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Authors' contributions Please specify the authors' role using this form.	All authors contributed to this work. Conceptualization: J.S. Kim and H. Tajudeen

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Abstract

The experiment was carried out to study the effect of Korean wild ginseng adventitious root supplementation on the laying performance, egg quality, cytokine expression, ginsenoside concentration, and microflora quantity of Institut de selection Animale (ISA) brown laying hens at 24 weeks old. A total of 90 laying hens were subjected to a completely randomized design at three treatments, five repetitions and six laying hens per replicate. The experiments were divided by diets into the basic feed (CON), basic feed + 0.1% wild ginseng (WG1), and basic feed + 0.5% wild ginseng (WG2). The feeding trial was carried out over a duration of 12 weeks after an initial acclimation period of 2 weeks. Feeds and water were administered *ad libitum* in mash form, and light was available for 16 hours per day. At the end of study, hen-day egg production (HDEP), average egg weight (AEW), and egg mass (EM) were increased (p <0.05) in WG2 at week 12. Feed conversion ratio (FCR) was decreased (p <0.05) in WG2 at week 12. The ginsenoside content in egg yolk was increased (p <0.05) in laying hens in the WG2 treatment at week 12. Relative expression of tumor necrosis factor alpha (TNF-α) was reduced (p <0.05) in the WG supplemented diets at week 12. The fecal microflora quantity of *Lactobacillus was increased* (p <0.05) *in WG2 at week 8 to week 12, and Escherichia coli* (*E. coli)* was significantly decreased (p <0.05) in the WG2 at week 12. We concluded that the result observed in the HDEP, AEW, EM and FCR was due to an increase in ginsenoside content, leading to an improvement in the TNF-α, and fecal microflora quantity such as *Lactobacillus and E. coli* in the WG2 supplemented diets. We therefore recommend the use of WG at application level 0.5% per basal diet for optimum laying performance in layer hens.

Keywords:

Ginsenoside, Saponin, Tumor necrosis factor alpha, Hen-day egg production, Inflammatory cytokine.

INTRODUCTION

The poultry sector struggled after the ban on antibiotics to achieve improved feed utilization, minimized rate of mortality, and growth of healthy livestock [1]. To enhance the health and quality of eggs produced by the poultry sector, reliable feed additives should be introduced to the rations [2-4]. Supplements used to improve the production or animal health should have negligible side effects. Different herbal remedies might be used as potential substitutes for antibiotics and growth promoters. They are beneficial for health and have naturally occurring growth-promoting and anti-oxidative properties [5,6]. Plants contain a wide variety of active ingredients. Essential oils extracted from aromatic and medicinal herbs have anti-inflammatory, antibacterial, and digestive-stimulating characteristics,

making them popular as poultry feed and other livestock supplements [7]. The ginseng plant and its extracts are natural growth stimulants that can replace antibiotics and have been used in Asian countries, such as Korea, Japan, and China, for over two millennia, leading to its global expansion in recent years [7]. Ginseng and its extracts have anti-allergy, anti-inflammatory, anti-cancer, anti-fatigue, anti-stress, and immunomodulatory properties in their essential oils, ginsenoside, polyacetylenic alcohol, saponin, peptides, polysaccharides, and vitamin contents, present in proportions based on the culture method used for growing the ginseng [8,9]. Based on the vegetative methods, ginseng can be classified as either wild or cultivated. Artificially grown ginseng is mostly collected after 5–6 years of methodical farming in an open field, while wild ginseng (WG) is sown in the deep mountain at a height between 800–1500 meters. WG grows more slowly, is cheaper to maintain, and is more susceptible to environmental changes, favoring daily temperatures and less exposure to sunlight when compared with cultured ginseng. WG root has more medicinal effects than cultivated ginseng, which could be the major factor leading to differences in both types of ginsengs [10]. However, the roots from ginseng have not been widely and regularly used due to its higher cost and challenging cultivation. Nonetheless, cells from the plant have been used commercially in various foods and cosmetics due to the growth of the contemporary industry [11]. Artificial vegetation approaches have served as a solution to the mass production of ginseng adventitious roots with the same chemical content ratios as the native roots [12]. The main goal of our study was to evaluate the effect of Korean WG adventitious root derived from artificial vegetation on the laying performance, egg quality, ginsenoside concentration of yolk, cytokine, and microflora of laying hens.

MATERIALS AND METHODS

Test animals, feed and experimental design

The experiment was carried out at the laying hen breeding facility of Kangwon National University with the approval of the University's Animal Experimental Ethics Committee (KW-220413-1). In order to evaluate the effect of WG powder in layers diet, 90 pieces of 24 weeks old laying hens (ISA Brown weighting 1,894 ± 0.12g) were tested in a completely randomized design with 3 treatments and 5 repetition with 6 numbers per replicate. The birds were initially subjected to an adaptation period of 14 days, and the feeding experiment was conducted for a total of 12 weeks (phase 1, 0-4 weeks; phase 2, 5-8 weeks; phase 3, 9-12 weeks). The experiments were divided into the CON (basic feed), WG1 (basic feed + 0.1% WG), and WG2 (basic feed + 0.5% WG). The wild ginseng root was dried and roughly grinded into a powder form to release the active ingredients in the root using the CE commercial

mini SUS304 electric 12-120 herb grinder model number LFJ-15B, and the inclusion level was based on economic reasons which is an important factor considered in our study. Previous researchers evaluated the effect of ginsengs between 1.0-5.0% [5,34]. However, we tried to evaluate its potency at a lower dosage, but with wild ginseng plant grown for a period of 10 years. Wild ginseng is generally believed to be more effective based on factors such as duration, specie, and their parts such as the leaves, root, or rhizomes supplemented in diets [34]. The lighting period was adjusted to 16 hours per day, and the chemical composition and mixing ratio of the experimental diet fed in this study were shown in Table 1, and the nutrient levels were formulated to meet or exceed the nutrient requirement as prescribed by Institut de selection Animale (ISA) brown commercial management guide [13]. The feeds and water were administered at *ad libitum* in mash form, and all hens were housed in a window-less and environmentally controlled room, with room temperature kept between 20-22℃, and each cage was equipped with individual nipples, feeders, and nest enrichments according to the EU laying down minimum standards for the protection of laying hen [14].

