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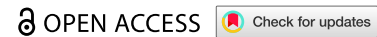


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RESEARCH ARTICLE



Influence of medium-chain fatty acid- or tannin-based herbal feed additive supplementation on the productive performance, egg quality, gut microbiota, jejunum morphology and immune response of laying hens after 57 wks of age

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ABSTRACT

This study aimed to evaluate the effects of two herbal-based feed additives – medium-chain fatty acids (MCFAs) and tannins (TANs) – on the productive performance, egg quality, gut health, immune response, and liver enzyme activity of Lohmann White hens during the late laying period (57–81 weeks). A total of 351 hens were randomly assigned to three dietary treatments: a control diet, a diet supplemented with MCFAs, and a diet supplemented with TAN. TAN supplementation significantly improved egg mass and the feed conversion ratio, while MCFAs supplementation reduced weight loss and increased eggshell breaking strength, likely due to improved calcium metabolism. Both additives positively influence gut health, as evidenced by increased villus height and, in the case of MCFAs, increased villus surface area and goblet cell numbers. Additionally, both MCFAs and TAN enhanced immune responses, as indicated by increased antibody titers against Newcastle disease virus. Notably, MCFAs improved the antioxidant defence system, maintaining or increasing the activities of liver enzymes such as catalase and superoxide dismutase. These findings suggest that MCFAs and TAN may enhance the health and productivity of hens during the late laying period, potentially offering sustainable solutions for extending egg production while maintaining egg quality and animal welfare.

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Medium-chain fatty acids; tannins; productive performance; egg quality; gut microbiota; immune response

Introduction

Eggs are important sources of protein, fats and micronutrients for humans for a long period of time and not only satisfy hunger but also improve the physical and mental well-being of consumers. Moreover, eggs are known to contain biologically active compounds beyond basic nutrients, and extensive research has been undertaken to identify and characterize these biologically active components (Kovacs-Nolan et al. 2005; Miranda et al. 2015). Recent advancements in laying hens' genetics have shown that laying hens have the capacity to lay approximately 500 eggs up to 100 weeks of age. However, increasing egg production and the ability to maintain this productivity in the later stages of life both create a metabolic burden on the animals and show the need for sustainable egg quality (Bain et al. 2016). In particular, during that period, improving nutrient availability and protecting intestinal health became more important; thus, laying hens can use the energy, amino acids and mineral substances they consume more effectively for laying and maintaining egg quality. Therefore, continuously increasing concern is now focused on improving the reproductive performance, feed efficiency, and egg quality of late laying period hens. Some herbal feed additives, especially after antibiotic restriction, have been used in layer feeds to optimize the gut environment for the optimal digestion and absorption of nutrients in a healthy manner. For this purpose, medium-chain fatty acids (MCFAs) and tannins, which are used as natural herbal feed additives, are employed in animal nutrition to maintain

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intestinal health because of their antimicrobial effects (Kimminau et al. 2022; Liu et al. 2020a; Soldado et al. 2021), and this study aimed to investigate the potential effects of these two herbal-based feed additives.

In general, MCFAs are defined as fatty acids with chain lengths varying from C6 to C12 (Bach and Babayan 1982). Caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0) and lauric acid (C12:0) are classified in this group, and milks (breast milk and bovine milk) and oils (palm kernel oil and coconut oil) are considered natural sources of these fatty acids. MCFAs have been identified as feed additives that can be used safely in livestock feed after being produced by glycerolysis of MCFA-rich fats such as palm kernel oil, coconut oil and cuphea oil at high temperatures and pressures (Nandi et al. 2004; Rimoldi et al. 2018). The intestinal microflora creates a very diverse environment, and the balance of microflora in this environment is related to maintaining a critical balance between animal health and susceptibility to diseases (Cui et al. 2017; Gong et al. 2007). MCFAs are defined as strongly antibacterial compounds, and their positive effects on health, nutrient digestibility, support of the integrity of the intestinal tissue, improvement in eggshell quality and mineral utilization, and modulation of immune responses, liver health and metabolism and production have been described by some researchers in poultry nutrition (Baltic et al. 2017; Ceylan et al. 2023; Liu et al. 2020b; Nawrot et al. 2025; van der Aar et al. 2017). In addition, MCFA supplementation in 44–52-week-old hens (Zhao et al. 2019) and 40–64-week-old hens (Liu et al. 2020a) has positive effects on performance, egg quality, and the gut microbiota. As mentioned previously, layer hens have the potential to expand their laying periods at 100 weeks of age. In those periods, animal requirements and the intestinal status for laying and egg quality become more important to evaluate.

