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The Impact of Dietary Supplementation with Fermented *Artemisia Vulgaris* and *Ipomoea Aquatica* on Egg Production, Egg Quality, Physiological Traits, and Gut Microbiota of Laying Hens

ABSTRACT

Artemisia vulgaris (mugwort) and *Ipomoea aquatica* (water spinach) are rich in nutrients and phytochemicals, yet their combined effects with multi-probiotics on laying hens remain unexplored. This study evaluated the effects of fermented mugwort and water spinach juice (FMW) on performance, physiological traits, and gut microbiota in laying hens. Sixty 67-week-old Lohmann Brown hens were randomly allocated to three dietary treatments for four weeks, with five replicates of four birds each: a control diet, 0.1% FMW, and 0.2% FMW. FMW supplementation had no significant effect on laying performance, organ weights, tibia traits, or cecal CFU counts. However, average egg weight (AEW) was higher in the control and 0.1% FMW groups than in the 0.2% group (quadratic, $p<0.01$). Yolk height was greater in the 0.1% group than in the control (quadratic, $p<0.05$), while the 0.2% group had the highest eggshell strength (linear, $p<0.05$; quadratic, $p<0.001$) and thicker shells than the 0.1% group (quadratic, $p<0.01$). Serum total cholesterol was higher in the 0.1% group than in the 0.2% group (quadratic, $p<0.05$), and HDL-cholesterol was also higher in the 0.1% group than in the control (quadratic, $p<0.05$). The 0.2% group showed reduced ileal villus height versus control and 0.1% (linear, $p<0.01$), and a lower VH/CD ratio than the 0.1% group (quadratic, $p<0.01$). Both FMW groups increased *Bacteroides* abundance (linear, $p<0.05$), and *Mediterraneibacter* tended to increase ($p=0.059$). These results suggest that FMW has potential as a functional feed additive for improving egg quality, enhancing HDL-cholesterol levels, and modulating gut microbiota in laying hens.

INTRODUCTION

Throughout the twentieth century, antibiotic production expanded exponentially, driven by the development of synthetic derivatives of naturally occurring antibiotic molecules and the creation of entirely synthetic compounds. However, the extensive use of antibiotics has led to the rapid emergence of bacterial resistance, increasing the prevalence of dangerous pathogens (Larsson, 2014). In recent years, many developed countries have enacted legislation prohibiting the use of antibiotics in animal feed for growth promotion. This legislative action reflects concerns about the role of such practices in exacerbating antibiotic resistance (Centner, 2016). Consequently, there is a growing market demand for clean and safe products, sparking interest in plant-based feed additives such as oils, extracts, and plant processing by-products. These additives have been shown to provide effective nutritional strategies, positively influencing poultry performance and production efficiency (Righi *et al.*, 2021).



The term “probiotic” was first introduced in 1965 to describe substances produced by microorganisms that promote the growth of beneficial organisms. Probiotics are now widely used to establish microbial balance and prevent disease in humans. For instance, *Lactobacillus rhamnosus* GG has been shown to significantly benefit intestinal immunity by increasing the number of IgA-secreting cells and other immunoglobulins in the intestinal mucosa. Additionally, it stimulates local interferon release and enhances antigen transport to lymphocytes, thereby boosting antigen expression in Peyer's patches (Gupta & Garg, 2009).

Artemisia vulgaris (mugwort) is a widespread distributed herbaceous plant characterized by significant morphological and phytochemical variability, depending on its geographic origin. Mugwort has a long history of human use and is valued for its essential oils, flavonoids, and sesquiterpenoid lactones, which have attracted considerable scientific interest. Research highlights the beneficial properties of mugwort, including its antioxidant, hepatoprotective, cytotoxic, antibacterial, and antifungal activities (Ekiert *et al.*, 2020). Similarly, *Ipomoea aquatica* (water spinach) is recognized for its high content of vitamins, minerals, carotenoids, and flavonoids, making it a valuable nutritional resource. Studies have shown that water spinach offers therapeutic benefits for various diseases, including cancer and diabetes (Manvar & Desai, 2013). Moreover, dietary inclusion of water spinach in broiler chickens has been shown to enhance growth performance and nutrient digestibility without negative effects, presenting a cost-effective alternative to expensive vitamin-mineral supplements (Maung *et al.*, 2020).

Fermented foods have been integral to diverse dietary traditions across cultures for centuries, valued for their numerous health benefits, such as antioxidant, antibacterial, antifungal, anti-inflammatory, antidiabetic, and antiatherosclerotic properties (Şanlier *et al.*, 2019). Fermentation of mugwort (*Artemisia annua* L.) has been shown to enhance its antimicrobial and antioxidant activities, with notable improvements observed in egg quality and Haugh units in poultry (Lee, 2015). However, the combined use of fermented mugwort and water spinach as a functional feed additive has yet to be explored. This study introduces a novel approach by integrating these two phytochemical-rich plant species to investigate their potential synergistic effects in poultry nutrition.

It is hypothesized that fermented mugwort and water spinach juice (FMW), due to their abundant bioactive compounds and functional properties, may provide substantial benefits to laying hens. The present study aims to evaluate the effects of FMW supplementation on laying performance, egg quality, blood parameters, visceral organ indices, intestinal histology, tibia characteristics, and gut microbiota composition.

MATERIALS AND METHODS

Ethical statement

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Konkuk University, Seoul, Republic of Korea (Approval number: KU24233).

Isolation and identification of bacteria from mugwort and water spinach

The mugwort and water spinach used in this experiment were sourced from a local market in Gia Lam, Hanoi, Vietnam. To isolate and identify bacteria from mugwort and water spinach, the plants were chopped and combined with molasses in a 4:1 ratio. Specifically, 400 mL of mugwort was mixed with 100 mL of molasses, and 400 mL of water spinach was mixed with 100 mL of molasses. The mixtures were incubated in a shaking incubator at 30°C for 24 hours, then stored at 4°C. Bacterial culture was prepared on de Man, Rogosa and Sharpe (MRS) agar (Man, Rogosa, and Sharpe, Difco, France) to isolate and identify bacterial strains from mugwort and water spinach. 100 µL of each molasses–mugwort and molasses–spinach mixture was spread evenly on culture plates and incubated at 30°C for 24 hours. A single bacterial colony was isolated from each plate and subcultured three times to ensure purity. The bacterial strains were then identified through 16S rRNA sequencing, conducted by at Macrogen Company in Seoul, South Korea.