Laying performance

The layer hens were weighed after adaptation, and at the end of each phase (4, 8, and 12 weeks). Feed conversion ratio (kg of feed/kg of eggs, FCR) for all periods was calculated on a cage basis from egg production (hen day egg production, HDEP), average egg weight (AEW), and average daily feed intake (ADFI). Eggs were collected daily and egg production was expressed on a hen-day basis (% hens day). Individual egg weights were recorded and then used to calculate the mean egg weight for all experimental periods. The egg mass (EM) was calculated by multiplying egg weights and egg production rate [15].

Egg and eggshell quality

Egg and eggshell quality examinations (Haugh units, yolk colour, yolk and albumin weights, yolk and albumin percentages, eggshell thickness, and hardness) were undertaken at the end of each phase. For measuring these parameters, eggs were collected on the last day of the phases. Haugh units, yolk colour, yolk, and albumin weights were measured by Egg multi-tester (Touhoku rhythm co., Ltd., Tokyo, Japan). Eggshell breaking strength was evaluated using a model II egg shell force gauge (Robotmation Co., Ltd., Tokyo, Japan). A dial pipe gauge (Ozaki MFG. Co., Ltd., Tokyo, Japan) was employed for measurements the egg shell thickness, which was determined on

the basis of the average thickness of the rounded end, pointed end, and the middle of the egg, excluding the inner membrane [15].

Blood immune substance

Blood withdrawal and PMBC isolation

Five birds per treatment were selected for this process. A minimum of 5 mL blood was collected from the median underwing coverts and was withdrawn slowly using a disposable syringe and a needle (21 gauge) and transferred into a heparin tube on ice.

PBMC isolation: To isolate a peripheral blood mononuclear cell (PBMC), a ficoll solution was dispensed into the tube containing the blood then centrifuged for 40 min at 4000 rpm at 20℃ using the Union 55R Refrigerated multipurpose centrifuge, Hanil Science Industrial Co., Ltd. with the break set at 0. After separation, the final solution was stored at -4℃.

Gene extraction analysis

The RNeasy Mini kit (Qiagen, Germany), was used for messenger ribonucleic acid (mRNA) extraction from the PBMC. In detailed, prior to homogenization using an Ultraturrax homogenizer (Polytron PT 1600E® , Kinematica, Luzern, Switzerland), the cells were trapped with 600 µl lysis-buffer (containing β-mercaptoethanol and RLT-buffer).

Reverse transcription polymerase reaction and qPCR analysis

250 ng of extracted mRNA and 100 l of TaqMan Reverse Transcription Reagents® (Life Technologies, Carlsbad, CA, USA) were used to reverse transcribe the mRNA. 5 micro liter of random hexamers, 2 micro liter oligo-DT, 10 micro liter 10x bufer, 25 mM MgCl2, 20 µl dNTP, 2 µl RNAse inhibitor, and 6.2 µl of MultiScribe Reverse Transcriptase were employed as the reagents. Reverse transcription was carried out in an Eppendorf flexid, nexus gradient master cycler, SN:6332kl132036, Germany with annealing (25℃, 10 min), enzyme inactivation (95℃, 5min). Relative tumor necrosis factor alpha (TNF-α) gene was quantified using 5 µl cDNA, 1.75 µl Aqua dest., 2 µl of TaqMan Master Mix and 0.25 µl of both forward and reverse primers, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was employed as the house keeping gene (Bioneer Corporation, Daedeok-gu, Daejeon, South Korea). The qPCR Rotor-Gene Qiagen with Serial Number 0312272 (Corbett Research) with cycling

condition 95℃ followed by cycles of melting at 95℃ for 15s; annealing for the specified times and temperatures according to the primers; and extension at 72℃ for a specified primer time and 40 cycles [16].

