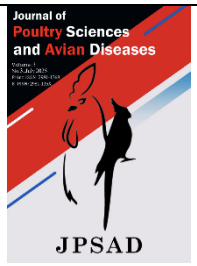
Journal of Poultry Sciences and Avian Diseases

Journal homepage: www.jpsad.com



Effects of Pennyroyal Essential Oil and *Heyndrickxia coagulans (Bacillus coagulans)* on Broiler Chicken Growth, Intestinal Microbiota, Intestinal Morphology, and Immune Response

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A r t i c l e I n f o

Article type:
Original Research

How to cite this article:
Hasani Sorkhani, E., Afsharmanesh, M., & Khajeh Bami, M. (2025). Effects of Pennyroyal Essential Oil and *Heyndrickxia coagulans (Bacillus coagulans)* on Broiler Chicken Growth, Intestinal Microbiota, Intestinal Morphology, and Immune Response. *Journal of Poultry Sciences and Avian Diseases, 3*(3), 20-28.
http://dx.doi.org/10.61838/kman.jpsad.3.3.4

A B S T R A C T

This experiment aimed to see how a probiotic containing *Heyndrickxia coagulans* (*Bacillus coagulans)* and different levels of pennyroyal essential oil (PEO) affected broiler chicken growth, intestinal microbiota, intestinal morphology, and immune response. In this study, 300 one-day-old Ross 308 broilers were used in a completely randomized design of 3×2 factorial arrangement, six treatments, five replications, and 10 chicks per replicate, with three levels of PEO (zero, 100, and 200 mg/kg) and two levels of probiotic (zero and 200 mg/kg). The interaction effects revealed that broilers fed only *H. coagulans* had the best body weight gain and feed conversion ratio ($p<0.05$). In addition, using *H. coagulans* alone caused increased lactic acid bacteria, villus height, villus height to crypt depth ratio, and antibody titer compared to sheep red blood cells compared to diet without additive. The main effects were observed, in which case the birds fed *H. coagulans* diets had higher villus height, villus height to crypt depth ratio, and antibody titer in contrast to sheep red blood cells, as well as lower intestinal coliform counts and pH than those not fed *H. coagulans* diets ($p<0.05$). In comparison, the number of intestinal coliform bacteria was smaller in chicks fed 200 mg/kg PEO than in chicks fed no PEO. In conclusion, using *H. coagulans* alone in broiler feeding is more effective than PEO in improving growth, immune response, intestinal microbiota, and intestinal morphology.

Keywords: *Heyndrickxia coagulans (Bacillus coagulans), Broilers, Pennyroyal essential oil, Performance, Probiotics.*

Article history:
Received 10 April 2025
Revised 02 June 2025
Accepted 06 June 2025
Published online 01 July 2025

1 Introduction

Probiotics are live microbial feed supplements that benefit the microbial equilibrium in the host's intestine (1). Several *Bacillus* species have recently been identified as potential probiotics. *Heyndrickxia coagulans* (*Bacillus coagulans)* is a spore-forming and lactic acid-producing bacterium probiotic strain (2). Studies in pigs (3) and humans (4) show that *H. coagulans* altered gastrointestinal microbial equilibrium by replenishing desirable microbes and antagonizing pathogenic microorganisms. According to a previous report, dietary supplementation of *H. coagulans* in broiler chicken feed improves their growth performance and intestinal microflora (5).

Volatile oils extracted from flowers, nuts, spices, vegetables, and roots are known as essential oils (6). Basic oils have been shown to increase growth performance and feed efficiency in poultry by improving gut ecological conditions, increasing digestibility, and simulating digestive enzyme secretion (7, 8). Pennyroyal (*Mentha pulegium L.*), a Labiatae family herb, is found worldwide. The Middle East, Europe, North Africa, Minor Asia, and the Near East are all home to pennyroyal (9, 10). Its essential oil has been shown to have antibacterial (11, 12) and antioxidant properties (11). Furthermore, pennyroyal had an important impact on the morphology of the intestinal tract of poultry. The intake of pennyroyal essential oil (PEO) (200, 300, and 400 mg/kg diet) in quail results in an improvement in the villi height and width of the intestine, according to Dehghani et al. (11). Additionally, the effects of pennyroyal on chicken performance have been shown (13).