Fermented mugwort and water spinach

The mugwort and water spinach were dropped into small pieces using a knife and blended with water at specific ratios. Mugwort was mixed with water in a 3:2 ratio (600 mL mugwort and 400 mL water), while water spinach was blended in a 2:1 ratio (700 mL of water



spinach and 350 mL of water). The resulting mixtures were stored separately in 1.5 L plastic bottles at 4°C. For fermentation, four microorganisms—SK6219 (*Pediococcus pentosaceus*), SK6129 (*Lactiplantibacillus plantarum*), SK6130 (*Lactiplantibacillus pentosus*), and SK6132 (*Pichia kudriavzevii*)—were isolated from mugwort and water spinach and specifically selected for the fermentation process with these vegetables. Four types of microorganisms were cultured in MRS broth at 30°C for 24 hours in a shaking incubator. Fermented mugwort juice was prepared by combining 1 L of mugwort juice, 1 L of distilled water, 10 mL of molasses, and 50 mL culture of the four microorganisms. Similarly, fermented water spinach juice was made using 1 L of spinach juice, 1 L of distilled water, 10 mL of molasses, and 50 mL culture of the four microorganisms. The fermentation mixtures were stored in 5 L Erlenmeyer flask, vigorously agitated, and incubated at 30°C with stirring (100 rpm) for 24 hours. The pH was adjusted to approximately seven prior to fermentation. After fermentation, the juices were transferred to glass jars and stored at 4°C.

Diets and design of animal experiments

A total of 60 67-week-old Lohman Brown laying hens were randomly assigned to three treatment groups, with five replicates of four birds per group. The hens were housed in a two-tier battery cage system, with two birds per cage (43 cm × 45 cm × 42 cm, length × width × height). The basal diet used was formulated to meet the nutritional requirements specified in the 2017 Korean Poultry Feeding Standard (Table 1). Fermented mugwort and water spinach juices were incorporated into the basal diet at levels of 0%, 0.1%, and 0.2%. Diets were mixed for 10 minutes using a food mixer (DKM-350SU, Daekwang, Hwaseong, Korea). The laying hens underwent a two-week adaptation period before the experimental diets were administered. The feeding trial lasted four weeks, from 69 to 73 weeks of age. Throughout the experiment, hens were provided *ad libitum* access to feed in mash form and water. Lighting conditions were maintained at 16 hours of light and 8 hours of darkness, with the cage temperature controlled at 24°C. On the final day of the experiment, 18 hens (six from each treatment group) were randomly selected and euthanized using CO₂ gas. Blood, tibia, and organ samples were collected for further analysis.

Table 1 – Ingredients and nutrient composition of the basal diet.

Items	Level
Ingredient, %	
Corn	56.93
Dried distillers' grains with solubles	15.0
Soybean meal (crude protein, 45%)	5.54
Wheat gluten	4.12
Rapeseed meal	2.96
Sesame oil meal	2.04
Beef tallow	0.48
Limestone	11.49
Monocalcium phosphate	0.51
Methionine	0.16
Lysine sulfate	0.30
Threonine	0.02
NaCl	0.24
Choline chloride	0.02
Vitamin Premix ¹	0.07
Mineral Premix ²	0.12
Total	100.0
Calculated chemical composition	
Crude protein, %	15.00
Crude fat, %	3.82
Crude fiber, %	2.73
Crude ash, %	12.86
Calcium, %	4.20
Available phosphorus, %	0.53
AMEn, kcal/kg ³	2,700

¹ Vitamin mixture provided the following nutrients per kg of diet: Vitamin A, 20,000 IU; vitamin D3, 4600 IU; vitamin E, 40 mg; vitamin K3, 4 mg; vitamin B1, 3.6 mg; vitamin B2, 8 mg; vitamin B6, 5.8 mg; vitamin B12, 0.04 mg.

² Mineral mixture provided the following nutrients per kg of diet: Fe, 70 mg; Cu, 7.5 mg; Zn, 60 mg; Mn, 80 mg; I, 1 mg; Co, 0.1 mg; Se, 0.2 mg.

³ AMEn: Nitrogen-corrected apparent metabolizable energy.

Egg productivity

The number of eggs laid by birds in each replicate was recorded daily at 10 a.m. and expressed as a percentage of egg production. The hen-day egg production rate (EPR) was calculated by dividing the total number of eggs collected by the number of live hens in each replicate on a given day (Xu *et al.*, 2006). Average egg weight (AEW) was determined by weighing all eggs laid within each treatment group on a single designed day each week. Egg mass was calculated by multiplying the EPR by the AEW. Feed intake (FI) was measured weekly by calculating the difference between the amount of feed provided and the amount of feed remaining in the feeders for each chicken. The feed conversion ratio (FCR) was determined by dividing FI by the daily egg mass (Bonekamp *et al.*, 2010).



Egg quality

Each week, 60 eggs were collected and subjected to various quality assessments, including egg weight, eggshell weight, albumen height, Haugh unit, yolk color, eggshell strength, eggshell color, and eggshell thickness. A digital egg tester (NABEL Co. Ltd., Japan) was used to measure all parameters except eggshell weight. For the latter, the eggshells were separated from the inner shell membranes (Kim *et al.*, 2004), dried for 24 hours, and weighed using a digital scale (SPX2202KR, OHAUS Co., USA). The Haugh unit was calculated using the formula (Haugh, 1937): $\text{Haugh unit} = 100 \times \log (H + 7.57 - (1.7 \times W^{0.37}))$, where H is the albumen height (mm) and W is the egg weight (g).

Blood biochemical characteristics

Blood samples were collected via cardiac puncture following CO₂ asphyxiation, yielding approximately 10 mL of blood from each bird. Clot activator tubes were used to preserve the blood samples, which were subsequently stored in a refrigerator before analysis. Serum was separated by centrifuging the samples at 1,500 rpm for 10 minutes using a centrifuge (HA-1000-3, Hanil Science Medical, Daejeon, South Korea). The separated serum was stored at –20°C until biochemical analysis was performed. Biochemical parameters analyzed included aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, glucose, total cholesterol (TC), total protein (TP), triglyceride (TG), inorganic phosphorus (IP), and high-density lipoprotein cholesterol (HDL-C). These parameters were measured using an automatic biochemical analyzer (CHEM 7000i, Fujifilm Corp., Tokyo, Japan).

Organ characteristics

Following blood sample collection, the internal organs of each bird were harvested. The intestines were divided into four sections for measurement: the duodenum, measured from the pancreatic ring; the jejunum, measured from the end of the pancreatic ring to Meckel's diverticulum; the ileum, measured from Meckel's diverticulum to the ileocecal junction; and the cecum, represented by the average length of the right and left ceca. Each segment was measured using a tape measure, and the lengths were recorded. Additionally, the liver and spleen were harvested alongside the intestinal samples and weighed using a digital scale (SPX2202KR, OHAUS Co., USA).

Tibia characteristics

At the end of the experiment, the left tibiae of randomly selected birds were collected. Nonbone tissues such as fat, tendons, and muscle were carefully removed. Cleaned tibiae were sealed in plastic bags to retain moisture and stored at 4°C for one day. The weight of each tibia was recorded, and its length and width were measured using a micrometer caliper. Tibial strength was assessed using a three-point bending test following ASAE Standard S459 (2001). The test was performed with an Instron universal tester (Model 3342, USA) set to a 50 kg load range and a crosshead speed of 200 mm/min. Each tibia was supported on a 4 cm span during the test (Ndazigaruye *et al.*, 2019).