Fecal microflora DNA

To measure fecal microflora differences according to each treatment, five fresh fecal samples per treatment were collected via the cloaca of layer hen at the end of each phase and immediately kept at -80℃ until analysis which was carried out on the same day. The cloaca was gently palpated to stimulate the discharge of fresh fecal samples void of external contaminations from the pen into airtight containers. Deoxyribonucleic acid (DNA) extraction protocol according to QIAamp fast DNA stool mini kit Germany, cat. No. 51604 2016 was carried out. In Detail (**Step 1**), 200 mg stool was weighted with a scalpel into a 2 mL microcentrifuge tube and placed on ice. 1 mL InhibitEX Buffer was added to each stool sample and Vortex continuously for 1 min until the stool sample was thoroughly homogenized to ensure maximum DNA concentration in the final eluate. The homogenized sample was heated in a water bath of 70℃ for 5 min and vortexed for 15 s for uniform lysis. Samples were then centrifuged at full speed (20,000 x *g*, 14,000 rpm) for 1 min to pellet stool particles. **Step 2**: 25 µl proteinase K was pipetted into a new 2 mL microcentrifuge tube and 600 µl supernatant from step 1 was pipetted into the 2 mL microcentrifuge tube containing proteinase K. We Added 600 µl Buffer AL and vortex for 15 s to form a homogeneous solution which was Incubated at 70℃ for 10 min and centrifugated briefly to remove drops within the tube lid. 600 µl of ethanol (96–100%) was added to the lysate, and mix by vertexing, followed by a brief Centrifugation to remove drops from the inside of the tube lid. 600 µl of the lysate above was transferred into the QIAamp spin column and centrifuged at full speed (20,000 x *g*, 14,000 rpm) for 1 min. The QIAamp spin column was placed in a new 2 mL collection tube, and the old tube containing the filtrate was discarded. The QIAamp spin column was carefully opened, 500 µl Buffer AW1 was added and centrifuge at full speed for 1 min. The QIAamp spin column was transferred to a new 2 mL collection tube, and old tube containing the filtrate was discarded. The QIAamp spin was carefully opened again, and 500 µl Buffer AW2 was added, and centrifuged at full speed for 3 min. The QIAamp spin column was placed in a new 2 mL collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 3 min to eliminate the chance of possible Buffer AW2 carryover. Lastly, The QIAamp spin column was transferred into a new, labeled 1.5 mL microcentrifuge tube and 200 µl Buffer ATE was pipetted directly onto the QIAamp membrane, incubated for 1 min at room temperature, then centrifuged at full speed for 1 min to elute DNA which is then later checked using the Spectrophotometer.

Real time pcr (QPCR)

For the quantification of fecal microflora such as *Lactobacillus* spp., *Bifidobacterium* spp., *Clostridium* spp., and *E. coli.,* in our experiment, 1x universal SsoAdvanced™ universal SYBR® Green Supermix, 2.5 ng/μL of each forward and reverse primers, and 10 ng of DNA was added in a 10 μL volume [17,18]. Enzyme activation was achieved at the cycling parameters at 95℃ followed by 40 cycles of melting at 95℃ for 15 s; annealing for the specified times and temperatures according to each primer (Bioneer Corporation, Daedeok-gu, Daejeon, South Korea) and the SYBR thermal cycling protocol carried out with extension at 72℃ for a specified time according to each primer, with beta actin (β-Actin) as the house keeping gene. At 72℃, the SYBR green fluorescent signals were recorded and known bacterial species were serially diluted 10-fold before being used to create PCR results. The qPCR Rotor-Gene Qiagen 2plex program with Serial Number 0312272, Corbett Research (Corbett Life Science Qiagen 2008) was used for the DNA quantification.

Ginsenosides in egg yolk

The ginsenoside concentration in egg yolk was measured using five egg samples for each treatment groups. 2 g of dry matter sample was extracted 3 times into 20 mL methanol while at 60℃ constant temperature in a water bath at 3-hour intervals. For the extracted sample, the solvent was removed under reduced pressure under a condition not exceeding 40℃ using a reflux concentrator, and the remaining residue was dissolved in 5 mL of distilled water. The dissolved residue was transferred to a separatory funnel, and the layers were separated with 50 mL of chloroform to remove non-polar components such as fat, and the organic solvent layer. At this time, the remaining residue was washed three times with 50 mL of ethyl ether. The ginsenoside layer dissolved by the aqueous layer was extracted using saturated n- butanol, and the solvent was removed under reduced pressure by observing 40℃ in an evaporator. It was tested gravimetrically to determine the total ginsenoside content (34).

Statistical analyses

Data generated in the present study were subjected to statistical analysis system [19] using the general linear model (GLM) procedure in a randomized complete block design. When significant differences were identified among treatment means, they were separated using Tukey's Honest Significant Difference test. Probability values of <0.05 were considered significant, and cages containing six laying hens per replicate was the experimental unit.

RESULTS

Laying performance

The effect of WG on laying performance is shown in **Table 4.** The supplementation of WG showed no significant difference in ADFI and body weight (BW) from phase 1 to phase 3 across all treatments. Supplementation of WG showed no significant difference in HDEP in phase 1 but showed a tendency towards significance in phase 2 and was significantly increased ($p < 0.05$) in phase 3 at WG2 compared with the CON. The addition of WG had no significant effect on AEW in phase 1 and phase 2 across all treatments, but AEW was significantly increased ($p < 0.05$) in phase 3 at WG2 compared with the CON. The effect of WG supplementation showed no significant difference in EM across all treatments in phase 1 but showed a tendency towards significance in phase 2 and was significantly increased ($p < 0.05$) in phase 3 at WG2 compared with the CON. FCR was not significant in phase 1 and phase 2 of our experiment across all treatments when WG was supplemented, but it was significantly decreased ($p < 0.05$) in phase 3 at WG2 compared with the CON.

Egg quality

The effect of WG on the egg quality of layer hen is shown in **Table 5.** There was no significant difference in Haugh unit, yolk color, yolk weight, albumin weight, yolk percentage, albumin percentage, eggshell thickness, and hardnes s across all treatments from phase 1 to phase 3.

Ginsenoside content in egg yolk

The effect of WG in the feed for laying hens on the ginsenoside content in egg yolk is shown in **Table 6**. There was no significant difference in phase 1 across all treatments when WG was supplemented, but the difference tended tow ards significant in phase 2, and there was a significant increase ($p < 0.05$) in the ginsenoside content in phase 3 at W G2 compared with the CON.