While individual probiotic strains and phytobiotic substances are beneficial, their synergism will improve their efficacy (14). The consumption of a mixture of probiotics and phytobiotic substances as functional feeds has not been extensively studied. The study showed that combining plant extracts with *lactobacillus* increases broiler growth performance (15). As a result, it was hypothesized that supplementing broiler development with PEO in conjunction with *H. coagulans* may have a positive impact. In light of this, we investigated the effects of a PEO blend containing *H. coagulans* on broiler growth performance, immune response, intestinal microbiota, and intestinal morphology in the current study.

2 Materials and Methods

2.1 Birds, Management, and Experimental Diets

Three hundred one-day-old male broiler chicks (Ross 308) were randomly assigned to 6 treatments with five replicate pens and 10 birds per replicate. The broilers were reared in pens of identical size (100×100 cm floor area and 80 cm height) for a 42-day experimental period. Temperature, light, and ventilation were controlled according to the Ross broiler breeding standards. Mash diets were prepared freshly daily and formulated to meet or top the Ross 308 management manual requirements for broiler chickens (Table 1). Feed and water were provided ad libitum to the birds throughout the 42-day experimental period. The experiment was conducted according to the animal welfare guidelines at the Veterinary Control and Research Institute of Kerman, Iran. Three levels of dietary supplemental PEO (0, 100, and 200 mg/kg of diet) and two levels of *H. coagulans* (0, 200 mg/kg) were pooled as an entirely randomized design with a 3×2 factorial arrangement. The *probiotic bacterium was used as the H. coagulans* ATCC 7050 (lyophilized *H. coagulans* powder, 10^{11} CFU/g, Pardis Roshd Mehregan Co., Iran). Before starting the experiment, total *H. coagulans* were confirmed in probiotics, and they were included and blended with diets every week. After mixing, the diets supplemented with *H. coagulans* were analyzed for spore counts weekly (16). Pennyroyal essential oil was purchased from Zahra Rosewater Company in Iran and kept in dark, glassy bottles until their use. The composition of the PEO was determined using gas chromatography (Agilent 7890B) with a mass detector (Model 5977A, Agilent Technology) (17). Table 2 demonstrates the chemical composition of pennyroyal essential oil. Pennyroyal essential oil was added to a quantity of soybean oil and homogenized using a mixer. The mixture was then pulverized with the corn. Corn with essential oil was added to the premixture. Finally, the premixture was added to the main mixture and stored in covered containers before feeding for blend stability.

Table 1. Ingredients and calculated nutrient composition of the basal diets

Items	Starter (1 to 14 d)	Grower (15 to 35 d)	Finisher (36 to 42 d)
Ingredients (%)			
Corn	30.0	30.24	30.00
Soybean meal (44% CP)	35.9	34.60	31.80
Wheat	28.0	27.98	30.25
Soybean oil	2.30	3.60	4.50
Calcium carbonate	1.35	1.352	1.361
Mono calcium phosphate	1.36	1.285	1.170
Salt	0.30	0.30	0.30
DL-methionine	0.16	0.176	0.089
L-Lysine HCl	0.10	0.148	0.20
Vitamin premix1	0.25	0.25	0.25
Mineral premix2	0.25	0.25	0.25
Xylanase enzyme	0.03	0.03	0.03
Calculated Analysis			
Metabolizable energy (kcal/kg)	2986	3010	3033
Crude protein (%)	22.1	20.1	18.7
Lysine (%)	1.27	1.2	1.07
Methionine + Cystine (%)	0.97	0.9	0.85
Calcium (%)	0.9	0.87	0.77
Available phosphorus (%)	0.68	0.61	0.61

[1]Supplied per kg of diet: vitamin A (retinol), 12000 IU; vitamin D3 (Cholecalciferol), 5000 IU; vitamin K3, 2.55 mg; thiamin, 3 mg; riboflavin, 7.5 mg; vitamin B6 (pyridoxine), 4.5 mg; vitamin B12 (cyanocobalamin), 0.02 mg; niacin, 51 mg; folic acid, 1.5 mg; biotin, 0.2 mg; pantothenic acid, 13.5 mg; choline chloride, 250 mg.