Intestinal histomorphology

Samples from the jejunum and ileum, each measuring 2 cm in length, were collected and fixed in 10% formalin solutions, respectively. After fixation, the samples were sent to a slide production company (KP&T, Cheongju, South Korea) for hematoxylin and eosin staining. The stained slides were examined under a microscope (BX43, Olympus, Tokyo, Japan), and the images were analyzed using eXcope X3 software (DIXI Science, Daejeon, South Korea). Villus height (VH) and crypt depth (CD) were measured at five distinct points in each sample, and the averages were calculated. The results were expressed as the VH and CD ratio.

Population size of bacteria in the cecum

The contents of the cecum were stored in a dry ice box before the experiment started. These contents were subsequently transferred to a 50 mL conical tube (Falcon, Arizona, USA). Various media were utilized for microorganism cultivation, including Reasoner's 2A agar (R2A; Difco) for total bacterial enumeration; de Man, Rogosa Sharpe (MRS; Difco) for lactobacilli isolation; MacConkey agar (Difco) for coliform bacteria identification, and Shigella and Salmonella agar (SS; Difco) to isolate Shigella and Salmonella. The samples were diluted in distilled water across a series of concentrations ranging from 10⁻¹ to 10⁻⁶. These dilutions were then spread onto the respective media for colony counting. The cultures were incubated at 30°C for 24–48 hours. After incubation, the colony numbers were counted, calculated, and expressed as log CFU/g.



Cecal microbiota

Three birds were randomly selected from each treatment group, and 1 g of cecal contents from each bird was collected and quenched in liquid nitrogen. PCR conditions, DNA extraction, bioinformatics, and NGS sequencing analysis were conducted as described (Niu *et al.*, 2019). Genomic DNA was extracted using the PowerSoil DNA Isolation Kit (Mobio Laboratories, Inc., Carlsbad, CA, USA). The V3–V4 region of the bacterial 16S rRNA gene was amplified using primers 341F and 785R. Sequencing was performed on the Illumina Miseq platform through Macrogen’s commercial service (Seoul, Korea). Alpha diversity indices, including amplicon sequence variants (ASVs), Chao1, Shannon, and Gini–Simpson indices, were calculated for comparison. Beta diversity was evaluated using principal coordinate analysis (PCoA) and unweighted pair group mean analysis (UPGMA) based on the UniFrac distance matrix.

Statistical analysis

A completely randomized design was employed for data analysis, which was conducted using the general linear model procedures in SAS 9.4 (SAS Institute, Cary, NC, USA). Orthogonal polynomial contrasts were applied to evaluate the linear and quadratic effects of fermented mugwort and water spinach juice supplementation on laying hens, according to the supplementation levels. Laying performance data were analyzed based on replicates, while egg quality assessments were conducted on an individual egg basis. Blood parameters, organ weights, tibia measurements, and intestinal microbiota were analyzed using each bird as the experimental unit. Differences between group means were determined using linear and quadratic models, with Duncan’s multiple range test at a significance level of $p<0.05$. A significant level of $0.05 < p < 0.01$ was indicated as a trend. The results are presented as means with their standard errors (SEM).

RESULTS AND DISCUSSION

Identification of bacteria in fermented mugwort and water spinach

The identification results for six bacteria isolated from fermented mugwort and fermented water spinach are summarized in Table 2. This research focused on selecting microorganisms with beneficial properties for fermented juices, emphasizing their antibacterial effects, immune-enhancing capabilities,

and toxin mitigation potential. The characteristics of the isolated bacteria are detailed below. *Lactiplantibacillus plantarum* is frequently observed in the fermentation of plant-based foods (Molin, 2001). It is recognized for its cholesterol-lowering properties and antibacterial effects (Nguyen *et al.*, 2007). *Lactiplantibacillus pentosus* is known for its health-promoting properties such as immunomodulatory, antiproliferative activities, making it a valuable probiotic source (Stergiou *et al.*, 2021). *Nakaseomyces glabratus* is a yeast species that can grow in a filamentous form and produce blastospores (Legrand *et al.*, 2016). However, it is also an infectious pathogen that can infect the urinary tract and, in some cases, cause systemic pineal infections through bloodstream dissemination (Xu *et al.*, 2013). *Pichia kudriavzevii* is a yeast strain commonly isolated from soil and fruit. It is characterized by its ability to form pseudohyphae and biofilms under nutrient-limited conditions (Wulan *et al.*, 2021). Additionally, *Pichia kudriavzevii* is notable for its ability to degrade mycotoxins and toxic compounds. Its high aflatoxin absorption capacity makes it an excellent candidate for use as a feed additive (Chu *et al.*, 2023). *Pediococcus pentosaceus* is used as a flavor-enhancing and preservation agent in animal products. *Pediococcus* strains also possess anti-inflammatory, anticancer, antioxidant, detoxifying and lipid-lowering properties (Jiang *et al.*, 2021). Based on these characteristics, four strains were selected for further use, including SK6129 (*Lactiplantibacillus plantarum*), SK6130 (*Lactiplantibacillus pentosus*), SK6132 (*Pichia kudriavzevii*), and SK6221 (*Pediococcus pentosaceus*). Due to the pathogenic potential of SK6131 (*N. glabratus*), it was excluded from consideration. Furthermore, SK6219 and SK6221 were identified as the same strain of *P. pentosaceus*, and SK6219 was selected as the representative strain for use in the study.

Table 2 – Identification of mugwort and water spinach inhabiting bacteria.

Stock #	Scientific name	Query cover (%)	Identity (%)	Isolated on media
SK6129	<i>Lactiplantibacillus plantarum</i>	98	99.79	MRS
SK6130	<i>Lactiplantibacillus pentosus</i>	92	100	MRS
SK6131	<i>Nakaseomyces glabratus</i>	98	99.79	MRS
SK6132	<i>Pichia kudriavzevii</i>	100	100	MRS
SK6219	<i>Pediococcus pentosaceus</i>	99	100	MRS
SK6221	<i>Pediococcus pentosaceus</i>	94	99.54	MRS



The incorporation of multistrain probiotics through the fermentation of mugwort and water spinach offers promising potential to enhance both the productivity and physiological traits of laying hens, offering an innovative and sustainable method for advancing poultry production systems.

Egg productivity

Table 3 presents the effects of supplementing fermented mugwort and water spinach on the laying performance of hens. The highest EPR across all three treatment groups occurred during the third week, although no significant differences were observed. Overall, there were no obvious differences in EPRs among the three groups, and the egg production rate remained consistently stable throughout the observation period. Regarding AEW, the FMW 0.1% group tended to show higher values than the FMW 0.2% group during the third week ($p=0.053$). Throughout the entire experimental period, both the CON and FMW 0.1% groups showed higher AEW than the FMW 0.2% group (quadratic, $p<0.01$). In the second week, FI was lower in the CON and FMW 0.1% groups compared to the FMW 0.2% group (quadratic, $p<0.01$), and the FMW 0.1% group had a lower FCR than the FMW 0.2% group (quadratic, $p<0.05$). However, no significant differences in laying performance were found between the treatment groups throughout the experiment. To assess the immediate impact of feed additives on laying performance in older laying hens prior to slaughter, a four-week feeding trial was conducted. It is anticipated that the practical and economic values will be better understood in the evaluation of a short-term trial such as this experiment.