Inflammatory cytokine

The effect of WG on the relative expression of TNF-α is shown in **Figure 1**. There was no significant difference in p hase 1 across all treatments when WG was added. However, phase 2 showed a tendency towards significance, and p hase 3 was significantly decreased ($p < 0.05$) in hens on the WG-supplemented diets compared with the CON.

Microflora

The effect of WG on the fecal microflora DNA of laying hens is shown in **Table 7.** The supplementation of WG had no significant effect on *Clostridium* spp. and *Bifidobacterium* spp**.** from phase 1 to phase 3 across all treatments. *La ctobacillus* spp. had no significant difference in phase 1 but was significantly increased ($p < 0.05$) in phase 2 and ph ase 3 at WG2 compared with the CON. Lastly, *E. coli* showed no significant difference in phase 1 and phase 2 but w as significantly decreased ($p < 0.05$) in phase 3 at WG2 compared with the CON.

DISCUSSION

Ginsenosides are the primary components of ginseng and positively affect reproductive organs and other tissues [20,22]. The supplementation of WG at WG2 in the current study improved FCR, HDEP, AEW, EM, TNF-α, yolk ginsenoside content, and microflora DNA in the last phase of our experiment but had no positive effect on ADFI, BW, and egg quality. Herbs and their extracts may help the digestive system by promoting the activity of the digestive enzymes in the gastric epithelium [23]. We propose this as the reason behind the amelioration of FCR in our study. However, the bioactive contents of ginseng, such as saponin, are typically associated with a bitter taste and unpleasant sensation, which might be the reason behind the indifference in ADFI and BW across all treatments.

The increased laying performance in hens on the WG-supplemented diets in our research agrees with the study of Kang et al.; Sook et al. [24,25], where the inclusion of fermented WG by-products enhanced the laying performance of layer hens. The enhanced laying performance can be attributed to the stimulating effect of ginsenosides on oocyte meiotic maturation proliferation through the cumulus cells. The ovary of a chicken accommodates a variety of hierarchical follicles according to their maturity. One of the crucial steps in the formation of follicles related to the laying performance is the selection of small yellow follicles as pre-ovulatory follicles from a group of follicles of comparable sizes [26]. This can be further illustrated using the study by Tan et al. [27] that demonstrated that the administration of ginsenoside significantly increased the number of granular cells, a major component of the ovarian follicle, in laying hens, leading to a more cuboidal shape of the granular cells in hens on the ginsenoside-supplemented diet. This might be the reason behind the increase in laying performance in our study.

In addition, the chicken gastrointestinal pathway is susceptible to *E. coli*, widely distributed in their excretes, making colibacillosis one of the most widespread diseases affecting the layer and poultry sector [28-29]. Commercial layer and breeder hens with this syndrome produce fewer eggs due to exudations in the peritoneal cavity due to the inflammatory cells covering the surfaces of several organs, such as the oviduct, ovary, and intestine

[30]. Additionally, the underdeveloped immune system of young layers, coupled with their high nutrient requirement, makes them more venerable to infections than the matured layers [31,32], which can also be the reason for the indifferent results in the early stages of our experiment. Therefore, we saw the effect of WG on mitigating the negative effect of *E. coli* and the modulation of inflammatory cytokine in the later phase of our study. This agrees with the study of Bi et al. [33], in which ginsenoside was able to lessen the stress caused by *E. coli* in broiler chickens. They explained that the impact was associated with the mammalian target of rapamycin, Hemeoxygenase-1, and superoxide dismutase overexpression, resistance against oxidative damage, and inflammatory suppression. We hypothesized from our results that the amelioration of the ginsenoside level of egg yolk in the current study could be linked to the improvement in the TNF-α and microflora DNA expression, leading to a reduction in the presence of negative bacteria *E. coli* and an increase in the positive *Lactobacillus* in the later phase of our experiment. In detail, ginsenoside's amphiphilic and hydrophilic carbohydrate nature enables their adhesion into membranes which also entails lamella collections of phospholipids with amphiphilic ends, allowing them to interact with the interfacial region membranes made up of multiple glycolipids and glycoprotein of the yolks allowing the steroid portion of the saponins to engage with sterol membrane. This enable the saponin osidic component to promote the development of intramolecular hydrogen bonding [34,35]. Furthermore, Fukuda et al. [35,36] revealed that ginsenosides shows a distinctive agglutinability to egg yolk vesicles which is phosphatidylcholine in nature, with three-dimensional structure serving as the final determining factor of its ability to agglomerate with other lipids to form a peripheral membrane domain [37-39]. We hypothesize this leads to an increase in O-glycosidic oligosaccharides enabling a better gut and immune system.

Lastly, our research showed no significant differences in egg qualities, such as haugh unit, yolk color, yolk weight, albumin weight, yolk percentage, albumin percentage, eggshell thickness, and hardness across all treatments and phases. This is contrary to the study of Jang et al. [40], in which red ginseng enhanced egg quality. We propose that such discrepancies may be attributed to different ginseng strains, sources, or preparation techniques used in the studies.

CONCLUSION

In conclusion, the supplementation of WG in diets led to an increase in yolk ginsenoside content and reduce TNF-α, ultimately leading to an improved microflora DNA quality and laying performances, such as HDEP, AEW, EM, and FCR in hens on the WG2-supplemented diets. Therefore, we recommend the use of 0.5 % WG per basal diet for improved laying performance in layer hens.

