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Original Article

Comparison of the Dietary Supplementation of Lactobacillus plantarum, and Fermented and Non-Fermented Artemisia Annua on the Performance, Egg Quality, Serum Cholesterol, and Eggyolk-Oxidative Stability During Storage in Laying Hens

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ABSTRACT

Artemisia annua L. is a widely distributed medicinal plant and well-known for treating malaria due to the artemisinin content. We previously found enhanced antioxidant and antibacterial activities of *Lactobacillus plantarum*-fermented *A. annua* dried leaves *in vitro*. The present study compared the effects of the dietary supplementation of *L. plantarum*, fermented (FA) or non-fermented (NFA) *A. annua* on laying performance, egg quality, serum cholesterol, and egg yolk oxidative stability during storage in 40-weeks-old Hy-Line Brown layers. In total, 180 layers were randomly allocated into four treatments for 6 weeks: basal diet (CON), basal diet + 0.5% *L. plantarum* only (LO), basal diet + 0.5% NFA, and basal diet + 0.5% FA. Each treatment comprised five replicates with nine birds each. Egg weight of NFA and FA groups were significantly higher as compared with the CON and LO groups (p<0.01). The FA group displayed higher Haugh unit (HU) compared with the NFA group (p<0.05). Eggshell color of the FA group was significantly increased compared with the other groups (p<0.01). There was no significant difference in triglyceride, total cholesterol, HDL-cholesterol, and VLDL+LDL cholesterol among the different groups. During egg storage, the HU of FA groups was significantly increased as compared with the CON group after 3 weeks storage (p<0.05). The malondialdehyde (MDA) content in the stored eggs was significantly lowered by feeding of FA as compared with the CON and LO groups (p<0.05). Altogether, the fermented *A. annua* displayed positive effects in promoting egg quality of layers.

INTRODUCTION

There has been an increasing interest in using phytogenic feed additives (PFA) in animal feed during the last two decades (Mohammadi Gheisar & Kim, 2018). To date, many studies have reported antimicrobial, antioxidant, anti-inflammatory, and growth-promoting effects of PFA in animals (Kim *et al.*, 2010; Abdel-Wareth & Lohakare, 2014; Qin & Hou, 2017; Abou-Elkhair *et al.*, 2018; Mohammadi Gheisar & Kim, 2018). Phytogenic sources contain abundant phytochemicals, which have been mainly classified into three categories as carotenoids, isothiocyanates, and polyphenols, and most polyphenols are recognized as having beneficial functions (Fraser, 2009).

A traditional medicinal herb, *Artemisia* has been used to treat a variety of diseases for a long time. The genus *Artemisia* comprises over 500 species and it is mainly distributed in the temperate zones of Europe, Asia, and North America. *A. annua*, also known as sweet wormwood or annual mug wort, is one of the most famous *Artemisia* species applied to treat several diseases, including malaria, due to its high artemisinin content (Bora & Sharma, 2011). At present, more

than 600 secondary metabolites have been identified and classified as terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols, and acetylenes (Brown, 2010; Abad *et al.*, 2012). The pharmacological effects of *A. annua* have already been well-studied, but its functional effects as feed additives are not well assessed so far. A few studies have investigated the effects of *Artemisia* supplementation in broiler diets. Dried *A. annua* leaves were reported to increase the feed conversion ratio and to reduce intestinal *Clostridium perfringens* counts in broilers (Engberg *et al.*, 2012). The dietary supplementation of dried *A. annua* leaves meal lowered lipid oxidation in the breast and thigh muscles of broilers (Cherian *et al.*, 2013). In addition, dried *A. annua* leaves also displayed anticoccidial effects in free-range broilers (Brisibe *et al.*, 2008; de Almeida *et al.*, 2012). Enzyme-treated *A. annua* has potential to improve growth performance, antioxidant capacity, tolerance to heat stress, and to alleviate the intestinal inflammatory response of broilers (Wan *et al.*, 2016; Song *et al.*, 2017). *A. annua* has also exhibited promising economic potential as a feed additive for broilers even before the ban of conventional coccidiostats in EU (Bosselmann & Gylling, 2013).