Proper fermentation can enhance the beneficial contents of foods, improving biological function and bioavailability (Kim *et al.*, 2016). In this study, diets containing fermented vegetable juice in the FMW 0.1% and FMW 0.2% groups had no effect on egg production, egg mass, FI, and FCR over the entire study period. This finding is consistent with research comparing fermented and nonfermented *Lactobacillus plantarum* and *Artemisia annua* (Lee *et al.*, 2019). However, the AEW was significantly higher in hens fed fermented vegetable juice, a result similar to studies involving black cumin seeds and White Leghorn chickens (Khan *et al.*, 2013). Additionally, research by Ekiert *et al.* (2020) indicated that *A. vulgaris* with its aromatic scent and bitter taste, stimulates digestive secretions in the treatment of anorexia, which aligns

with this observed difference in FI in this study. The hens fed FMW 0.2% exhibited higher FI in the second week. During the second week, FI appeared to increase; however, this did not seem to have a notable impact on overall feed consumption across the entire experimental period. Another study on broiler diets using water spinach (Maung *et al.*, 2020) found no effect on weight gain and FCR when chopped spinach was used. Given the relatively short duration of the present study, conducted over a four week period, the observed production outcomes may not fully reflect the long term effects of dietary FMW supplementation. Accordingly, extended feeding trials encompassing distinct phases of the laying cycle, including early, peak, and late periods, are necessary to verify the sustained efficacy of fermented mugwort and water spinach juice and to further elucidate their potential as functional feed additives in commercial poultry production systems.

Table 3 – Effect of dietary supplementation with fermented mugwort and water spinach on the productivity of laying hens.

	Items ¹	Treatment ²			SEM ³		p-value	
		CON	FMW 0.1%	FMW 0.2%			Linear	Quadratic
1 Week	EPR, %	93.57	87.14	91.43	1.69	0.606	0.139	
	AEW, g	61.10	62.04	59.94	0.56	0.403	0.208	
	Egg mass, g/hen/day	57.13	54.28	54.89	1.18	0.448	0.496	
	FI g/hen/day	129.46	134.43	130.39	2.55	0.884	0.414	
	FCR, g feed/g egg	2.30	2.69	2.44	0.10	0.561	0.130	
2 Week	EPR, %	92.14	92.86	92.14	1.42	1.000	0.816	
	AEW, g	61.18	61.72	60.43	0.61	0.617	0.485	
	Egg mass, g/hen/day	56.35	57.06	55.80	1.09	0.839	0.679	
	FI g/hen/day	135.5 ^b	126.18 ^b	142.89 ^a	1.93	0.089	0.001	
	FCR, g feed/g egg	2.46 ^{ab}	2.27 ^b	2.64 ^a	0.07	0.267	0.047	
3 Week	EPR, %	94.29	94.29	92.86	1.37	0.677	0.810	
	AEW, g	61.43 ^{ab}	62.86 ^a	58.77 ^b	0.68	0.105	0.053	
	Egg mass, g/hen/day	58.08	58.62	54.77	1.14	0.242	0.368	
	FI g/hen/day	147.11	148.96	145.22	1.90	0.690	0.495	
	FCR, g feed/g egg	2.59	2.63	2.70	0.07	0.500	0.913	
4 Week	EPR, %	93.57	90.71	91.43	1.74	0.622	0.636	
	AEW, g	61.79	62.64	60.19	0.65	0.313	0.231	
	Egg mass, g/hen/day	57.94	56.18	54.87	1.24	0.320	0.933	
	FI g/hen/day	129.04	129.54	134.21	2.20	0.345	0.659	
	FCR, g feed/g egg	2.28	2.40	2.58	0.09	0.174	0.872	
Overall period	EPR, %	93.39	91.25	91.96	0.78	0.457	0.390	
	AEW, g	61.37 ^a	62.31 ^a	59.83 ^b	0.31	0.040	0.009	
	Egg mass, g/hen/day	57.38	56.79	55.16	0.58	0.122	0.674	
	FI g/hen/day	135.28	134.78	138.18	1.15	0.306	0.427	
	FCR, g feed/g egg	2.41	2.50	2.59	0.04	0.101	0.259	

^{a, b} Means within a same row with different letters differ significantly at $p<0.05$. ¹Abbreviations: EPR, egg production rate; AEW, average egg weight; FI, feed intake; FCR, feed conversion ratio. ²CON, basal diet; FMW 0.1%, basal diet with 0.1% FMW; FMW 0.2%, basal diet with 0.2% FMW. ³SEM, standard error of the means.



Egg quality

The effects of treatment groups containing fermented mugwort and fermented water spinach on egg quality are presented in Table 4.

Table 4 – Effect of dietary supplementation with fermented mugwort and water spinach on egg quality.

	Items	Treatment ¹			SEM ²	p-value	
		CON	FMW 0.1%	FMW 0.2%		Linear	Quadratic
1 Week	Egg weight, g	61.1	62.04	59.94	0.56	0.403	0.208
	Eggshell weight, g	5.91	5.82	6.10	0.09	0.414	0.379
	Yolk height, mm	7.46	7.77	7.25	0.15	0.567	0.194
	Haugh unit	85.73	87.39	84.19	1.02	0.542	0.266
	Yolk color	8.04	7.81	7.92	0.06	0.379	0.163
	Eggshell strength, kg/cm ²	4.45 ^{ab}	3.85 ^b	4.80 ^a	0.14	0.261	0.006
	Eggshell thickness	0.41	0.40	0.43	0.01	0.323	0.227
	Eggshell color	25.39	25.18	24.16	0.66	0.458	0.780
2 Week	Egg weight, g	61.18	61.72	60.43	0.61	0.617	0.485
	Eggshell weight, g	5.98	5.91	6.11	0.09	0.532	0.479
	Yolk height, mm	7.58 ^b	8.16 ^a	7.72 ^{ab}	0.10	0.536	0.013
	Haugh unit	85.4	88.99	86.79	0.79	0.466	0.084
	Yolk color	7.84	7.71	7.69	0.07	0.410	0.726
	Eggshell strength, kg/cm ²	4.32	3.97	4.57	0.15	0.512	0.149
	Eggshell thickness	0.42	0.41	0.43	0.01	0.254	0.165
	Eggshell color	25.39	25.18	24.16	0.66	0.981	0.845
3 Week	Egg weight, g	61.43 ^{ab}	62.86 ^a	58.77 ^b	0.68	0.105	0.053
	Eggshell weight, g	5.84	5.60	5.90	0.10	0.788	0.220
	Yolk height, mm	7.72	7.97	8.04	0.16	0.418	0.801
	Haugh unit	86.61	87.97	89.93	1.09	0.221	0.838
	Yolk color	7.80	7.88	7.86	0.07	0.736	0.771
	Eggshell strength, kg/cm ²	4.12	3.82	4.55	0.14	0.219	0.095
	Eggshell thickness	0.41	0.40	0.42	0.01	0.616	0.168
	Eggshell color	24.92	24.64	23.88	0.55	0.448	0.839
4 Week	Egg weight, g	61.79	62.64	60.19	0.65	0.313	0.231
	Eggshell weight, g	5.86	5.92	5.97	0.09	0.612	0.994
	Yolk height, mm	7.76	8.11	7.86	0.14	0.770	0.313
	Haugh unit	86.95	89.4	88.42	0.85	0.484	0.346
	Yolk color	7.74	7.60	7.85	0.06	0.420	0.113
	Eggshell strength, kg/cm ²	4.08	4.03	4.62	0.12	0.067	0.211
	Eggshell thickness	0.41	0.40	0.42	0.01	0.504	0.280
	Eggshell color	24.69	25.34	23.98	0.53	0.586	0.373
Overall period	Egg weight, g	61.37 ^a	62.31 ^a	59.83 ^b	0.31	0.040	0.009
	Eggshell weight, g	5.89	5.81	6.02	0.05	0.268	0.146
	Yolk height, mm	7.58 ^b	7.97 ^a	7.68 ^{ab}	0.07	0.571	0.029
	Haugh unit	86.17	88.39	87.33	0.47	0.315	0.102
	Yolk color	7.85	7.75	7.83	0.03	0.757	0.175
	Eggshell strength, kg/cm ²	4.24 ^b	3.92 ^b	4.63 ^a	0.07	0.017	<0.001
	Eggshell thickness	0.41 ^{ab}	0.40 ^b	0.42 ^a	0.003	0.099	0.010
	Eggshell color	24.71	24.69	23.96	0.29	0.292	0.571