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REFERENCES

1 Yuan L, Li W, Huo Q, Du C, Wang Z, Yi B, et al. Effects of xylo-oligosaccharide and flavomycin on the immune function of broiler chickens. PeerJ. 2018; 6:4435. https://doi.org/10.7717/peerj.4435

2 Olobatoke RY, Mulugeta SD. Effect of dietary garlic powder on layer performance, fecal bacterial load, and egg quality. Poult Sci. 2011; 90:665-670. https://doi.org/10.3382/ps.2010-00736

3 Yildırım A, Sekeroglu A, Eleroglu H, Duman MI. Effects of Korean ginseng (Panax ginseng C.A. Meyer) root extract on egg production performance and egg quality of laying hens. South Afr J Anim Sci. 2013; 43:194-207. https://doi.org/10.4314/sajas.v43i2.10

4 Tajudeen H, Hosseindoust A, Ha SH, Moturi J, Mun JY, Lee CB, et al. Effects of dietary level of crude protein and supplementation of protease on performance and gut morphology of broiler chickens. Europ Poul Sci. 2022; 86:1612-9199. http://dx.doi.org/10.1399/eps.2022.355

5 Yan L, Meng QW, X AO, Wang JP, Jang HD, Kim IH. Evaluation of dietary wild-ginseng adventitious root meal on egg production, egg quality, hematological profiles and egg yolk fatty acid composition in laying hens. Livest Sci. 2011; 140:1-3. https://doi.org/10.1016/j.livsci.2011.03.033

6 Lee JI, Park KS, Cho IH. Panax ginseng: a candidate herbal medicine for autoimmune disease. JGR. 2019; 43:342-348. https://doi.org/10.1016/j.jgr.2018.10.002

7 Mutlu MI, Yildirim A. Effect of dietary supplementation of Panax ginseng leaf extract on production performance and egg quality of hens at the beginning of their laying period. Large Anim Rev. 2015; 26:341-348. https://doi.org/10.5713%2Fajas.15.0664

8 Lee JH, Cho SH. Korean red ginseng extract ameliorates skin lesions in NC/N ga mice: an atopic dermatitis model. J Ethnopharmacol. 2011; 133:810-817. https://doi.org/10.1016/j.jep.2010.11.020

9 Wee JJ, Park MK, Chung AS. Biological activity of ginseng and its application to human health. In: Benzie IFF, Wachel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. CRC Press; Boca Raton, FL, USA. 2011; 157-174. https://www.ncbi.nlm.nih.gov/books/NBK92776

10 Jung CH, Seog HM, Choi IW, Park MW, Cho HY. Antioxidant properties of various solvent extracts from wild ginseng leaves. LWT-Food Sci. Technol. 2006; 39:266-274. https://doi.org/10.1016/j.lwt.2005.01.004

11 Han JY, Jung SJ, Kim SW, Kwon YS, Yi MJ, Yi JS, et al. Induction of adventitious roots and analysis of ginsenoside content and the genes involved in triterpene biosynthesis inPanax ginseng. Journal of Plant Biology. 2006; 49:26-33. https://doi.org/10.1007/BF03030785

12 Yan L, Meng QW, Lee JH, Wang JP, Kim IH. Effects of dietary wild-ginseng adventitious root meal on growth performance, blood profiles, relative organ weight and meat quality in broiler chickens. Asian-Australas J Anim Sci. 2010; 24:258-263. https://doi.org/10.5713/ajas.2011.10222

13 The ISA brown commercial management guide. 2014. https://cpif.org/wp-content/uploads/2014/04/ISA-Alternative-Productions-Management-Guide-copy.pdf

14 Directive EU. Council Directive 99/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. OJEC. 1999; 203:53-7. http://data.europa.eu/eli/dir/1999/74/oj

15 Ko HS, Choi YH, Khun S, Cho ES, Kim YY, Pi JS, et al. Laying performance, egg quality, haematological traits, and faecal noxious gas emission of laying hens fed with Tenebrio molitor meal. Europ Poul Sci. 2020; 84:1612-9199. https://doi.org/10.1399/eps.2020.307

16 Langjahr M, Schubert AL, Sommer C, Uceyler N. Increased pro-inflammatory cytokine gene expression in peripheral blood mononuclear cells of patients with polyneuropathies. J Neurol. 2018; 265:618-627. https://doi.org/10.1007/s00415-018-8748-4

17 Stringer AM, Gibson RJ, Logan RM, Bowen JM, Yeoh AS, Keefe DM. Faecal microflora and β-glucuronidase expression are altered in an irinotecan-induced diarrhea model in rats. Cancer biol. 2008; 7: 1919-1925. https://doi.org/10.4161/cbt.7.12.6940

18 Muhammad AI, Mohamed DA, Chwen LT, Akit H, Samsudin A. Effect of selenium sources on laying performance, egg quality characteristics, intestinal morphology, microbial population and digesta volatile fatty acids in laying hens. Animals. 2021; 11:1681. https://doi.org/10.3390/ani11061681.