[2] Supplied per kg of diet: Mn, 120 mg; Cu, 16 mg; I, 1mg; Fe, 40 mg; Zn, 100 mg, Se, 0.3 mg.

Table 2. Phytochemical composition of pennyroyal essential oil

Compounds	% Total
Pulegone	39.22
Menthone	35.82
Piperitone	14.76
γ-Terpinene	3.34
Other compounds	6.86

2.2 Growth Performance

Birds and feed were weighed on days 1, 21, and 42 on a pen basis, and the body weight gain (BWG), feed intake (18), and feed conversion ratio (FCR) were calculated. Birds were checked twice daily for mortality and weighed.

2.3 Intestinal Microbiota and pH

On day 42, a bird was randomly chosen from each pen, and one gram of exudative material from their lower ileum was collected. The ileal digesta were serially diluted in phosphate-buffered saline for lactic acid bacteria and coliform counts. To cultivate lactic acid, bacteria were used from dilutions 10^{-3} to 10^{-6}; for coliforms, bacteria were used from the culture of dilutions 10^{-2} to 10^{-5}. The lactic acid bacteria were cultivated on MRS agar (Merck-110661) at 37 °C for 48 h, and the coliforms were cultivated on MacConkey agar (Merck-105465) at 37 °C for 24 h. (5). The pH of the intestinal contents was measured using a digital pH meter (Elmetron, CP103 model) after mixing digesta with distilled water at a ratio of 1: 1.

2.4 Intestinal Morphology

At 42 days of age, one bird was randomly selected from each replicate, weighed, and sacrificed. To evaluate the structure of the ileum tissue, 1 cm segments of the lower ileum were fixed in 10% buffered formaldehyde after evacuation and washing. Then, each sample was embedded in paraffin waxes, and finally, 5-µm sections of each sample were stained with hematoxylin and eosin. The slides were examined using an optical microscope (Micromaster, Fisher Scientific, Cat. No. 12-562-27, Fisher Scientific, Waltham, MA), and the intestinal criteria, including villus height, villus width, and crypt depth (n = 5/bird) were measured

using the Image Pro Plus v 4.5 software package (Media Cybernetics, Silver Spring, MD, USA) (5). For histomorphological analysis, villus height and crypt depth were measured from five randomly selected villi and their corresponding crypts, taken from one intestinal section per bird.

2.5 Immune Response

On days 21 and 35, two birds per replicate were injected brachial with 1 mL of 0.5% sheep red blood cells (SRBC) suspension into a brachial vein. For SRBC preparation, first, the blood of a male sheep was collected into tubes with EDTA. Then, the samples were centrifuged at 2000 rpm for 15 min. Finally, they were washed 3 times in phosphate-buffered saline. Blood samples were collected seven days after injection, and sera were frozen until antibody titers were determined using hemagglutination assays (5). For hemagglutination titration, 50 µL of PBS was added to the first row of a 96-well V-bottom microtiter plate, followed by 50 µL of heat-inactivated serum. Twofold serial dilutions were performed after incubation at 37°C for 30 minutes. Subsequently, 50 µL of a 2.5% SRBC suspension was added to each well, and the plates were incubated for 30 minutes. The IgG titers were determined using a similar protocol, adding 2-mercaptoethanol to assess mercaptoethanol-resistant antibodies. Titers were recorded as the $\log_2$ of the reciprocal of the last dilution, showing agglutination.