Probiotics are also well-studied feed additives, with reported benefits on laying performance, egg quality, and immune response of laying hens (Zhang *et al.*, 2012; Forte *et al.*, 2016; Abd *et al.*, 2017; Mazanko *et al.*, 2018). *Lactobacillus plantarum* is one of the most common probiotic strains, and possesses strong antioxidant and antimicrobial activities (Tsai *et al.*, 2012; Niu *et al.*, 2018). The dietary supplementation of *L. plantarum* metabolites increased egg production, reduced plasma and yolk cholesterol, and improved fecal volatile fatty acids content (Loh *et al.*, 2014) in layers. Plant materials may be used as probiotic carriers by fermentation, and, in turn, fermented plants with selected autochthonous probiotics may improve its functional properties (Hossain *et al.*, 2012; Lokaewmanee *et al.*, 2012; Peres *et al.*, 2012; Di cagno *et al.*, 2013; Zhao *et al.*, 2013). Phenolic and flavonoid compounds and their antioxidant capacity in herbal teas were increased by lactic acid fermentation (Ibrahim *et al.*, 2014). Enhanced antioxidant and antibacterial capacities were also reported in a fungi-fermented medicinal plant (*Bletilla striata*) (Dong *et al.*, 2015). The supplementation of *Lactobacillus*-fermented *Artemisia princeps* has been reported to improve the growth performance, meat lipid stability, and gut health of broilers (Kim *et al.*, 2012a).

A suitable probiotic strain is essential to achieve synergetic effects in phytogenic fermentation. We had previously observed enhanced antioxidant and antibacterial activities of *L. plantarum*-fermented *A. annua* L. (Lee *et al.*, 2017). To the best of our knowledge, very few studies regarding the dietary effects of fermented *A. annua* L. in laying hens have been reported. Therefore, we compared the effects of the *L. plantarum,* fermented and non-fermented *A. annua* L., in layer diets on laying performance, egg quality, serum cholesterol, and egg yolk oxidative stability during storage.

MATERIALS AND METHODS

Experimental diets, birds, and management

In the study, the experimental feed additives were prepared as follows. *L. plantarum* SK3494 was cultured in MRS (deMan, Rogosa and Sharpe, Difco, USA) medium at 37°C for 24 h to achieve 1.0×10^9 CFU/mL cells and used as a probiotic feed additive (LO). A volume of 10% (w/v) *A. annua* L. dried leaves powder in 10-fold diluted MRS medium was used as phytogenic feed additive (NFA). The fermented *A. annua* L. (FA) was prepared by inoculating 2% (v/v) of *L. plantarum* SK3494 in 10-fold diluted MRS containing 10% (w/v) *A. annua* L. dried leaves powder and cultured at 37°C for 24 h to achieve 1.0×10^9 CFU/mL cells. Finally, 0.5% (w/v) of each additive was evenly mixed with the basal diet (Table 1) in a feed blender and used as experimental diets. The supplemented diets were prepared freshly each 2 weeks to maintain the shelf life. The basal diet not supplemented with feed additives was used as a control feed (CON). The feeding experiment was conducted for 6 weeks.

The procedures with animals were performed according to the guidelines of the animal policy of the Institutional Animal Care and Use Committee at Konkuk University, Seoul, South Korea.

One hundred eighty 40-week-old Hy-Line Brown layers were randomly assigned to four treatments with five replicates of nine birds each. Hens were housed in a two-tier iron cage (length, 90 cm; width, 90 cm; space allowance, 735 cm^2) with nine birds each.

Hens were housed at 22 ± 3°C and 76.4 ± 15.7% humidity, with 16 h of light per day, and were fed the experimental diets and water *ad libitum*.

Determination of laying performance

All the eggs laid, including intact and broken eggs, were collected daily to determine hen-day egg

Table 1 – Feedstuffs and calculated chemical composition of the basal diet.

Ingredients	Composition (%)
Corn	50.00
Tallow	0.90
Corn dried distiller's grains with solubles (DDGS)	21.24
Soybean meal	7.42
Rapeseed meal	5.00
Sesame seed oil meal	2.00
Feather meal	1.50
Syn. Lysine-sulfate	0.38
Syn. Methionine (liq.)	0.108
Syn. Threonine	0.026
Limestone	10.20
Mono dicalcium phosphate (MDCP)	0.59
Salt	0.20
Sodium bicarbonate	0.10
Vitamin premix[1]	0.11
Mineral premix[2]	0.18
Choline-chloride (50%)	0.049
Total	100.00
Calculated nutritional composition	
Crude protein, %	17
Crude fat, %	5.3
Crude fiber, %	3.7
Ca, %	4.0
Available P, %	0.27
Lys, %	0.83
TSAA[4], %	0.72
TMEn[3], kcal/kg	2,800

[1]Vitamin premix provided followings per kg of diet: vitamin A, 40,000 IU; vitamin D3, 8,000 IU; vitamin E, 10 IU; vitamin K3, 4 mg; vitamin B1, 4 mg; vitamin B2, 12 mg; vitamin B6, 6 mg; vitamin B12, 0.02 mg; pantothenic acid, 20 mg; folic acid, 2 mg; nicotinic, 60 mg.