Phenolics are defined as products of the secondary metabolism of plants. Phenolic compounds can be defined as any compound having a benzene ring with one (phenol) or more (polyphenol) hydroxyl group, such as esters or methylesters (Christaki et al. 2020). As a member of phenolics, tannins are identified as being the fourth most abundant compound in plant vascular tissue, after cellulose, hemicellulose, and lignin, and they are quantitatively dominant in plant secondary metabolites (Adamczyk et al. 2017). Additionally, tannins are divided into three main groups according to their chemical structure. These include hydrolysable tannins (HTs), condensed tannins (CTs), and phlorotannins (PTs) (Koleckar et al. 2008). Tannins might be defined as an anti-nutritive because their ability to precipitate proteins results in lower nutrient retention in monogastric animals (Liu et al. 2020b; Smulikowska et al. 2001), but, owing to their ability to enrich specific phenolic compounds found in tannins, they have potential antimicrobial, antibacterial, antioxidant, and antiviral properties in farm animals, including poultry (Choi and Kim 2020; Dakheel 2018; Schiavone et al. 2008; Shao et al. 2015; Smeriglio et al. 2017; Soldado et al. 2021; Viveros et al. 2011). In the literature, tannins have the potential to improve the gut health of broilers, depending on the dose used in the feed (Choi et al. 2022), and improve the egg shell quality of young layer hens without affecting their performance (Englmaierová et al. 2022). However, there are very few or no experiments on the use of tannin-based herbal feed additives considering the late laying periods in laying hens.

In our previous study (Ceylan et al. 2023), the positive effects of using MCFA-based feed additives at levels of 1.2 and 0.7 g/kg in broiler chickens at different periods were observed, and in the current study, the 1.1 g/kg level was preferred for laying hens. In addition, there are few studies on the use of tannic acid-based feed additives in poultry, and in studies conducted with broiler chickens, the dose used ranged from 0.5 to 2.5 g/kg (Choi et al. 2022). In our study, the tannic acid dose in laying hens was determined to be 1 g/kg, which is approximately the optimal dose (0.5–0.9 g/kg) reported in the literature (Choi et al. 2022). In this trial, we aimed to evaluate the effects of different herbal-based feed additives (MCFAs or HT-CT based) on productive performance, egg quality, the gut microbiota, jejunum morphology, immune response, and liver enzymes during late laying periods (57–81 weeks).

Materials and methods

Ethical approval

All procedures involving animals in this study were approved by the Ankara University Animal Research Ethics Committee (Approval number: 2024-14-115) and conducted in accordance with the National Institutes of Health guidelines.

Birds, diets, and experimental design

A total of 351 Lohmann White laying (Lohmann LSL Classic) hens (57 weeks old) were randomly divided into three treatment groups (9 hens per replicate and 13 replicates per group) and fed with basal diets (CON) or basal diets supplemented with medium-chain fatty acids (MCFAs) or tannins (TAN) based on different herbal feed additives. The CON diet was formulated to meet the recommended nutrient requirements of Lohmann White (Lohman LSL-Classic Layers) according to the breeder breeding company's nutrition specification guide. MCFAs and TAN were provided by Agrifirm Apeldoorn, The Netherlands (Shellbiotic® - blend of MCFA and SCFA, Vitanox® - blend of hydrolysable tannins and condensed tannins and low-molecular-weight phenolic acids) and were added at concentrations of 1.1 and 1 g/kg, respectively. Before the experimental period, hens fed a common diet for two weeks were used to examine the uniformity of the hens in terms of body weight and egg mass output. After 56 weeks of age, the hens were distributed to the 3 treatment groups to obtain uniform conditions for each treatment according to their preliminary egg production and body weight. The research was then started and conducted according to a completely randomized block design, and the trial lasted for 24 weeks (57–81 weeks). The birds were housed in cages (9 hens each) at the Layer Production Farm of the Agriculture Faculty in Haymana, Ankara University. Each pen was equipped with nipple drinkers and separate feeders in front. The size of each cage was 75 cm wide by 60 cm deep by 50 cm high. The room temperature was controlled by automatic fans. Water was provided ad libitum. The treatment feed to each cage was 110 g per day. Sixteen hours of light was provided per day (incandescent lighting, between 10 and 20 lux). The feeds were served in mash form. After conducting nutrient analyses (Weende & amino acid analysis) on raw materials according to the procedures stated in AOAC (2005), the formulation of the diets was adapted based on the results (Table 1).