2.6 Statistical Analysis

The data were analyzed using a two-factorial design with three PEO and two *H. coagulans* levels using the GLM procedure of SAS (SAS Institute, Cary, NC). Tukey's test was used to determine the effect of treatments, and differences were considered significant at $p<0.05$. Means were presented with their standard error of means.

3 Results and Discussion

3.1 Growth Performance

Table 3 demonstrates the effects of different amounts of PEO and *H. coagulans* supplementation on broiler chicken growth performance. During the entire time, there was a significant interaction ($p<0.05$) between these two supplements on BWG and FCR. As a result, broilers fed only *H. coagulans* had the best BWG and FCR. Feeding 200 mg/kg of PEO to the birds had a negative impact on their performance, lowering BWG and worsening FCR ($p<0.05$).

Table 3. Effects of pennyroyal essential oil (PEO) and *Heyndrickxia coagulans* on the growth performance of broilers

Items		Body weight gain (g/b/d)	Feed intake (g/b/d)	Feed conversion ratio
PEO (mg/kg)	*H. coagulans*			
0	–	53.83^{b,c}	96.96	1.85^{a,b}
	+	58.42^{a}	96.31	1.69^{d}
100	–	55.99^{a,b}	94.69	1.70^{c,d}
	+	50.38^{c,d}	94.07	1.82^{b,c}
200	–	50.49^{c,d}	95.89	1.94^{a}
	+	48.43^{d}	93.92	1.89^{a,b}
SEM		0.937	1.453	0.025
Main effect				
0		56.12^{a}	96.63	1.77^{b}
100		53.19^{b}	94.38	1.76^{b}
200		49.46^{c}	94.90	1.91^{a}
SEM		0.663	1.027	0.018
	–	53.44	95.84	1.83
	+	52.41	94.77	1.80
SEM		0.541	0.839	0.014
p-value				
PEO		<0.001	0.291	<0.001
H. coagulans		0.196	0.377	0.168
PEO × *H. coagulans*		<0.001	0.870	<0.001

[a–d] Means with different superscripts within each column are significantly different ($p<0.05$)

"–" *H. coagulans* without 200 mg/kg diet; "+" *H. coagulans* with (+) (200 mg/kg diet)

Broilers fed diets containing *Lactobacillus acidophilus, Bacillus subtilis, and Clostridium butyricum* had higher BWG and lower FCR than those fed a control diet in another experiment (19). Furthermore, Wealleans et al. (20) found

that supplementing the diet with multi-*bacillus*-strains improves BWG and FCR substantially. Preserving beneficial microbial populations in the intestine, which improves feed digestion and absorption, is one potential explanation for this change (21, 22). In addition, other studies have shown that supplementing with probiotics will increase growth efficiency by improving intestinal morphology, which leads to better nutrient consumption and optimal growth (21-23). As a result, the improved FCR of the broilers fed *H. coagulans* in our experiment may be attributed to improved intestinal microbiota and intestinal morphology, as seen in the current experiment's intestinal microbiota and intestinal morphology results (Table 5 and Table 6). Pennyroyal powder does not seem to have a positive effect on broiler growth efficiency, according to Ghalamkari et al. (24). Oregano essential oils (400 mg/L) had little impact on broiler BWG and FCR from 1 to 40 days in a study (25). In another study, dietary supplementation with PEO (200, 300, and 400 mg/kg) did not affect quail BWG and FCR (11). A study found that pennyroyal at a concentration of 0.07 percent reduced broiler efficiency, which may be attributed to its toxic effect at higher doses (26), by the present study's findings. In addition, this result may be explained by a reduction in beneficial microbiota at higher PEO doses, which counteracts the typical benefits of antimicrobial activity.

3.2 Intestinal Microbiota and pH

Table 4 shows the effects of various amounts of PEO and *H. coagulans* supplementation on the intestinal bacteria of birds. There was a significant interaction (p<0.05) between these two additives on the population of lactic acid bacteria. As a result, using only *B. coagulants* or 100 mg/kg, PEO caused increased lactic acid bacteria compared to a diet without additives (p<0.05). Intestinal coliform counts and pH were smaller in *H. coagulans*-fed chicks than in non-*H. coagulans*-fed chicks (p<0.05).