19 SAS. SAS software for PC. Release 9.3.Cary NC: SAS Institute. 2012

20 Zhang DL, Wang KM, Zhang CQ. Ginsenosides stimulated the proliferation of mouse spermatogonia involving activation of protein kinase C.J Zhejiang Univ Sci B. 2009; 10:87–92. https://doi.org/10.1631%2Fjzus.B0820133

21 Hong JK, Bong MH, Park JC, Moon HK, Lee SC, Lee JH, et al. Effect of feeding red ginseng marc on vital reaction in laying hens under stress task. Korean J. Poult. Sci. 2012; 39:63-70. https://doi.org/10.5536/KJPS.2012.39.1.063

22 Hamid MM, Moon J, Yoo D, Kim H, Lee YK, Song J, et al. Rumen fermentation, methane production, and microbial composition following in vitro evaluation of red ginseng byproduct as a protein source. J Anim Sci Technol. 2020; 62:801. https://doi.org/10.5187/jast.2020.62.6.801

23 Chrubasik S, Pittler MH, Roufogalis BD. Zingiberis rhizome: a comprehensive review on the ginger effect and efficacy profiles. Phytomedicine. 2005; 12: 684–701. https://doi.org/10.1016/j.phymed.2004.07.009

24 Kang HK, Park SB, Kim CH. Effect of dietary supplementation of red ginseng by-product on laying performance, blood biochemistry, serum immunoglobulin and microbial population in laying hens. Asian-australas J Anim Sci. 2016; 29:1464. https://doi.org/10.5713/ajas.15.0664

25 Sook CH, Lee SY. Modification of ginseng flavors by bitter compounds found in chocolate and coffee. J Food Sci. 2012; 77:202-210. https://doi.org/10.1111/j.1750-3841.2012.02716.x

26 Johnson AL, Woods DC. Dynamics of avian ovarian follicle development: cellular mechanisms of granulosa cell differentiation. Gen Comp Endocrinol. 2009; 163:12–17. https://doi.org/10.1016/j.ygcen.2008.11.012

27 Tan TQ, Ge C, Mi Y, Jin Y, Zhang C. Ginsenosides promote proliferation of granulosa cells from chicken prehierarchical follicles through PKC activation and up-regulated cyclin gene expression. Cell Bio Int. 2010; 34:769-775. https://doi.org/10.1042/cbi20090244

28 Do KH, Byun JW, Lee WK. Virulence genes and antimicrobial resistance of pathogenic Escherichia coli isolated from diarrheic weaned piglets in Korea. J Anim Sci Technol. 2020; 62:543. http://doi.org/10.5187/jast.2020.62.4.543

29 Manjunatha V, Rout M, Satish R, Muniyellappa HK, Byregowda SM. Diagnosis of various pathological conditions causing mortality in Asian koels (Eudynamys Scolopaceus). Indian J Anim Res. 2019;53(10):1367-71. http://dx.doi.org/10.18805/ijar.B-3645

30 Nolan LK, Barnes HJ, Vaillancourt JP, Abdul-Aziz T, Logue CM. Colibacillosis. Diseases of poultry. 2013; 751-805. https://doi.org/10.1002/9781119421481.ch18

31 Jazi V, Mohebodini H, Ashayerizadeh A, Shabani A, Barekatain R, Fermented. soybean meal ameliorates Salmonella Typhimurium infection in young broiler chickens. Poult Sci 2019; 98:5648-60. https://doi.org/10.3382/ps/pez338

32 Da RG, Alba DF, Silva AD, Gris A, Mendes RE, Mostardeiro VB, et al. Impact of Escherichia coli infection in broiler breeder chicks: the effect of oxidative stress on weight gain. Microb Pathog. 2020; 139:103861. https://doi.org/10.1016/j.micpath.2019.103861

33 Bi S, Qu Y, Shao J, Zhang J, Li W, Zhang L, et al. Ginsenoside Rg3 Ameliorates Stress of Broiler Chicks Induced by Escherichia coli Lipopolysaccharide. Front Vete Science. 2022; 9. https://doi.org/10.3389/fvets.2022.878018

34 Hou G, Niu J, Song F, Liu Z, Liu S. Studies on the interactions between ginsenosides and liposome by equilibrium dialysis combined with ultrahigh performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Biomed Appl. 2013; 1-7:923-924. https://doi.org/10.1016/j.jchromb.2013.01.035

35 Fukuda K, Utsumi H, Shoji J, Hamada A. Saponins can cause the agglutination of phospholipid vesicles. Biochim Biophys Acta-Bioenerg. 1985; 820:199-206. https://doi.org/10.1016/0005-2736(85)90113-0

36 Fukuda K, Utsumi H, Soda S, Shoji J, Hamada A. Specific interaction of arabinose residue in ginsenoside with egg phosphatidylcholine vesicles. Biochim Biophys Acta-Bioenerg. 1987; 900:267-274. https://doi.org/10.1016/0005-2736(87)90341-5

37 Lorent J, Le DC, Quetin LJ, Mingeot MP. Induction of highly curved structures in relation to membrane permeabilization and budding by the triterpenoid saponins, alpha-and delta-Hederin. J Biol Chem. 2013; 288:14000-14017. https://doi.org/10.1074/jbc.M112.407635

38 Keukens EA, De VT, Van BC, De WP, Plasman HH, Thiel F, et al. Molecular basis of glycoalkaloid induced membrane disruption. Biochim Biophys Acta-Bioenerg. 1995; 1240:216228. https://doi.org/10.1016/0005-2736(95)00186-7

39 Lorent J, Lins L, Domenech O, Quetin-Leclercq J, Brasseur R, Mingeot Leclercq MP. Domain formation and permeabilization induced by the saponin alpha-hederin and its aglycone hederagenin in a cholesterolcontaining bilayer. Langmuir. 2014; 30:4556-4569. https://doi.org/10.1021/la4049902

40 Jang HD, Kim HJ, Cho JH, Chen YJ, Yoo JS, Min BJ, et al. Effects of dietary supplementation of fermented wild-ginseng culture by-products on egg productivity, egg quality, blood characteristics and ginsenoside concentration of yolk in laying hens. Korean J Poult Sci. 2007; 34:271-278. https://doi.org/10.5536/KJPS.2007.34.4.271

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Table 1. Experimental feed mixing ratio (air dry basis)