[2]Mineral premix provided followings per kg of diet: Fe, 30 mg; Zn, 25 mg; Mn, 20 mg; Co, 0.15 mg; Cu, 5 mg; Se, 0.1 mg.

[3]TMEn: true metabolizable energy corrected for nitrogen.

[4]TSAA: Total sulfur amino acids.

production ratio (EPR). Average egg weight (AEW) was determined using only intact eggs to divide the egg number. Daily egg mass was calculated by multiplying EPR with AEW on the same day. The feed intake was recorded weekly and expressed as g/day/hen. The feed conversion ratio (FCR) was determined as grams of feed intake per grams of egg mass.

Determination of egg quality

Egg quality was measured weekly in three eggs per replicate. The eggs were individually weighed and exposed to a breaking force in an eggshell strength tester (FHK, Fugihira, Ltd, Japan). After breaking, egg contents were poured into a glass plate to measure albumen height. Haugh unit (HU) was calculated using the records of albumen height (*H*) and egg weight (*W*) following the formula [HU = 100 Log (H + 7.57 – 1.7 $W^{0.37}$)] as described in Haugh (1937). Egg yolk color was determined by comparing the color with egg yolk color fan (Yolk color fan, Roche, Switzerland). Eggshell color was determined by comparing the color with an eggshell color fan (Eggshell color fan, Samyang, Korea). Eggshell thickness was measured at the center of eggshell fragments using a micrometer (Digimatic Micrometer, Series 293~330, Mitutoyo, Japan).

Determination of serum cholesterol

At the end of the experiment, eight laying hens per treatment were selected for blood sampling. Birds were sacrificed using carbon dioxide and then cardiac blood was collected. The collected blood was stored at 4°C for 1 day in EDTA-treated blood collection tubes. Serum was collected by centrifugation at 1,500 g for 10 min and stored at -20°C until use. Serum total cholesterol, total triglyceride, and high-density lipoprotein (HDL) cholesterol levels were determined in a biochemical analyzer (HITACHI 7600, Japan), using the corresponding diagnostic kits (Youngdong Medical Corporation, Korea) according to the manufacturer's direction. VLDL (very low-density lipoprotein) +LDL (low-density lipoprotein)-cholesterol was calculated by subtracting HDL-cholesterol from the total cholesterol as described earlier (Nishizawa & Fudamoto, 1995).

Determination of egg freshness and lipid oxidation during storage

Haugh units and egg yolk oxidative stability were used to determine egg storage quality. At the end of the experiment (6th week), four intact eggs per replicate (20 eggs/treatment) were randomly collected and stored at 18°C for 4 weeks to determine HU change (Haugh, 1937). Additional 5 intact eggs per replicate (25 eggs/treatment) were stored at room temperature (15 ± 5°C) for 4 weeks, then they were broken and the egg yolks were stored for 2 more weeks (total 6 weeks). Egg yolk oxidative stability was evaluated by thiobarbituric acid reactive substances (TBARS) assay with some modifications (Botsoglou *et al.*, 1994). Egg yolk samples (1.5 g) were homogenized in hexane with 5% (v/v) aqueous trichloroacetic acid (TCA) and 0.8% (v/v) butylated hydroxytoluene, and then the solution was centrifuged at 1,500g for 5 min. A 2.5-mL aliquot of the bottom layer was removed and mixed with 1.5 mL of 0.8% 2-thiobarbituric acid. After incubation at 70°C for 30 min, absorbance was measured at a range

of 400-650 nm using a spectrophotometer (Shimadzu, Model UV-1601, Tokyo, Japan) by producing a third-derivative spectrum. Malondialdehyde (MDA) concentration in the analyzed samples was directly quantified by referring a standard calibration curve using tetraethoxypropane (an MDA precursor).

Statistical analysis

All data obtained in this study were subjected to one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS statistical software (SAS Institute, 2002). Cage was used as the experimental unit for laying performance data, whereas individual birds as experimental unit for serum cholesterol data. The egg was an experimental unit for egg quality data and storage quality data. Dietary treatment was a fixed factor in all statistical models and the LSMEANS procedure was used to calculate mean values. Significant differences among treatments were determined using Duncan's multiple range test at the level of $p<0.05$. The data were presented as mean ± standard deviation (SD).