Table 4. Effects of pennyroyal essential oil (PEO) and *Heyndrickxia coagulans* on intestinal microbiota (log cfu/g) and pH of broilers

Items		Lactic acid bacteria	Total Coliform bacteria	Intestinal pH
PEO (mg/kg)	*H. coagulans*			
0	−	7.365^{b}	6.183^{a}	6.143^{a,b}
	+	8.205^{a}	5.713^{a,b}	5.67^{b,c}
100	−	8.235^{a}	5.965^{a,b}	6.43^{a}
	+	7.445^{a,b}	5.217^{a,b}	5.30^{c}
200	−	7.947^{a,b}	5.357^{a,b}	5.85^{b}
	+	7.362^{b}	4.500^{b}	5.65^{b,c}
SEM		0.185	0.259	0.112
Main effect				
0		7.785	5.498^{a}	5.90
100		7.840	5.591^{ab}	5.86
200		7.655	4.928^{b}	5.75
SEM		0.131	0.204	0.079
	−	7.849	5.835^{a}	6.14^{a}
	+	7.671	5.143^{b}	5.54^{b}
SEM		0.107	0.161	0.060
p-value				
PEO		0.601	0.013	0.364
H. coagulans		0.255	0.013	0.001<
PEO × *H. coagulans*		0.001	0.039	0.002

$^{a-c}$Means with different superscripts within each column are significantly different (p<0.05)

"–" *H. coagulans* without 200 mg/kg diet; "+" *H. coagulans* with (+) (200 mg/kg diet)

In comparison, the number of intestinal coliform bacteria was smaller in chicks fed 200 mg/kg PEO than in chicks fed no PEO (p <0.05). According to interaction effects, birds fed diets supplemented with PEO plus *H. coagulans* had lower coliform counts than those without additives. These findings are consistent with those of Hung et al. (21), who found that feeding *H. coagulans* to broilers reduces the number of coliform bacteria in the duodenum. Furthermore, a study found that using *H. coagulans* causes the intestinal pH to change from alkaline to acidic (27). The use of *B. subtilis* selectively inhibits the proliferation of pathogens such as salmonella by producing reactive fatty acids and lowering the pH of the digestive tract, according to Park and Kim (28). In agreement, Erhan et al. (13) reported that adding

pennyroyal levels (0.25 or 0.50%) reduced *the E. coli* count in the intestines of broilers. Mentha species had antimicrobial properties that were effective against microorganisms believed to be toxic to broilers (29). Compounds found in Mentha plants, such as pulegon, menthole, menthone, and piperitenone oxide, have antimicrobial properties (29). PEO had a bactericidal activity against many bacteria, according to Dehghani et al. (11). Different levels of PEO, on the other hand, had little impact on the population of lactic acid bacteria. Furthermore, interaction effects show that PEO plus *H. coagulans* have the lowest coliform counts and pH. Previous research into the mechanisms of action of phytobiotic compounds and probiotic strains has shown that these compounds can work together to improve animal health (18).

3.3 Intestinal Morphology

Table 5 shows the influence of various amounts of PEO and *H. coagulans* on the intestinal morphology of broiler chickens. There was an interaction between PEO and *H. coagulans* for villus height and width, with the highest villus height and width associated with 200 mg/kg of PEO plus *H. coagulans* (p<0.05). In addition, broilers fed only *H. coagulans* had the highest villus height to crypt depth ratio. The main effects were observed, with birds feeding *H. coagulans* having higher villus height and a higher villus height to crypt depth ratio than birds not feeding *H. coagulans* (p<0.05). In this study, we may hypothesize that combining dietary PEO with *H. coagulans* will synergistically affect intestinal morphology change. Hung et al. (21)observed that birds feeding *H. coagulans* had more villi in the jejunum than control birds, but no major variations in crypt depth were found. Supplemental probiotics can increase villus height by lowering intestine pH, which inhibits the growth of many pathogenic intestinal microbes and, as a result, reduces inflammatory processes in the intestinal mucosa, which raises villus height (30). As seen in the current experiment's immune response results, a healthy intestine significantly improves the bird's immune status (Table 6).