Productive performance, egg quality measurement and data collection

Body weights were recorded at the beginning and the end of the experiment. During the experimental period (57–81 weeks), egg production, cracked, deformed, or soft shell eggs were recorded daily. Egg weight was recorded weekly, and feed intake and the feed conversion ratio were recorded and calculated biweekly. Egg shell (shell weight, shell thickness, and shell breaking strength) and egg internal (albumen height, Haugh unit, and yolk color) qualities were measured every 4 weeks, during which a minimum of seven eggs were collected from each pen for measurement. The collected eggs were weighed, and the eggshell breaking strength (Newton) was determined via a digital eggshell force reader (ADCON–1, Türkiye) with a constant head speed of 10 mm/min. The albumen height (mm) was subsequently measured using a tripod micrometer, and the yolk color was measured using the Roche yolk color fan scale. Eggshell thickness (µm) was measured by a digital micrometer on a shell part without a membrane, taken from the equatorial region of the egg. The eggshell ratio (% of egg weight) was calculated after all the shells were washed and dried for 5 days in a drying oven at 55 °C. The Haugh unit was calculated according to the following formula: $100 \times \log (H + 7.57 - 1.7 * W^{0.37})$, where H is the height of the albumen (mm) and W is the weight of the egg (g). For further examination (histological, volatile fatty acid, microbiota), 2 hens per replicate were killed by cervical dislocation to obtain an initial intestinal section.

Histological analysis

For the histological analysis, jejunum segments (towards the proximal duodenum) were taken, each 5 cm long, and flushed with a saline solution. These segments were fixed in 10% neutral buffered formalin at room temperature for 72 h. After routine histological procedures, the samples were embedded in paraffin wax. Sections with a thickness of 4 µm were cut on glass slide using a microtome. Images were taken with a computer-supported imaging system (ImageJ; Laboratory for Optical and Computational Instrumentation, University of Wisconsin-Madison, Madison, WI, USA) connected to a light microscope (Zeiss Axio Lab. A1 Microscope and Zeiss AxioCam ICc 5 camera; Carl Zeiss AG, Oberkochen, Germany). The variables measured were the villus height and width, the V/C depth ratio, and the villus surface area. Villus height was measured

Table 1. Control diet composition and calculated nutrient values (as-fed basis, %).

Ingredients		Amount, g/kg
Corn 6.2% CP		441,42
Wheat 13% CP		200,00
Soybean meal 47%CP		143,51
Sunflower meal 33.5% CP		100,00
Limestone		93,05
Soy oil		8,58
Dicalcium phosphate		2,85
Salt		2,53
Vitamin mineral premix ^a		2,50
Phytase and xylanase enzyme mixture		2,00
DL-methionine		1,49
Sodium bicarbonate		1,30
L-Lysine HCL		0,77
Total		1000,00
Nutrients	Unit	Value
Crude protein	%	16,25
Metabolisable energy (ME)	kcal/kg	2755,00
Crude fat	%	3,16
Crude fiber	%	4,21
Crude ash	%	12,62
Ca	%	4,00
P available	%	0,33
Na	%	0,17
Cl	%	0,21
Dig. Methionine	%	0,40
Dig. Lysine	%	0,70
Dig. Met + Cys	%	0,64
Dig. Arginine	%	0,95
Dig. Threonine	%	0,49
Dig. Isoleucine	%	0,58
Dig. Valine	%	0,66
Linoleic acid C18:2	%	1,71
Electrolyte balance	mEq/Kg	187,28

^a Supplied per kg diet: 10,000 U vitamin A, 3000 U vitamin D3, 30 mg vitamin E, 2.5 mg vitamin B1, 6.0 mg vitamin B2, 5.0 mg vitamin B6, 0.015 mg vitamin B12, 4.0 mg vitamin K3, 8.0 mg pantothenic acid, 30 mg niacin, 0.050 mg biotin, 1.0 mg folic acid, 50 mg vit C and 149 mg choline, 3.75 mg canthaxanthin, 1.75 mg B-Apo-8-carotenoic acid ester; 20 mg Fe, 1.25 mg Cu, 45 mg Zn, 62 mg Mn, 0.62 mg I, 0.9 mg Co, 0.01 mg Se.

from the tip of the villus to the junction of the villus and the crypt, and the crypt depth was measured from its base to the region of transition between the crypt and the villus. The surface area of the villi was calculated using the following formula: $(2\pi) * (\text{villus width}/2) * (\text{villus length})$ (Solis de los Santos et al. 2005).

Microbiota determination of caecal contents

At the end of the treatment, the samples of caecal contents were collected in sterile plastic bottles and immediately placed on ice until analysis was conducted. The cecal microbiota was analysed using a culture technique, as described previously (Choi et al. 2009). One gram of intestinal contents was diluted in 9 ml of butterfield phosphate buffer solution, after which further serial dilutions were made using the same buffer system. Duplicate plates were inoculated with 0.1 ml of sample and incubated. The bacterial populations were transformed ($\log^{10}$) before statistical analysis and expressed as $\log^{10}$ cfu/g of intestinal contents. The microbial groups analysed were coliform bacteria (MacConkey agar (105465, Merck, Darmstadt, Germany)), lactic acid bacteria enumerated on MRS agar (110660, Merck, Darmstadt, Germany), and total aerobic bacteria enumerated on nutrient agar (105450, Merck, Darmstadt, Germany).