RESULTS

Laying performance

The effects of the dietary supplementation of the probiotic *L. plantarum* (LO), fermented (FA), and non-fermented *A. annua*L (NFA) on laying performance were compared. There were no significant differences ($p>0.05$) in performance-related parameters, such as egg production rate, egg mass, feed intake, and FCR with the administration of different feed additives (Table 2). However, egg weight of the groups fed NFA and FA groups was significantly higher compared with the CON and LO groups ($p<0.01$).

Table 2 – Effects of the supplementation of the evaluated feed additives on laying performance.

Item	Treatment[1]				*p*-value
	CON	LO	NFA	FA	
Egg production, %	85.89 ± 6.35	87.60 ± 8.06	85.50 ± 5.97	86.27 ± 6.92	0.66
Egg weight, g	63.18 ± 1.69^{b}	62.89 ± 1.37^{b}	64.55 ± 2.16^{a}	64.19 ± 1.48^{a}	<0.01
Egg mass, g/hen/d	54.33 ± 5.02	55.08 ± 5.18	55.14 ± 3.39	55.40 ± 4.67	0.83
Feed intake, g/hen/d	113.05 ± 9.77	116.28 ± 6.29	116.35 ± 6.27	115.97 ± 5.48	0.22
FCR[2], g feed/g egg	2.08 ± 0.23	2.12 ± 0.19	2.11 ± 0.11	2.12 ± 0.23	0.86

1CON: basal diet; LO: basal diet + 0.5% *L. plantarum*; NFA: basal diet + 0.5% non-fermented *A. annua* L.; FA: basal diet + 0.5% fermented *A. annua* L.

2FCR: feed conversion ratio, measure of an animal's efficiency in converting feed mass into increases of the desired output.

a,bDifferent superscripts (a, b) in the same row indicate significantly ($p<0.05$) different values in mean score (Mean ± SD) among treatment groups.

Egg quality

The effects of the dietary treatments on the egg quality of laying hens are shown in Table 3. Haugh unit a of the NFA group was significantly lower compared with the CON and FA groups ($p=0.03$). Eggshell color was significantly increased in the FA group compared to the other groups ($p<0.01$). No significant difference was found in egg yolk color, eggshell breaking strength, and eggshell thickness with the supplementation of different additives. The FA diet was likely to show a trend on increasing the eggshell breaking strength ($p=0.08$).

Table 3 – Effects of the supplementation of the evaluated feed additives on egg quality.

Item	Treatment[1]				*p*-value
	CON	LO	NFA	FA	
Haugh unit	93.07 ± 6.48^{a}	92.95 ± 7.67^{ab}	90.69 ± 8.32^{b}	94.01 ± 7.35^{a}	0.03
Eggshell color	10.75 ± 1.13^{b}	10.98 ± 0.97^{b}	11.03 ± 1.18^{b}	11.36 ± 0.90^{a}	<0.01
Egg yolk color	7.17 ± 0.66	7.25 ± 0.73	7.19 ± 0.75	7.22 ± 0.72	0.89
Eggshell breaking strength, kg/cm^2	3.20 ± 0.79	3.42 ± 0.79	3.22 ± 0.95	3.45 ± 0.69	0.08
Eggshell thickness, mm	0.36 ± 0.03	0.36 ± 0.02	0.36 ± 0.03	0.37 ± 0.03	0.25

1CON: basal diet; LO: basal diet + 0.5% *L. plantarum*; NFA: basal diet + 0.5% Non-fermented *A. annua* L.; FA: basal diet + 0.5% fermented *A. annua* L.

a,bDifferent superscripts (a, b) in the same row indicate significantly ($p<0.05$) different values in mean score (Mean ± SD) among treatment groups.

Serum cholesterol

The effects of the dietary treatments on serum cholesterol of laying hens are shown in Table 4. There were no significant differences in triglyceride, total cholesterol, HDL-cholesterol and VLDL+LDL-cholesterol among the different diet groups.

Table 4 – Effects of the supplementation of the evaluated feed additives on serum cholesterol (mg/dL).