Table 5. Effects of pennyroyal essential oil (PEO) and *Heyndrickxia coagulans* on intestinal morphology of broilers

Items		Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Villus height/crypt depth
PEO (mg/kg)	*H. coagulans*				
0	–	1383^{e}	198.1^{a,b}	82.38	17.10^{c}
	+	1509^{b}	185.6^{d}	78.38	19.31^{a}
100	–	1414^{d}	194.8^{b,c}	81.50	17.35^{b,c}
	+	1451^{c}	195.6^{b}	81.25	17.87^{b,c}
200	–	1499^{b}	187.5^{c,d}	83.59	17.90^{b,c}
	+	1557^{a}	205.0^{a}	84.50	18.43^{a,b}
SEM		5.018	1.638	1.757	0.264
Main effect					
0		1446^{b}	191.9^{b}	80.37	18.21
100		1432^{b}	195.2^{a,b}	81.38	17.61
200		1528^{a}	196.3^{a}	84.05	18.17
SEM		3.542	1.158	1.242	0.187
	–	1432^{b}	193.5	82.49	17.45^{b}
	+	1505^{a}	195.4	81.37	18.54^{a}
SEM		2.897	0.945	1.014	0.152
p-value					
PEO		<0.001	0.039	0.125	0.069
H. coagulans		<0.001	0.167	0.447	<0.001
PEO × *H. coagulans*		<0.001	<0.001	0.365	0.007

$^{a–d}$Means with different superscripts within each column are significantly different (p<0.05)

"–" *H. coagulans* without 200 mg/kg diet; "+" *H. coagulans* with (+) (200 mg/kg diet)

Table 6. Effects of pennyroyal essential oil (PEO) and *Heyndrickxia coagulans* on immune response (log2) of broilers

Items		Primary (28 day)	Secondary (42 day)
PEO (mg/kg)	*H. coagulans*		
0	−	3.667^{c}	5.750^{b}
	+	8.500^{a}	8.500^{a}
100	−	3.750^{c}	4.665^{b}
	+	4.000^{b,c}	5.500^{b}
200	−	3.500^{c}	4.938^{b}
	+	5.500^{b}	7.625^{a}
SEM		0.363	0.588
Main effect			
0		6.083^{a}	7.125^{a}
100		3.875^{b}	5.083^{b}
200		4.500^{b}	6.28^{a,b}
SEM		0.256	0.416
−		3.639^{b}	5.117^{b}
+		6.000^{a}	7.208^{a}
SEM		0.221	0.339
p-value			
PEO		<0.001	0.020
H. coagulans		<0.001	0.001
PEO × *H. coagulans*		<0.001	0.034

$^{a-c}$Means with different superscripts within each column are significantly different (p<0.05)

"–" *H. coagulans* without 200 mg/kg diet; "+" *H. coagulans* with (+) (200 mg/kg diet)

3.4 Immune Response

Table 6 shows the effects of various amounts of PEO and *H. coagulans* on the immune response of broiler chickens. In contrast to SRBC, there was a significant association (p<0.05) between various amounts of PEO and *H. coagulans* on antibody titer at 28 and 42 days. With and without 200 mg/kg of PEO, the highest antibody titer is related to *H. coagulans*. At 28 and 42 days, the antibody titer against SRBC in birds fed *H. coagulans* was higher than in birds fed no *H. coagulans* (p <0.05). PEO feeding has a negative impact on the immune response of the birds at 28 and 42 days (p<0.05). In agreement with the present study, Kabir et al. (31)found that feeding probiotics to broilers improved antibody synthesis in response to SRBC. In a study, pennyroyal powder did not affect antibody titers against SRBC in broilers (24). Furthermore, Nobakht et al. (12) found that different amounts of pennyroyal did not affect broiler Heterophil/Lymphocyte ratio. Unfortunately, no research has been done on the impact of PEO on broiler immune responses. Hossain et al. (18) found that feeding pigs green tea plus probiotics enhanced immunity by the synthesis of IL-6 and TNF-a

4 Conclusions

Individual *H. coagulans* (200 mg/kg) dietary supplementation may enhance broiler growth, immune response, intestinal microbiota, and intestinal morphology; however, the combination of *H. coagulans* with PEO did not vary the measured parameters. Future studies should examine lower inclusion levels of PEO or explore alternative essential oil combinations to optimize efficacy.