Immunity measurement

Hens were vaccinated with Newcastle disease vaccine (NDV), Hipraviar Clon, at 79 weeks of age for immunological examination. Blood samples (13 samples from each treatment) were collected 14 days after vaccination, and the serum was separated on the same day and stored at 4 °C. The day after the serum

samples were collected, they were evaluated for the presence of any antibody response, specifically against NDV, using Enzyme-Linked Immunosorbent Assay (ELISA). Indirect ELISA (ProFLOK NDV Plus, Location), specific for the IgG anti-NDV isotype, was performed according to the manufacturer's recommendations (Synbiotics, USA). The antibody titer was calculated through software, which correlates the optical density of the samples with the positive control optical density (i.e., the sample/positive ratio). The antibody titers are presented as log₁₀ values.

Volatile fatty acid determination of caecal contents

Volatile fatty acids (VFAs) concentrations were determined at the end of the treatment, and for this purpose, caecal contents were collected and pooled per replicate pen. The SCFA concentrations of frozen samples from the cecal digesta were measured via gas chromatography. Approximately 1 g of thawed digesta was mixed with 0.2 mL of 24% metaphosphoric acid solution, diluted with deionized water (4 mL), and centrifuged at 25,000 × g for 20 min at 4 °C. The supernatants were analyzed to determine the concentrations of acetic, propionic, and *n*-butyric acids using a Varian CP-3800 gas chromatograph (Varian Analytical Instruments, Middelburg, the Netherlands) equipped with a 30 m × 0.53 mm i.d. column (Teknokroma TRB-FFAP, Barcelona, Spain) and a flame ionization detector. The temperature of the column was 110 °C, and that of the detector and injector was 250 °C. Nitrogen was used as the carrier gas with a flow rate of 60 mL/min. The internal standard was 4-methyl-*n*-valeric acid.

Bone measurements

The right tibia of each hen was removed to measure the amount of bone ash. To determine the tibia ash content, bones were oven-dried at 105 °C for 24 h and ashed in a muffle furnace at 600 °C for 14 h according to the procedure of Liu et al. (2003). The percentage of ash was determined relative to the dry weight of the tibia. Before performing tibia ash analysis, the tibias were subjected to the tibia breaking test. A three-point bending test was used to measure the force (Newtons (N)) needed to break the bone using a materials testing machine from Amatek (Lloyd Instruments) equipped with a load cell capacity of 500 N and a crosshead speed of 5 mm/min and a 35.9 mm span.

Liver enzymes of blood samples

Blood samples (approximately 5–6 ml) were taken from the wing vein of each pen, with 13 per replicate, during the 60th, 70th, and 81 st weeks of age in the hens. Each sample was subsequently stored at room temperature, subsequently centrifuged and stored as a serum sample at –20 °C for further analysis. The liver enzymes catalase (CAT-ELISA kit: BT Labs, Bioassay Technology Laboratory, China, Catalog No. E0118Ch), glutathione peroxidase (GSH-Px-ELISA kit: BT Labs, Bioassay Technology Laboratory, China, Catalog No. E0298Ch) and superoxide dismutase (SOD-ELISA kit: BT Labs, Bioassay Technology Laboratory, China, Catalog No. E0011Ch) in the serum samples were assayed with commercial reagent kits.

Statistical analysis

The data for all response variables of each experiment were analyzed in a completely randomized block design, which consisted of three dietary treatments and 13 replicate blocks, using the general ANOVA/MANOVA procedure of the MINITAB 18 software (Minitab Ltd.). The cage pens were the experimental units for all analyses. When significant differences ($P < 0.05$) among groups were found, the means were separated using the Tukey HSD test. Mortality results were assessed via the chi-square test.

Results

Productive performance and egg quality

The effects of different herbal feed additives on productive performance are presented in Table 2. No significant differences ($P > 0.05$) were observed in egg production, egg weight, daily feed intake or mortality measurements, but egg mass and the feed conversion ratio were significantly improved ($P < 0.05$) by TAN supplementation. Weight loss during the experimental period was significantly decreased by MCFA supplementation ($P < 0.05$).

The effects of different herbal feed additives on egg external and internal quality are presented in Table 3. While cracked egg, shell-less egg, and dirty egg ratio and egg shell weight, egg shell ratio, shell thickness, and internal egg quality parameters (Haugh unit, albumen height and yolk color) were not significantly ($P > 0.05$) affected by the addition of any feed additives, considered over the whole period, egg shell breaking strength only improved ($P < 0.05$) by the MCFA-based feed additive supplementation.