Item	Treatment[1]				*p*-value
	CON	LO	NFA	FA	
Triglycerides	1,174.63 ± 419.73	1,557.38 ± 192.98	1,128.25 ± 471.41	1,351.13 ± 434.05	0.14
Total cholesterol	133.63 ± 31.08	159.50 ± 12.92	136.75 ± 32.15	148.88 ± 30.51	0.25
HDL-cholesterol	10.22 ± 3.05	11.70 ± 2.19	12.05 ± 3.57	10.36 ± 2.37	0.48
VLDL+LDL-cholesterol[2]	123.40 ± 28.41	147.80 ± 11.67	124.70 ± 29.36	138.52 ± 28.49	0.20

[1]CON: basal diet; LO: basal diet + 0.5% *L. plantarum*; NFA: basal diet + 0.5% Non-fermented *A. annua* L.; FA: basal diet + 0.5% fermented *A. annua* L.

[2]VLDL+LDL-cholesterol: value calculated by subtracting HDL-cholesterol from the total cholesterol.

[a,b]Different superscripts (a, b) in the same row indicate significantly (p<0.05) different values in mean score (Mean ± SD) among treatment groups.

Egg freshness and lipid oxidation during storage

The effects of the dietary treatments on Haugh units and egg yolk peroxidation during storage are shown in Table 5. Significantly higher HU was only observed in 3-week stored eggs of hens fed FA compared with the CON group (p=0.02) (Table 5).The addition of feed additives did not significantly affect egg yolk MDA concentration compared with the CON group after storage for 4 weeks, but lower MDA values were found in the FA group after storage for 6 weeks (p=0.04).

Table 5 – Effects of the supplementation of the evaluated feed additives on Haugh unit and egg yolk oxidative stability during storage.

Period	Treatment[1]				*p*-value
	CON	LO	NFA	FA	
Haugh unit (1 week)	88.77 ± 4.89	85.39 ± 7.60	85.12 ± 10.44	84.28 ± 4.55	0.27
Haugh unit (2 weeks)	69.88 ± 11.91	73.30 ± 10.69	73.10 ± 7.36	72.78 ± 10.23	0.69
Haugh unit (3 weeks)	60.21 ± 6.47[b]	62.38 ± 7.50[ab]	64.28 ± 6.91[ab]	66.78 ± 3.93[a]	0.02
Haugh unit (4 weeks)	59.25 ± 8.67	61.24 ± 4.28	61.24 ± 5.22	60.49 ± 6.31	0.79
Haugh unit (Overall)	69.81 ± 14.44	71.47 ± 12.58	71.41 ± 12.05	71.69 ± 10.91	0.79
MDA[2] (4 weeks) (μg/g yolk)	0.0122 ± 0.0044	0.0119 ± 0.0040	0.0127 ± 0.0020	0.0123 ± 0.0019	0.98
MDA[3] (4+2 weeks) (μg/g yolk)	0.0687 ± 0.0285[a]	0.0641 ± 0.0247[a]	0.0419 ± 0.0143[ab]	0.0313 ± 0.0034[b]	0.04

[1]CON: basal diet; LO: basal diet + 0.5% *L. plantarum*; NFA: basal diet + 0.5% Non-fermented *A. annua* L.; FA: basal diet + 0.5% fermented *A. annua* L.

[2]MDA: Malondialdehyde concentration after storage for 4 weeks.

[3]MDA: Malondialdehyde concentration after storage for 4 weeks and additional 2 weeks with open-contamination condition without eggshell.

[a,b]Different superscripts (a, b) in the same row indicate significantly (p<0.05) different values in mean score (Mean ± SD) among treatment groups.

DISCUSSION

Fermentation is a common method to produce biological resources with enhanced beneficial properties (Ng *et al.*, 2011). The present study compared the effects of LO, NFA, and FA supplementation in layer diets. No significant differences in laying performance and egg quality were found by LO addition compared with the CON group, which is consistent with a previous study (Forte *et al.*, 2016). The dietary inclusion of NFA and FA significantly increased the egg weight. This finding agrees with those observed by using fennel and cumin seed to increase egg weight (Aydin *et al.*, 2008; Yalcin *et al.*, 2009; Khan *et al.*, 2013; Abou-Elkhair *et al.*, 2018). Conversely, a high inclusion of *A. annua* leaves did not significantly increase egg weight, but dramatically decreased FCR (Baghban-Kanani *et al.*, 2018). The inclusion of *Artemisia capillaris* was reported to increase egg production of laying hens (Kim *et al.*, 2010). Positive effects on body weight gain and feed efficiency were also reported by supplementing *Lactobacillus*–fermented *Artemisia princeps* for broilers (Kim *et al.*, 2012a). There are many factors that could lead to the difference in the mentioned results *viz.*, dietary level, processing conditions, surveying purpose, and some others. However, the clear reason regarding the mechanism has not been found yet.