Item	basic feed
Raw material feed mixing ratio, %	
Corn	62.20
Rice bran	1.53
Soybean meal, crude protein 45%	24.00
Animal fat	1.50
Limestone	8.55
Tricalcium phosphate	1.40
Vitamin-mineral additive	0.32
Sodium chloride	0.31
DL -Methionine	0.19
Sum	100.00
Nutrient content	
Metabolizable energy, kcal/kg	2,750
Crude protein, %	16.00
Calcium, %	3.50
Total P, %	0.48
Available P %	0.32
Lys dig	0.84
Met dig	0.41
Met-Cys dig	0.66
Thr	0.61
Trp	0.19

Content in kg: Vitamin A, 10,000 IU; Vitamin D, 2,000 IU; Vitamin E, 0.25 IU; vitamin K_3,2 mg; Vitamin B_{12}, 10 mg; Choline, 250 mg; folic acid, 1 mg; niacin, 30 mg; pantothenic acid, 1 0 mg; pyridoxine, 3 mg; Riboflavin, 6 mg; Thiamine, 2 mg; ethoxyquin, 125 mg; cobalt,0.3 mg; copper, 10 mg; iron, 60 mg; iodine, 0.5 mg; manganese, 40 mg; selenium, 0.2 mg; Zinc, 50 mg.

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Table 2 Cycling details of primers used for TNF alfa in this study.

Cytokine	Primer sequence	Anneal Temperature	Cycles
TNF-a NM_204267 F	DNA-GCC CCT GTA ACC AGA TG	57℃	40
TNF-a NM_204267 R	DNA-ACA CGA CAG CCA AGT CAA CG	60.2℃	40
GAPDH NM_204305 F	DNA-AGA ACA TCA TCC CAG CGT CC	58.8℃	40
GAPDH NM_204305 R	DNA-CGG CAG GTC AGG TCA ACA AC	60.6℃	40

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Table 3 Cycling details of primers used for fecal microflora DNA in this study

Microflora	Primer sequence	Anneal/Extension Temperature	Cycles
Lactobacillus spp.	F:DNA-AGC AGT AGG GAA TCT TCC A	54	40
	R:DNA-CAC CGC TAC ACA TGG AG	53.6	
Bifidobacterium spp.	F:DNA-TCG CGT CYG GTG TGA AAG	59.4	
	R:DNA-CCA CAT CCA GCR TCC AC	55.9	
Clostridium spp.	F:DNA-GGC GGC YTR CTG GGC TTT	62.1	
	R:DNA-CCA GGT GGA TWA CTT ATT GTG TTA A	56.1	
E.Coli	F:DNA-AAA ACG GCA AGA AAA AGC AG	55	
	R:DNA-GCG TGG TTA CAG TCT TGC G	58.6	
β.Actin	F:DNA-CTC CTT CCT GGG CAT GGA	57.3	
	R:DNA-CGC ACT TCA TGA TCG AGT TGA	57.8	

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Table 4. Effect of wild ginseng on laying performance

Treatments[1]	CON	WG1	WG2	SEM	*p*-value
ADFI, g/d/bird					
4 weeks	110.31	111.90	112.63	2.730	0.694
8 weeks	110.71	108.87	111.23	2.157	0.535
12 weeks	112.64	110.40	109.94	2.926	0.626
BW, kg					
4 weeks	1.86	1.91	1.88	0.051	0.594
8 weeks	1.90	1.93	1.90	0.031	0.617
12 weeks	1.93	1.94	1.92	0.030	0.674
HDEP, %					
4 weeks	92.68	93.30	93.86	0.532	0.128
8 weeks	91.98	92.36	93.08	0.461	0.092
12 weeks	91.23 [b]	92.80 [ab]	93.66 [a]	0.530	0.002
AEW, g					
4 weeks	59.14	59.82	59.46	0.908	0.760
8 weeks	60.08	60.78	61.32	0.740	0.282
12 weeks	60.75 [b]	61.46 [ab]	62.80 [a]	0.711	0.040
Egg mass, g/bird/d					
4 weeks	54.82	55.81	55.82	1.048	0.560
8 weeks	55.54	56.14	57.07	0.629	0.087
12 weeks	55.43 [b]	57.05 [ab]	58.82 [a]	0.926	0.011
FCR					
4 weeks	2.32	2.22	2.18	0.102	0.372
8 weeks	2.00	1.94	1.94	0.043	0.266
12 weeks	2.03 [a]	1.94 [a]	1.87 [b]	0.031	0.001

[1]CON, basal diet; WG1, 0.1% wild ginseng + basal diet; WG2, 0.5% wild ginseng + basal diet;.SEM, standard error of means; BW, body weight; ADFI, average daily feed intake; HDEP, hen day egg production; AEW, average egg weight; FCR, feed conversion ratio.