Histological analysis of jejunum

The effects of different herbal feed additives on histological parameters are presented in Table 4. Both experimental groups had significant ($P < 0.05$) positive effects on jejunum histomorphology. Compared

Table 2. Effects of different herbal feed additive supplementation on overall productive performance of old aged laying hens.

Parameters	CON	MCFA	TAN	P-value
EP, %	89,07 ± 0,82	88,16 ± 0,85	90,53 ± 0,77	0,183
EW, g	64,04 ± 0,34	64,03 ± 0,45	64,62 ± 0,30	0,431
EM, g	57,02 ± 0,61 ^b	56,41 ± 0,42 ^b	58,46 ± 0,43 ^a	0,048
DFI, g	113,61 ± 0,23	113,09 ± 0,38	112,75 ± 0,47	0,174
FCR, g:g	2,000 ± 0,021 ^a	2,009 ± 0,015 ^a	1,934 ± 0,014 ^b	0,014
Mortality, %	9,61 ± 2,88	6,73 ± 2,29	9,38 ± 3,48	0,907
IBW, g	1577,10 ± 9,79	1580,10 ± 9,22	1573,30 ± 8,79	0,957
FBW, g	1494,60 ± 11,55 ^{ab}	1529,70 ± 17,74 ^a	1483,70 ± 8,67 ^b	0,048
BWG, g	-82,50 ± 10,83 ^b	-50,40 ± 14,82 ^a	-89,60 ± 9,36 ^b	0,015

CON: Control group without any herbal supplement, MCFA: Medium-chain fatty acid-based herbal additive supplemented group; TAN: hydrolysable tannins and condensed tannin-based herbal additive supplemented group; EP: Egg production; EW: Egg weight; EM: Egg mass; DFI: Daily feed intake; FCR: Feed conversion ratio; IBW: Initial live body weight; FBW: Final live body weight; BWG: Body weight gain between the initial and final stages;

The data are expressed as the means ± standard error of the mean (SEM).

a,b: Means within a row with different superscripts are significantly different at $P < 0.05$.

Table 3. Effects of different herbal feed additive supplementation on overall egg quality of old aged laying hens.

Parameters	CON	MCFA	TAN	P-value
External egg quality parameters				
CE, %	0,92 ± 0,17	1,02 ± 0,22	1,12 ± 0,20	0,694
SHL, %	0,44 ± 0,07	0,53 ± 0,08	0,57 ± 0,30	0,131
DE, %	0,55 ± 0,09	0,67 ± 0,18	0,38 ± 0,07	0,287
ESW, g	6,10 ± 0,03	6,16 ± 0,04	6,15 ± 0,05	0,804
ESR, %	9,34 ± 0,04	9,48 ± 0,05	9,41 ± 0,07	0,230
ST, mm	360,20 ± 2,01	361,52 ± 2,64	360,90 ± 2,16	0,818
ESBS, N	40,69 ± 0,67 ^b	42,49 ± 0,60 ^a	42,09 ± 0,51 ^{ab}	0,048
Internal egg quality parameters				
HU	81,31 ± 0,65	81,68 ± 0,56	82,32 ± 0,83	0,711
AH, mm	6,96 ± 0,08	7,02 ± 0,08	7,13 ± 0,12	0,531
YC	11,27 ± 0,05	11,16 ± 0,07	11,21 ± 0,06	0,399

CON: Control group without any herbal supplement, MCFA: Medium chain fatty acid based herbal additive supplemented group, TAN: hydrolysable tannins and condensed tannins based herbal additive supplemented group.

CE: Cracked eggs; SHL: Shell-less eggs; DE: Dirty eggs; ESW: Egg shell weight; ESR: Egg shell ratio; ST: Shell thickness; ESBS: Egg shell breaking strength; HU: Haugh unit; AH: Albumen height; YC: Yolk color; Number given according to the Roche Yolk Color Fan.

The data are expressed as means ± standard error of the mean (SEM).

a,b: Means within a row with different superscripts are significantly different at $P < 0.05$.

Table 4. Effects of different herbal feed additive supplementation on jejunum histomorphology parameters of old aged laying hens.

Parameters	CON	MCFAs	TAN	P-value
VH, μm	1098,84 $\pm$ 15,02c	1189,75 $\pm$ 10,58a	1143,97 $\pm$ 11,30b	<0,001
CD, μm	197,26 $\pm$ 7,32	177,65 $\pm$ 14,14	183,64 $\pm$ 14,16	0,457
VV, μm	187,65 $\pm$ 5,34	203,08 $\pm$ 6,27	186,32 $\pm$ 4,60	0,100
VH/CD	5,65 $\pm$ 0,20b	7,20 $\pm$ 0,59a	6,68 $\pm$ 0,55ab	0,050
VSA, mm^2	0,648 $\pm$ 0,018b	0,758 $\pm$ 0,022a	0,669 $\pm$ 0,017b	0,003
NGC	185,69 $\pm$ 9,55b	218,75 $\pm$ 9,92a	187,34 $\pm$ 11,28b	0,042

CON: Control group without any herbal supplement, MCFAs: Medium-chain fatty acid-based herbal additive supplemented group, TAN: hydrolysable tannins and condensed tannins-based herbal additive supplemented group.