Haugh unit is a very important measure of internal egg quality (Monira *et al.*, 2003). The lower egg HU in NFA group compared with the FA and CON groups is inconsistent with the findings in Baghban-Kanani *et al.* (2018), which reported inclusion of *A. annua* leaves did not significantly influence HU. We speculate that some original compounds in *A. annua* may affect albumen synthesis, and these compounds may be converted into others during fermentation. It was earlier reported that fermented herbs (*Artemisia capillaris* and *Acanthopanax senticosus*) increased

polyunsaturated fatty acid content in the *Longissimus dorsi* muscle of pigs compared with non-fermented herbs (Lei *et al.*, 2018). The brown coloration of the eggshell is considered as an important eggshell quality parameter and has a positive impact on consumer preference (Samiullah *et al.*, 2015). The supplementation of phytochemicals has been reported to increase eggshell biliverdin (one eggshell pigment) content (Butler & McGraw, 2013). Thus, the enhanced eggshell coloration in FA diet may be caused by more produced active compounds after fermentation.

For serum cholesterol, dietary supplementation of 1% and 2% *Artemisia vulgaris* L. leaf powder significantly increased HDL-cholesterol and decreased LDL-cholesterol in broilers (Kim *et al.*, 2012b). In contrast, no impact on reducing serum cholesterol was reported by feeding with 1% *Artemisia sieberi* leaf extract (Khalaji *et al.*, 2011). *Artemisia* spp. are well known for their pharmacological, antioxidant, anti-inflammation, and antibacterial activities (Choi *et al.*, 2013; van der Kooy & Sullivan, 2013; Kim *et al.*, 2015). In our study, 0.5% of fermented and non-fermented *A. annua* L. were used and showed little effects on serum cholesterol. The inconsistent results reported among the studies may be ascribed to the difference in supplement concentration, additive form, and additive self-characteristics.

During storage, comparatively higher HU value was observed in the eggs of FA-fed hens stored for 3 weeks. Lee *et al.* (2017) reported increased total antioxidant activity with decreased content of polyphenolic and flavonoid in *L. plantarum*-fermented *A. annua* L. Some substances newly-synthesized during fermentation may be more effective to prevent egg albumen loss during storage at room temperature (Ibrahim *et al.*, 2014; Dong *et al.*, 2015). Fermented *Ginkgo*-leaves have been reported to increase monounsaturated fatty acids, polyunsaturated fatty acids (PUFA), and the ratio of PUFA/saturated fatty acids in the egg yolk (Zhao *et al.*, 2013). The increased content of unsaturated fatty acids is considered to be more efficient to improve egg oxidative stability and extend egg freshness period. In the present study, both NFA and FA additives were effective in reducing MDA concentration in egg yolk, which is consistent with previous studies that verified MDA reduction in the meat of broilers fed different *Artemisia* species (Kim *et al.*, 2012a; Kim *et al.*, 2012b; Cherian *et al.*, 2013). In addition, the dietary supplementation of plant extract mixture from *A. capillaris*, *Camellia sinensis*, *Schizandrachinensis*, and *Viscum album* var. *coloratum* has increased HU of egg stored for 2 and 3 weeks, coupled with a decrease in yolk MDA concentration. In addition of its most well-known compound, artemisinin, 80 natural products have been identified in *Artemisia annua*, out of which phenolics are the most abundant active compounds, which have diverse antioxidant, antimicrobial, and anti-inflammatory effects (Czechowski *et al.*, 2018, Lee *et al.*, 2017; Kim *et al.*, 2015). Overall, the effects of the supplemented additives did not influence laying performance, except for egg weight. In comparison with *L. plantarum* and non-fermented *A. annua,* fermented *A. annua* showed better effect on changing eggshell color. In addition, both FA and NFA supplementation showed oxidative protective effect on egg yolk during storage.

CONCLUSION

This is the first study determining the effects of fermented and non-fermented *A. annua* dried leaves as phytogenic feed additives on laying performance, egg quality, serum cholesterol, and lipid oxidation in 40-week-old Hy-Line Brown layers. A synergistic beneficial effect was observed by the supplementation of fermented *A. annua* L. with *L. plantarum* (FA) compared to non-fermented *A. annua* L. (NFA) and *L. plantarum* only (LO) in the basal diet. In the future work, it is worthy to investigate the dietary effect of non-fermented and fermented *A. annua* L. on egg lipid composition as well as on the immunity of laying hens.

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CONFLICT OF INTEREST

We certify that there is no conflict of interest in the study.

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