoid identification and hypoglycaemic studies of the butanol fraction from *Gynura procumbens*. Pharm Biol 2002; 40: 405-410.

19. Iskander MN, Song Y, Coupar IM, Jiratchariyakul W. Antiinflammatory screening of the medicinal plant Gynura procumbens. Plant Foods Hum Nutr 2002; 57: 233-244.

20. Mahmood AA, Mariod AA, Al-Bayaty F, Abdel-Wahab SI. Anti-ulcerogenic activity of *Gynura procumbens* leaf extract against experimentally-induced gastric lesions in rats. J Med Plant Res 2010; 4: 685-691.

21. Nisa F, Hermawan A, Murwanti R, Meiyanto E. Antiproliferative effect of *Gynura procumbens* (Lour.) Merr. leaves etanolic extract on 7, 12-dimethylbenz(a)antracene induced male rat liver. Adv Pharm Bull 2012; 2: 99-106.

22. Tomoda M, Kato S, Onuma M. Water-soluble constituents of rehmanniae radix. I. Carbohydrates and acids of *Rehmannia glutinosa* f. *hueichingensis*. Chem Pharm Bull 1971; 19: 1455-1460.

23. Liang AH, Xue BY, Wang JH, Hao JD, Yang H, Yi H. A study on hemostatic and immunologic actions of fresh- and dry Dihuang. China J Chin Mater Med 1999; 24: 663-666.

24. Tan W, Yu KQ, Liu YY, Ouyang MZ, Yan MH, Luo R, Zhao XS. Anti-fatigue activity of polysaccharides extract from *Radix Rehmanniae Preparata*. Int J Biol Macromol 2012; 50: 59-62.

25. Gao XD, Wu WT. Biological function of five Chinese traditional drugs on proliferation and IL-2 production of the mice lymphocytes. Chin Pharm J 1990; 21: 43-45.

26. Chao JC, Chiang SW, Wang CC, Tsai YH, Wu MS. Hot water extracted *Lycium barbarum* and *Rehmannia glutinosa* inhibit proliferation and induce apoptosis of hepatocellular carcinoma cells. World J Gastroenterol 2006; 12: 4478-4484.

27. Oh HL, You BR, Kim HJ, Lee JY, Kim NY, Song JE, Kim MR. Quality characteristics and antioxidant activities of *Rehmanniae radix* paste. J Korean Soc Food Sci Nutr 2011; 40: 1518-1524.

28. Hole AS, Rud I, Grimmer S, Sigl S, Narvhus J, Sahlstrøm S. Improved bioavailability of dietary phenolic acids in whole grain barley and oat groat following fermentation with probiotic *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, and *Lactobacillus reuteri*. J Agric Food Chem 2012; 60: 6369-6375.

29. Cho KM, Hong SY, Math RK, Lee JH, Kambiranda DM, Kim JM, Islam SMA, Yun MG, Cho JJ, Lim WJ et al. Biotransformation of phenolics (isoflavones, flavanols and phenolic acids) during the fermentation of *cheonggukjang* by *Bacillus pumilus* HY1. Food Chem 2009; 114: 413-419.

30. Huynh NT, Van Camp J, Smagghe G, Raes K. Improved release and metabolism of flavonoids by steered fermentation processes: a review. Int J Mol Sci 2014; 15: 19369-19388.