VH: Villus height, CD: Crypt depth, VV: Villus width, VSA: Villus surface area, NGC: Number of goblet cells.

The data are expressed as the means $\pm$ standard error of the mean (SEM).

a–c: Within a row with different superscripts, significantly different at $P < 0.05$.

Table 5. Effects of different herbal feed additive supplementation on antibody titer and cecal bacterial counts of old aged laying hens.

Parameters	CON	MCFAs	TAN	P value
NDV antibody titers, $\log_{10}^*$	4,153 $\pm$ 0,009b	4,196 $\pm$ 0,012a	4,202 $\pm$ 0,013a	0,050
TAB, $\log$ (cfu/g)	7,76 $\pm$ 0,30b	8,60 $\pm$ 0,30a	7,81 $\pm$ 0,22ab	0,024
Lab. $\log$ (cfu/g)	8,52 $\pm$ 0,24b	9,29 $\pm$ 0,30a	8,82 $\pm$ 0,27ab	0,047
Colf. $\log$ (cfu/g)	7,62 $\pm$ 0,39	7,43 $\pm$ 0,21	7,43 $\pm$ 0,25	0,942

CON: control group without any herbal supplement; MCFAs: medium-chain fatty acid-based herbal additive-supplemented group; TAN: hydrolysable tannin- and condensed tannin-based herbal additive-supplemented group;

TAB: total aerobic bacteria; Lab: *Lactobacillus*; Colf: Coliform.

The data are expressed as the means $\pm$ standard errors of the means (SEMs).

a,b: Means within a row with different superscripts are significantly different at $P < 0.05$.

* NDV titer against the Hipriavir Clon.

with the control treatment, MCFA and TAN supplementation significantly improved the villus height ($P < 0.05$). Villus surface area and goblet cell numbers were also positively influenced by the inclusion of MCFAs compared to the control treatment ($P < 0.05$), while the effect of TAN was not significant ($P > 0.05$).

Microbiota contents of caecal and antibody titer result

The effects of different herbal feed additives on the microbial content of caecal and the antibody titre against NDV are presented in Table 5. Compared with the control treatment, both MCFA and TAN supplementation improved the antibody titers of laying hens vaccinated with the NDV ($P < 0.05$). In addition, compared with the control treatment, MCFA supplementation also significantly ($P < 0.05$) increased the number of *Lactobacillus* and total aerobic bacteria in the cecum.

Volatile fatty acid contents of caecal

The effects of different herbal feed additives on the volatile fatty acid contents of the cecum contents are presented in Table 6. While propionate, butyrate and total short-chain fatty acid concentrations were significantly improved ($P < 0.05$) by MCFA and TAN supplementation, the acetate concentration was significantly ($P < 0.05$) improved only by MCFAs, and the isobutyrate and branched-chain fatty acid concentrations were significantly ($P < 0.05$) improved only by TAN supplementation. Both valerate and isovalerate concentrations were not significantly ($P > 0.05$) affected by herbal feed additive supplementation.

Tibia parameters

The effects of different herbal feed additives on tibia parameters are presented in Table 7. While the tibia ash and tibia weights were not significantly affected ($P > 0.05$) by the treatments, the tibia breaking strength was significantly improved ($P < 0.05$) by the MCFAs supplementation.

Table 6. Effects of different herbal feed additive supplementation on concentration of ceca short-chain fatty acids (SCFAs) (mmol/l) of old aged laying hens.

Parameters	CON	MCFAs	TAN	P-value
Acetate	29,91 ± 1,29 ^b	33,33 ± 0,65 ^a	31,04 ± 0,45 ^{ab}	0,037
Propionate	10,18 ± 0,32 ^b	11,65 ± 0,49 ^a	11,34 ± 0,32 ^a	0,016
Butyrate	7,08 ± 0,42 ^b	10,12 ± 0,53 ^a	10,95 ± 0,40 ^a	<0,001
Isobutyrate	1,02 ± 0,04 ^b	1,11 ± 0,03 ^{ab}	1,32 ± 0,08 ^a	0,006
Valerate	1,20 ± 0,03	1,21 ± 0,06	1,22 ± 0,02	0,956
Isovalerate	1,20 ± 0,04	1,21 ± 0,04	1,19 ± 0,05	0,984
BCFA	3,42 ± 0,05 ^b	3,52 ± 0,06 ^{ab}	3,73 ± 0,12 ^a	0,048
T-SCFAs	50,59 ± 1,52 ^b	58,62 ± 0,77 ^a	57,05 ± 0,78 ^a	<0,001

CON: Control group without any herbal supplement; MCFAs: Medium-chain fatty acid-based herbal additive supplemented group; TAN: hydrolysable tannins and condensed tannin-based herbal additive supplemented group.