^{a, b} Means within a same row with different letters differ significantly at $p < 0.05$. ¹CON, basal diet; FMW 0.1%, basal diet with 0.1% FMW; FMW 0.2%, basal diet with 0.2% FMW. ²SEM, standard error of the means.

A significant difference in overall egg weight was observed between the CON and the FMW 0.1% group, with the latter having a higher weight than the FMW 0.2% group (linear, $p < 0.05$; quadratic, $p < 0.01$). In week 3, the FMW 0.1% group tended to have a higher egg weight than the FMW 0.2% group (quadratic, $p = 0.053$). However, no differences were observed between the experimental groups in terms of the eggshell weight, Haugh unit, yolk color, and eggshell color. In the first week, the FMW 0.2% group exhibited stronger eggshell strength compared to the FMW 0.1% group (quadratic, $p < 0.01$). In the second week, the yolk height index of the FMW 0.1% group was higher than the control group (quadratic, $p < 0.05$). Over the entire experimental period, the FMW 0.1% group maintained higher yolk height compared to the CON group (quadratic, $p < 0.05$). Additionally, the FMW 0.2% group demonstrated stronger eggshell strength than both the CON and FMW 0.1% groups (linear, $p < 0.05$; quadratic, $p < 0.001$), and increased eggshell thickness compared to the FMW 0.1% group (quadratic, $p < 0.01$).

In this study, the inclusion of fermented vegetable juice in the diet of laying hens did not affect egg weight, eggshell weight, Haugh units, yolk color, eggshell thickness, and eggshell color. However, there were significant differences in the FMW 0.1% group regarding yolk height and in the FMW 0.2% group with eggshell strength. Previous studies have shown that mugwort contains phenols, flavanols, and flavonoids that exhibit antioxidant activity, which may be helpful for treating diseases related to oxidative stress (Temraz & El-Tantawy, 2008). Additionally, water spinach contains amino acids such as threonine, leucine, lysine, histidine and arginine, which support development and production in laying hens (Rao & Tuhina Vijay, 2002). The fermented mugwort and water spinach had no adverse effects on overall egg quality. Instead, the bioactive compounds produced during fermentation suggest potential benefits, particularly in enhancing eggshell strength and yolk height, highlighting their potential as functional feed additives in poultry production.

Blood chemical characteristics

Table 5 presents the results of blood biochemical characteristics. No statistically significant differences were observed in the indicators of calcium, IP, glucose, creatine, TP, aspartate aminotransferase, alanine aminotransferase, and triglyceride. However, the FMW 0.1% group exhibited a higher TC level compared to



the FMW 0.2% group (quadratic, $p < 0.05$). Additionally, the HDL-cholesterol level was significantly higher in the FMW 0.1% group than in the control group (quadratic, $p < 0.05$).

Previous studies have shown that adding mugwort to the diet of rats has a hypolipidemic effect (Ekiert *et al.*, 2020). Khan (2015) reported that mugwort root extract significantly normalized lipid profiles and notably increased HDL-cholesterol levels by 68 mg/dL. Similarly, Sivaraman & Muralidaran (2010) observed hypoglycemic effects using 200 and 400 mg/kg water spinach extract in Swiss albino rats over 30 days. Mugwort contains steroidal saponins that increase the activity of lipoprotein lipase, helping to remove free fatty acids from circulation. In addition, steroidal saponins increase biliary cholesterol secretion and reduce cholesterol absorption (El-Tantawy, 2015). Earlier studies that used mugwort alone in rodents yielded promising results, and this study achieved similar outcomes by combining fermented mugwort with water spinach. Building on previous findings, this study used a combination of fermented mugwort and water spinach to assess their impact on blood characteristics. Despite differences in treatment and animal subjects, similar results were observed, with the FMW 0.1% group of laying hens showing a significant increase in HDL-cholesterol levels. This suggests no adverse effects on blood health and a positive influence on HDL levels, suggesting that FMW may have potential as a feed additive with beneficial effects on blood health.

Table 5 – Effect of dietary supplementation with fermented mugwort and water spinach on blood chemical characteristics.

Items ¹	Treatment ²			SEM ³	p-value	
	CON	FMW 0.1%	FMW 0.2%		Linear	Quadratic
Calcium, mg/dL	33.36	36.47	31.96	0.98	0.543	0.070
IP, mg/dL	4.80	5.67	3.49	0.68	0.446	0.310
Glucose, mg/dL	255.17	255.33	252.50	2.42	0.677	0.786
Creatine, mg/dL	0.18	0.14	0.16	0.01	0.355	0.182
TC, mg/dL	160.83 ^{ab}	199.33 ^a	139.17 ^b	10.77	0.366	0.027
TP, g/dL	7.07	7.25	7.70	0.32	0.448	0.852
AST, U/L	181.5	162.33	187.00	7.91	0.782	0.214
ALT, U/L	10.50	10.17	20.50	3.64	0.283	0.504
TG, mg/dL	2716.67	3096.67	1908.33	259.92	0.199	0.153
HDL-cholesterol, mg/dL	67.83 ^b	82.50 ^a	71.00 ^{ab}	2.85	0.621	0.029

^{a, b} Means within a same row with different letters differ significantly at $p < 0.05$.