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Table 5. Effect of wild ginseng on egg quality

Treatments [1]	CON	WG1	WG2	SEM	*p*-value
Haugh units					
4 weeks	79.72	82.14	82.10	2.704	0.606
8 weeks	78.74	77.45	79.01	5.762	0.204
12 weeks	80.60	84.01	82.70	1.688	0.167
Yolk color					
4 weeks	7.88	7.94	8.44	0.269	0.114
8 weeks	7.44	8.20	7.74	0.447	0.270
12 weeks	7.86	7.93	8.11	0.223	0.538
Yolk weight, g					
4 weeks	14.36	15.14	15.65	0.538	0.092
8 weeks	14.85	15.00	15.63	0.757	0.570
12 weeks	16.36	16.61	17.12	0.445	0.254
Albumin weight, g					
4 weeks	38.25	39.03	39.08	1.194	0.741
8 weeks	39.68	40.21	40.11	1.283	0.910
12 weeks	38.39	38.86	39.44	0.654	0.316
Yolk percentage, %					
4 weeks	24.29	25.29	26.36	1.027	0.176
8 weeks	24.73	24.67	25.51	1.302	0.775
12 weeks	26.93	27.02	27.27	0.692	0.885
Albumin percentage, %					
4 weeks	65.97	65.33	64.28	1.255	0.421
8 weeks	66.05	66.17	65.37	1.729	0.885
12 weeks	63.19	63.22	62.80	0.704	0.803
Eggshell thickness, mm					
4 weeks	0.43	0.41	0.41	0.035	0.763

8 weeks	0.41	0.40	0.42	0.014	0.632
12 weeks	0.41	0.42	0.44	0.032	0.722
Eggshell hardness					
4 weeks	4.56	4.74	4.84	0.255	0.556
8 weeks	4.04	4.26	4.16	0.251	0.689
12 weeks	4.74	4.43	4.52	0.380	0.709

[1]CON, basal diet; WG1, 0.1% wild ginseng + basal diet; WG2, 0.5% wild ginseng + basal diet; SEM, standard error of means.

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Table 6. Effect of ginsenoside content in egg yolk by the addition of wild ginseng in the feed for laying hens.

Treatments	CON	WG1	WG2	SEM	*p*-value
Ginsenosides (mg/g)					
4 weeks	1.17	1.22	1.24	0.039	0.265
8 weeks	1.22	1.26	1.34	0.045	0.084
12 weeks	1.19 [b]	1.31 [b]	1.48 [a]	0.056	0.002

[1]CON, basal diet; WG1, 0.1% wild ginseng + basal diet; WG2, 0.5% wild ginseng + basal diet; SEM, standard error of means.

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Table 7. Effect of wild ginseng on fecal microflora DNA of laying hens.

Treatments	CON	WG1	WG2	SEM	*p*-value
Clostridium spp.					
4 weeks	0.54	0.48	0.51	0.047	0.468
8 weeks	0.57	0.54	0.54	0.037	0.721
12 weeks	0.53	0.52	0.50	0.039	0.710
Bifidobacterium spp.					
4 weeks	0.79	0.79	0.77	0.053	0.956
8 weeks	0.68	0.82	0.76	0.172	0.738
12 weeks	0.79	0.77	0.78	0.094	0.972
Lactobacillus spp.					
4 weeks	1.23	1.26	1.30	0.034	0.192
8 weeks	1.33 [b]	1.40 [ab]	1.44 [a]	0.033	0.021
12 weeks	1.26 [b]	1.36 [ab]	1.51 [a]	0.056	0.006
E. coli					
4 weeks	0.35	0.37	0.35	0.036	0.296
8 weeks	0.39	0.36	0.33	0.025	0.115
12 weeks	0.38 [a]	0.34 [ab]	0.31 [b]	0.024	0.049

[1]CON, basal diet; WG1, 0.1% wild ginseng + basal diet; WG2, 0.5% wild ginseng + basal diet; SEM, standard error of means.

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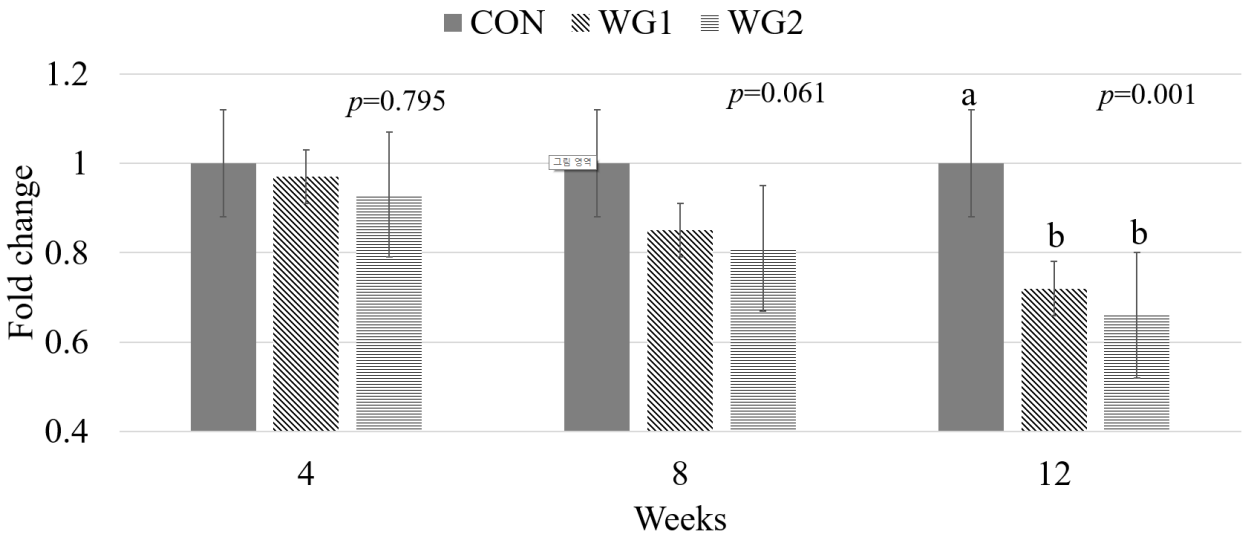

Figure 1. Effects of Wild ginseng supplementation on relative expression of TNF-α.