BCFA: Branched-chain fatty acids (iso-butyric12-me-butyric1iso-valeric); T-SCFAs: Total short-chain fatty acids.

The data are expressed as the means ± standard error of the mean (SEM).

a,b: Means within a row with different superscripts are significantly different at $P < 0.05$.

Table 7. Effects of different herbal feed additive supplementation on tibia parameters of old aged laying hens.

Parameters	CON	MCFAs	TAN	P-value
Tibia ash, %	50,68 ± 0,52	50,65 ± 0,74	50,92 ± 0,55	0,621
Tibia breaking strength, N	205,42 ± 9,78 ^b	221,86 ± 9,11 ^a	207,17 ± 2,53 ^b	0,041
Tibia weight, g	5,65 ± 0,14	5,77 ± 0,16	5,73 ± 0,18	0,099

CON: Control group without any herbal supplement, MCFAs: Medium-chain fatty acid-based herbal additive supplemented group; TAN: hydrolysable tannins and condensed tannins-based herbal additive supplemented group.

The data are expressed as the means ± standard error of the mean (SEM).

a,b: Means within a row with different superscripts are significantly different at $P < 0.05$.

Table 8. Effects of herbal feed additive supplementation on liver enzymes of old aged laying hens at different ages.

	CAT (ng/mL)	GSH-Px (ng/mL)	SOD (U/L)
60 wks of age			
CON	16,405 ± 2,54	3,387 ± 0,351	87,52 ± 6,15
MCFAs	15,54 ± 1,98	2,972 ± 0,225	95,74 ± 10,26
TAN	10,62 ± 1,28	2,031 ± 0,270	72,95 ± 8,17
P-value	0,090	0,088	0,518
70 wks of age			
CON	11,99 ± 2,42	1,872 ± 0,249	64,67 ± 11,76 ^b
MCFAs	15,88 ± 2,52	1,910 ± 0,350	100,63 ± 20,72 ^a
TAN	13,75 ± 2,54	1,612 ± 0,214	84,13 ± 14,91 ^{ab}
P-value	0,521	0,360	0,055
81 wks of age			
CON	8,09 ± 1,03 ^b	1,841 ± 0,231	60,09 ± 6,25 ^b
MCFAs	19,66 ± 3,34 ^a	2,348 ± 0,129	103,01 ± 6,58 ^a
TAN	13,49 ± 2,78 ^{ab}	2,445 ± 0,297	103,41 ± 15,58 ^a
P-value	0,010	0,390	0,042

CON: Control group without any herbal supplementation; MCFAs: Medium-chain fatty acid-based herbal additive-supplemented group; TAN: hydrolysable tannin- and condensed tannin-based herbal additive-supplemented group.

CAT, catalase, GSH-Px, glutathione peroxidase, SOD; superoxide dismutase.

The data are expressed as the means ± standard error of the mean (SEM).

a,b: Values within a row with different superscripts are significantly different at $P < 0.05$.

Liver enzymes of serum samples

The effects of different herbal feed additives on liver enzymes are presented in Table 8. Compared with those at the beginning of the experiment, liver enzymes (CAT, GSH-Px and SOD) at different ages significantly changed after the middle of the experiment. While the control treatment had a decreasing trend in activity throughout the experimental period, both herbal additive treatment groups maintained their level of activity or increased it somewhat throughout the trial period. SOD enzyme activity in the liver was significantly increased by the addition of MCFAs compared to the control group, as observed in blood

samples from 70- and 81-week-old subjects ($P < 0.05$). In addition, while CAT enzyme activity was significantly improved by supplementation with MCFAs, and SOD activity was significantly improved by supplementation with both herbal additives at the end of the experiment ($P < 0.05$) compared to the control group.

Discussion

The present study evaluated the effects of supplementing the diet of laying hens with MCFAs or TAN-based herbal feed additives on performance parameters, egg quality, gut health, and immune parameters after 57 weeks of age. The results demonstrate several key outcomes associated with dietary interventions, offering insights into the potential benefits of these supplements in poultry nutrition.