¹Abbreviations: EPR, egg production rate; AEW, average egg weight; FI, feed intake; FCR, feed conversion ratio.

²CON, basal diet; FMW 0.1%, basal diet with 0.1% FMW; FMW 0.2%, basal diet with 0.2% FMW.

³SEM, standard error of the means.

Organ characteristics

The intestinal length and internal organ weight of laying hens fed diets supplemented with fermented mugwort and fermented water spinach are presented in Table 6. No significant differences were observed in the lengths of the duodenum, jejunum, and cecum, nor in the weight of the liver and spleen.

Diet and its components can cause changes in the structure and size of internal organs. An increase in gut length may lead the bird to require more maintenance energy, while reducing energy input. Additionally, digestive substances are stored longer in the intestine (Marume *et al.*, 2020). Therefore, a reduction in intestinal length can lead to more efficient food utilization and better nutrient digestibility (Reis *et al.*, 2017). In this study, the inclusion of FMW for 4 weeks did not affect the intestinal length or organ weight between groups. This finding contrasts with Chen *et al.* (2005), who reported significant effects from using dietary prebiotic chicory. Variations in results may be attributed to differences in the type of additive, the fermentation process, and the age of the chickens.

The liver is a vital organ involved in detoxification, digestion, metabolism, and immunity, while the spleen plays a critical role in the immune system. An increase in the size of both the liver and spleen may indicate infections, heightened immune responses, or liver conditions such as fatty liver, hepatitis, or cancer (Julian, 2005, Whitehead, 1979, William & Corazza, 2007). The results of this experiment suggest that including FMW in the diet of laying hens had no noticeable impact on organ size or function. This indicates that FMW supplementation does not adversely affect organ health, supporting its safety for laying hens. Further studies are recommended to explore the broader effects of FMW on organ characteristics and overall health.

Table 6 – Effect of dietary supplementation with fermented mugwort and water spinach on organ characteristics.

Items	Treatment ¹			SEM ²	<i>p</i> -value	
	CON	FMW 0.1%	FMW 0.2%		Linear	Quadratic
Relative intestinal length						
Duodenum, cm	30.50	28.83	31.67	0.69	0.489	0.135
Jejunum, cm	66.83	65.83	64.50	1.73	0.610	0.966
Ileum, cm	66.00	64.83	67.83	1.07	0.505	0.384
Cecum, cm	15.83	16.75	17.58	0.47	0.149	0.967
Visceral organ weight						
Liver, g	45.92	43.92	41.56	1.58	0.288	0.958
Spleen, g	1.98	1.83	1.72	0.10	0.336	0.923

¹CON, basal diet; FMW 0.1%, basal diet with 0.1% FMW; FMW 0.2%, basal diet with 0.2% FMW.

²SEM, standard error of the means.



Tibia characteristics

Table 7 presents the tibia characteristics of laying hens. No statistical differences were observed in tibial characteristics, including bone weight, bone length, bone width, and breaking strength, across the experimental groups.

Bones are essential for supporting movements, productivity, and overall health in chickens. They are rich in calcium, a critical nutrient necessary for maintaining bone strength and quality (Olgun & Aygun, 2016, Zhao *et al.*, 2020). Additionally, decreased marrow bone quality may be linked to the age of the laying hen (Yamada *et al.*, 2021). Świątkiewicz *et al.* (2018) found that laying hens fed diets supplemented with probiotic bacteria, herbal extracts, or chitosan had higher fracture strength, load-bearing capacity, and mineralization compared to hens that were not supplemented. Additionally, Angel *et al.* (2005) discovered that bone mineralization and strength were improved in parallel with increased calcium and phosphorus retention when supplemented with *Lactobacillus* probiotics. Conversely, Leskovec *et al.* (2018) reported no positive effects of plant extracts on the bone characteristics of broilers. In the present study, no significant differences in tibial indices were found among the experimental groups, which may be attributed to factors such as the duration of supplementation or specific experimental conditions. Further research under varying conditions may help clarify the effects of FMW supplementation on tibial characteristics.

Table 7 – Effect of dietary supplementation with fermented mugwort and water spinach on tibia characteristics.

Items	Treatment ¹			SEM ²	p-value	
	CON	FMW 0.1%	FMW 0.2%		Linear	Quadratic
Bone weight, g	12.69	13.08	12.62	0.21	0.893	0.369
Bone length, cm	11.92	12.20	12.00	0.08	0.660	0.153
Bone width, mm	7.60	7.83	7.81	0.10	0.436	0.581
Breaking strength, kgf	21.76	20.63	20.25	0.79	0.135	0.569

¹CON, basal diet; FMW 0.1%, basal diet with 0.1% FMW; FMW 0.2%, basal diet with 0.2% FMW. ²SEM, standard error of the means.

Intestinal histomorphology

Table 8 presents the effects of fermented mugwort and fermented water spinach on the intestinal histomorphology of laying hens. No statistical difference was observed in the intestinal histomorphology of the jejunum, including the height of the villus, crypt depth, and the ratio between VH and CD. However, the ileal villus height in the FMW 0.2% group was lower than that in both the CON and FMW 0.1% groups (linear,

$p < 0.01$), and the VH/CD ratio was also lower in the FMW 0.2% group compared to the FMW 0.1% group (quadratic, $p < 0.01$).

The digestive tract can adapt and respond morphologically to dietary changes (Perić *et al.*, 2010). VH is important for gastrointestinal health due to its role in nutrient absorption, which increases with VH (Fonseca-García *et al.*, 2017); while optimal CD enhances the absorption ability of the villus (He *et al.*, 2017). In some probiotic products, prebiotics and phytochemicals have been shown to positively impact the VH/CD ratio (Nguyen *et al.*, 2021). Improvements in the VH/CD and villus height in both the duodenum and ileum have been observed with synbiotic or probiotic supplementation and increased epithelial cell turnover due to feeding of direct-fed microbials (Awad *et al.*, 2009). The present experimental results are consistent with those of Wang *et al.* (2019), who found no differences in the histomorphology parameters of the jejunum. In the ileum, only VH was affected by the addition of 300 ppm of essential oils and organic acids. However, Mousavi *et al.* (2018) showed that the addition of 100 or 200 ppm of an oil mixture containing thymol resulted in increased villus height and VH/CD ratio in the jejunum. Previous studies suggest that essential oils, which contain bioactive substances, can alter the histology of the gastrointestinal tract, thereby improving digestive processes. Mugwort also contains essential oils (Ekiert *et al.*, 2020); Therefore, while the improvement in the VH/CD ratio observed in this study with FMW 0.1% supplementation did not show statistically significant differences compared to the control group, the numerical increase suggests a potential positive effect of the bioactive compounds present in FMW.

Table 8 – Effect of dietary supplementation with fermented mugwort and water spinach on intestinal histomorphology.