Supplementation with TAN significantly improved egg mass and the feed conversion ratio (FCR), indicating more efficient feed utilization. However, there were no significant differences in overall egg production, egg weight, daily feed intake, or mortality rates across all groups. These findings suggest that while TAN enhances certain aspects of production efficiency, it does not broadly affect primary productivity measures. These results are consistent with previous studies reporting that tannins can improve nutrient utilization and metabolic efficiency, thereby increasing the FCR and egg mass without significantly affecting egg production or weight (Lee et al. 2021). MCFA supplementation resulted in a significant reduction in weight loss during the experimental period, which could indicate that MCFAs may help maintain the body condition of older hens. As shown in different studies (Ee et al. 2024; Roopashree et al. 2021), MCFAs not only serve as an energy source but also regulate glucose and lipid metabolism and have a direct role in inhibiting cell signaling.

Regarding egg quality, neither MCFAs nor TAN supplementation in the conditions of this study significantly affected cracked eggs, shell-less eggs, dirty eggs, or internal egg quality parameters (Haugh unit, albumen height, and yolk color). However, MCFA supplementation significantly improved eggshell breaking strength, likely because of the positive effects of MCFA on calcium metabolism and deposition, as observed in other studies (Świątkiewicz et al. 2010). This finding is consistent with the results for the tibia parameters, as the tibia breaking strength was notably improved by MCFA supplementation. This suggests that MCFAs contribute to bone strength as well, possibly through enhanced calcium and mineral metabolism, which is crucial for maintaining skeletal integrity in laying hens (Świątkiewicz & Arczewska-Włosek, 2012), which is especially important during later stages of production (Alfonso-Carrillo et al. 2021).

At the gut level, we found that both MCFAs and TAN had significant positive effects on jejunum histomorphology, with increased villus height observed in both treatment groups compared to the control. An increased villus height is indicative of improved nutrient absorption capacity, which could contribute to improved feed efficiency in the TAN group (Ravindran & Abdollahi, 2021). MCFA supplementation also significantly increased the villus surface area and number of goblet cells, further supporting the positive influence of MCFAs on gut health by enhancing the absorptive surface and mucosal barrier of the intestine (Jia et al., 2020). In the literature, similar to our study, Wang et al. (2024) reported that MCFA supplementation improved the gut morphology of laying hens by increasing the intestinal VH, and the possible effects of MCFAs on chick histomorphology were associated with influencing the gut microbiota function and community (Amer et al. 2020). The study also examined the impact of MCFAs and TAN on short-chain fatty acids (SCFAs) production in the gut. MCFA supplementation led to an increase in acetate production, whereas TAN supplementation increased the production of isobutyrate and branched-chain fatty acids. These endogenous SCFAs are crucial for gut health, serving as energy sources for enterocytes, modulating the gut pH, and inhibiting pathogenic bacteria (Zhan et al. 2022). The specific increases in endogenous SCFAs observed with MCFAs and TAN supplementation suggest that these additives may selectively modulate the gut microbiota, promoting beneficial fermentation profiles. In addition, both MCFAs and TAN significantly increased antibody titers against NDV, indicating an enhancement of the immune response in laying hens. These findings are in line with recent work by Mellouk et al. (2024), where he demonstrates that MCFAs in the diet enhance the humoral and cellular immune response to infectious bronchitis virus. The improved immune response may also be linked to the enhanced gut health observed, as a healthier gut environment is often associated with a more robust immune system. In literature Wang

et al. (2024) reported that the use of glycerol monolaurate as an MCFA simultaneously reduced the mRNA expression of proinflammatory cytokines and that TLR4 in the intestine improved the immune response and reduced the occurrence of inflammation.

Another aspect of this study was the evaluation of liver enzyme activity, particularly that of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px). The results revealed significant changes in liver enzyme activities, particularly after the middle of the experiment. While the control group showed a declining trend in enzyme activity throughout the experimental period, both MCFA and TAN supplementation helped maintain or even increase the enzyme activity levels. MCFAs significantly increased SOD enzyme activity at 70 and 81 weeks, as well as CAT enzyme activity at the end of the experiment, compared to the control group. The improvement in SOD activity caused by both MCFA and TAN supplementation at the end of the trial period suggests that these additives may enhance the antioxidant defense system in laying hens. This enhancement could be crucial in protecting the liver from oxidative stress, which is particularly important in older hens where oxidative damage tends to accumulate (Oke et al. 2024; Surai et al. 2019).

Conclusion

In conclusion, supplementing the diets of laying hens with MCFAs or TAN-based herbal feed additives offers several benefits. The increased egg mass, feed efficiency, eggshell and strength, tibia strength, and liver antioxidant capacity observed with MCFAs and TAN suggest that these additives could be valuable in improving the performance and health of laying hens, especially during the later stages of production, although overall productivity measures such as egg production and weight remain unaffected. Future research should focus on the long-term effects of these supplements and their interactions with other dietary ingredients to optimize their use in poultry nutrition.

Author contributions

None.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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