Items	Treatment ¹			SEM ²	p-value	
	CON	FMW 0.1%	FMW 0.2%		Linear	Quadratic
Jejunum						
VH3, μm	1090.25	1223.8	1197.15	45.11	0.466	0.411
CD4, μm	167.24	165.32	157.34	6.65	0.659	0.838
VH/CD1, μm/μm	6.59	7.55	7.55	0.30	0.333	0.455
Ileum						
VH, μm	798.84 ^a	795.44 ^a	624.34 ^b	26.91	0.007	0.091
CD, μm	208.71	180.14	216.04	7.25	0.680	0.030
VH/CD1, μm/μm	3.90 ^{ab}	4.59 ^a	3.05 ^b	0.21	0.082	0.007

^{a,b} Means within a same row with different letters differ significantly at $p < 0.05$.

¹CON, basal diet; FMW 0.1%, basal diet with 0.1% FMW; FMW 0.2%, basal diet with 0.2% FMW.

²SEM, standard error of the means.

³VH, villus height.

⁴CD, crypt depth.



CFU measurement in cecal content

Table 9 presents the CFU measurements of the cecal content from the three experimental groups. No statistically significant differences were observed among the treatments in the counts of *Lactobacillus*, coliform, *Shigella*, and *Salmonella*. Research by Loh *et al.* (2007), which used a diet supplemented with fermented products and vinegar containing *Lactobacillus plantarum* as starter culture for laying hens, found a reduction in the number of intestinal bacteria in feces. This decrease was attributed to the high concentration of lactic acid bacteria, which lowers the pH and creates an unfavorable environment for acid-sensitive, gram-negative bacteria. Similar studies in pigs, supplementing fermented liquid feed (mixing meal and water), also reported a reduction in intestinal bacteria (Canibe & Jensen, 2003; van Winsen *et al.*, 2001).

The current study did not show statistically significant differences in the CFU counts of cecal contents. This outcome may be due to insufficient colonization of the intestinal mucosa by the microorganisms used in the experiment. To achieve more pronounced effects, future studies should focus on using higher concentrations of microorganisms to improve their colonization. Additionally, combining probiotics and prebiotics may offer a more comprehensive understanding of the intestinal microbiota in laying hens. However, increasing the amount of fermented feed containing microorganisms could affect feed palatability and intake, potentially causing stress. Therefore, it is crucial to determine the optimal combination and concentration of microbial supplementation to effectively colonize the intestinal mucosa, promote the growth of beneficial gut microbes, and ultimately improve the productivity and health of laying hens.

Table 9 – Effect of dietary supplementation with fermented mugwort and water spinach on CFU measurement in cecal content.

Items (log CFU/g)	Treatment ¹			SEM ²	p-value	
	CON	FMW 0.1%	FMW 0.2%		Linear	Quadratic
Microbes on R2A	7.39	7.16	7.25	0.12	0.644	0.542
Lactobacilli on MRS	8.29	8.08	8.13	0.10	0.564	0.580
Coliform bacteria on MacConkey	5.94	5.75	5.22	0.27	0.277	0.826
<i>Shigella</i> and <i>Salmonella</i> on SS	6.06	5.56	4.82	0.45	0.468	0.937

¹CON, basal diet; FMW 0.1%, basal diet with 0.1% FMW; FMW 0.2%, basal diet with 0.2% FMW. ²SEM, standard error of the means.

Cecal microbiota

Table 10 presents the alpha diversity indices (Shannon, Gini–Simpson, and ASVs) for the cecal

microbiota of laying hens. No significant differences were observed in the values of the Shannon index, Gini–Simpson diversity, and ASVs. The PCoA indicates that the bacterial communities in the FMW group differed from those in the CON group (Figure 1A). The samples from the FMW 0.1% and FMW 0.2% group showed greater clustering than that of the CON group (Figure 1B). Figure 2A illustrates the cecal microbiota and the four most dominant phyla identified in the gut microbiota of hens: Bacillota, Bacteroidota, Actinomycetota, and Thermodesulfobacteriota. However, no significant differences were observed in the relative abundance of these phyla between the three groups (Figure 2A). Furthermore, no significant differences were noted in bacterial relative abundance at the family level (Figure 2B), but there was a significant predominance of *Bacteroides* at the genus level (Figure 2C). The relative abundance of *Bacteroides* at the genus level increased in the FMW 0.1% and 0.2% group compared to the CON group (linear, $p < 0.05$). *Mediterraneibacter* tended to increase with FMW supplementation, showing a trend toward a linear effect ($p = 0.059$) (table 11).

The intestinal microbiota is diverse and balanced, consisting of a highly varied microbial community that supports intestinal health, restore imbalances, and improve immune function and metabolism (Barko *et al.*, 2018). Several studies have shown that specific gut bacteria play a crucial role in maintaining gut microbiota balance, which is directly linked to improved gut health. At the genus level, bacteria such as *Bacteroides*, *Phocaeicola*, and *Mediterraneibacter* were identified in the ceca of laying hens, with these three genera comprising the highest proportion in this study. These bacteria help ferment carbohydrates to produce volatile fatty acids, which are reabsorbed in the intestine and contribute to gut health (Hooper *et al.*, 2002). *Bacteroides*, *Phocaeicola*, and *Mediterraneibacter* specifically maintain a beneficial relationship between the host and its microbiota, reducing the risk of disease (Yoshida *et al.*, 2018). Additionally, the production of butyrate by these bacteria provides further benefits to intestinal health by promoting gut barrier function and reducing inflammation (Kim *et al.*, 2019).

The findings of this study indicate that FMW supplementation may impact the gut microbiota composition in laying hens, particularly through the increased relative abundance of *Bacteroides* in the FMW 0.1% group. The increase in *Bacteroides* in the gut microbiota composition of laying hens supplemented with FMW is considered a positive



outcome. *Bacteroides* play a key role in the digestion of complex carbohydrates, as well as the synthesis and transport of metabolic products such as vitamins and short-chain fatty acids. Moreover, they contribute to immune enhancement and host protection against pathogens (Zafar & Saier Jr, 2021). Therefore, the elevated abundance of *Bacteroides* may support the development and health of laying hens. These changes suggest a potential benefit to gut health. However, further investigation is necessary to understand the mechanisms underlying these changes and to assess the long-term effects of FMW supplementation on intestinal microbiota and overall poultry health.

Table 10 – Alpha diversity indices of cecal contents in laying hens.

Items	Treatment ²			SEM ³	p-value	
	CON	FMW 0.1%	FMW 0.2%		Linear	Quadratic
ASVs ¹	704.67	680.67	708.33	28.38	0.965	0.720
Shannon	8.23	8.10	8.20	0.06	0.867	0.418
Gini-Simpson	0.99	0.99	0.99	0.00	0.730	0.779

¹ASVs, amplicon sequence variants. ²CON, basal diet; FMW 0.1%, basal diet with 0.1% FMW; FMW 0.2%, basal diet with 0.2% FMW. ³SEM, standard error of the means.

The increase in *Bacteroides* in the gut microbiota composition of laying hens supplemented with FMW is considered a positive outcome. *Bacteroides* play a key role in the digestion of complex carbohydrates, as well as the synthesis and transport of metabolic products such as vitamins and short-chain fatty acids. Moreover, they contribute to immune enhancement and host protection against pathogens. Therefore, the elevated abundance of *Bacteroides* may support the development and health of laying hens.

Table 11 – The five most dominant relative abundances at the genus level.

Genus	Treatment ¹			SEM ²	p-value	
	CON	FMW 0.1%	FMW 0.2%		Linear	Quadratic
<i>Bacteroides</i>	7.34 ^b	9.59 ^a	9.24 ^a	0.44	0.047	0.098
<i>Phocaeicola</i>	7.82	7.77	7.21	0.49	0.667	0.832
<i>Mediterraneibacter</i>	5.32	6.19	7.67	0.49	0.059	0.742
<i>Faecalibacterium</i>	3.72	4.04	4.08	0.45	0.786	0.901
Other	10.22	8.58	9.63	0.60	0.718	0.359

^{a, b} Means within a same row with different letters differ significantly at $p < 0.05$.
¹CON, basal diet; FMW 0.1%, basal diet with 0.1% FMW; FMW 0.2%, basal diet with 0.2% FMW.
²SEM, standard error of the means.

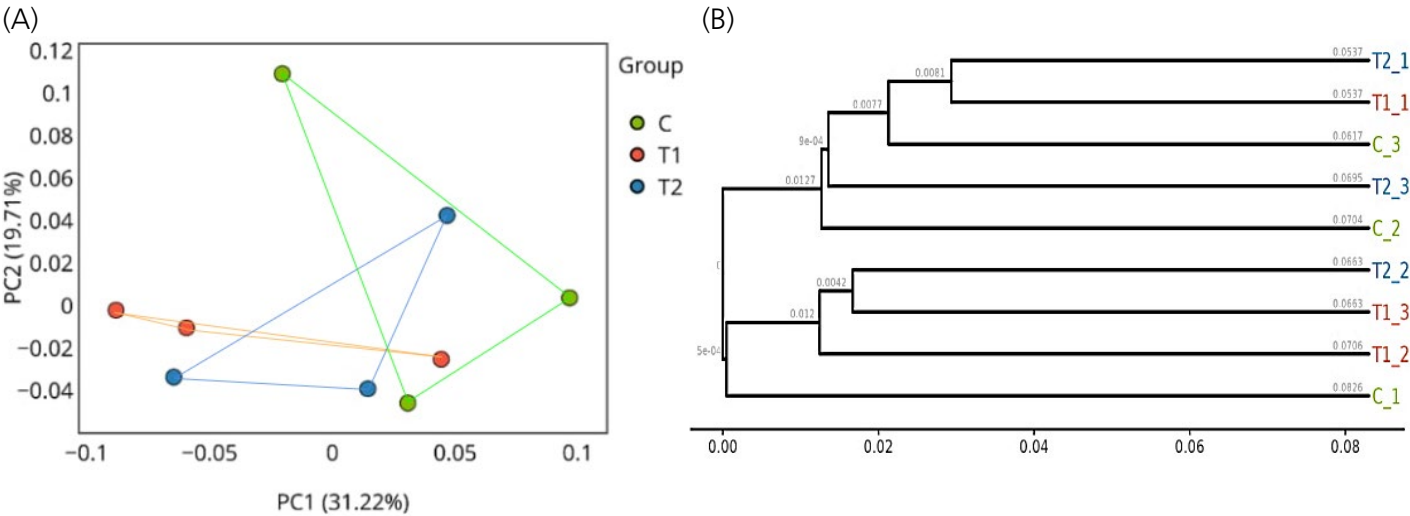


Figure 1 – Beta diversity of microbiota in cecal contents. (A) Principal coordinate analysis (PCoA) plot based on the weighted UniFrac distance matrix (B) UPGMA tree based on weighted UniFrac distance matrix. C, basal diet; T1, basal diet with 0.1% FMW; T2, basal diet with 0.2% FMW.

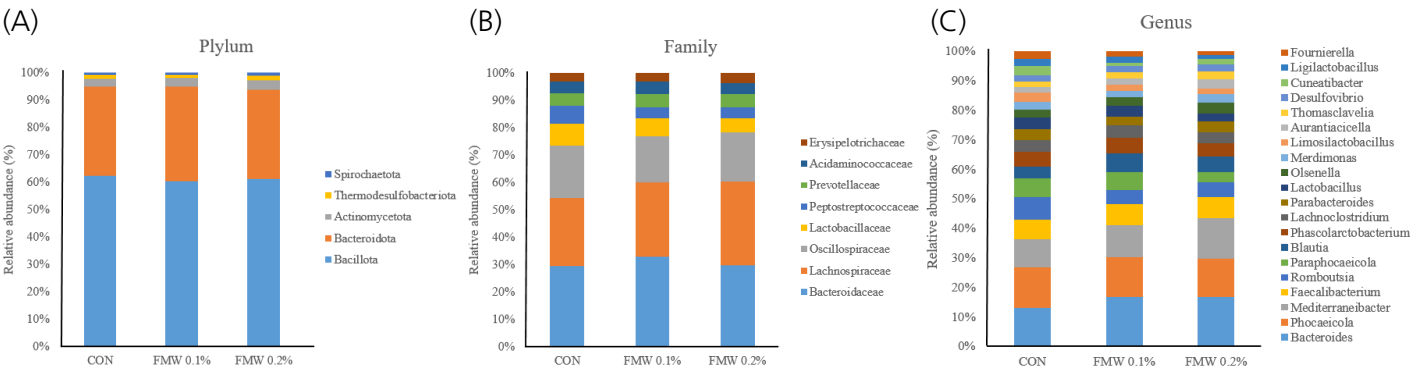


Figure 2 – Relative abundances of the cecal microbiota are presented at the phylum level (A), family level (B), and genus level (C). CON, basal diet; FMW 0.1%, basal diet with 0.1% FMW; FMW 0.2%, basal diet with 0.2% FMW.



CONCLUSION

The findings of this study suggest that dietary supplementation with FMW may offer practical benefits for poultry producers, particularly in improving egg quality parameters such as yolk height, shell strength, increasing HDL-cholesterol levels, and modulating gut microbiota. These advantages, combined with the accessibility of FMW as a natural plant-based resource, indicate its potential as a functional feed additive to support laying hen health and enhance product quality in organic, antibiotic-free production systems. Overall, FMW represents a promising candidate for the development of sustainable and health-oriented feeding strategies in poultry nutrition.

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AUTHOR CONTRIBUTIONS

Conceptualization, TH and SK; data curation, SJH; software, PJH; formal analysis, TH; investigation, TH and PJH; methodology, TH, SJH; visualization, AR; project administration, SK; writing—original draft preparation, TH and AR; writing—review and editing, AR, TV, KD, and SK; All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

The research data will be available upon request.

CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

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