Acknowledgements

We thank the Shahid Bahonar University of Kerman for help during the experiment.

Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

EHS: Methodology, Investigation. MA: Conceptualization, Methodology, Formal analysis, Writing – review and editing. MKB: Methodology, Writing-original draft, Formal analysis.

Data Availability Statement

All data analyzed during this study are included in this article. Any other data are available from the corresponding author upon reasonable request.

Ethical Considerations

All animal experiments were performed in accordance with guidelines for the care and use of laboratory animals and were approved by the Veterinary Faculty of Shahid Bahonar University of Kerman (approval number: IR.UK.VETMED.REC 2019-03-05).

Funding

This research did not receive a specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Mafe AN, Iruoghene Edo G, Akpoghelie PO, Gaaz TS, Yousif E, Zainulabdeen K, et al. Probiotics and Food Bioactives: Unraveling Their Impact on Gut Microbiome, Inflammation, and Metabolic Health. Probiotics and Antimicrobial Proteins. 2025;14:1-42. [DOI]
2. Zhou X, Wang Y, Gu Q, Li W. Effect of dietary probiotic, Bacillus coagulans, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. Poultry science. 2010;89(3):588-93. [PMID: 20181878] [DOI]
3. Adami A, Cavazzoni V. Occurrence of selected bacterial groups in the faeces of piglets fed with Bacillus coagulans as probiotic. Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms. 1999;39(1):3-9. [DOI]
4. Nyangale EFS, Cash HA, Keller D, Chernoff D, Gibson GR. Bacillus coagulans GBI-30, 6086 modulates Faecalibacterium prausnitzii in older men and women. The Journal of nutrition. 2015;145(7):1446-52. [PMID: 25948780] [DOI]
5. Khajeh Bami M, Afsharmanesh M, Salarmoini M, Tavakoli H. Effect of zinc oxide nanoparticles and Bacillus coagulans as probiotic on growth, histomorphology of intestine, and immune parameters in broiler chickens. Comparative Clinical Pathology. 2018;27:399-406. [DOI]
6. Brenes A, Roura E. Essential oils in poultry nutrition: Main effects and modes of action. Animal feed science and technology. 2010;158(1-2):1-4. [DOI]
7. Jang IS, Ko YH, Kang SY, Lee CY. Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Animal Feed Science and Technology. 2007;134(3-4):304-15. [DOI]
8. Zeng Z, Zhang S, Wang H, Piao X. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. Journal of animal science and biotechnology. 2015;6(1):1-10. [PMID: 25774291] [PMCID: PMC4359495] [DOI]
9. Chalchat JC, Gorunovic MS, Maksimovic ZA, Petrovic SD. Essential oil of wild growing Mentha pulegium L. from Yugoslavia. Journal of Essential Oil Research. 2000;12(5SP - 598):600. [DOI]
10. Domingues PM, Santos L. Essential oil of pennyroyal (Mentha pulegium): Composition and applications as alternatives to pesticides-New tendencies. Industrial Crops and Products. 2019;139:111534. [DOI]
11. Dehghani N, Afsharmanesh M, Salarmoini M, Ebrahimnejad H. Characterization of pennyroyal (Mentha pulegium) essential oil as an herbal, antibacterial, and antioxidant substance. Comparative Clinical Pathology. 2018;27:1575-81. [DOI]
12. Nobakht A, Norani J, Safamehr A. The effects of different amounts of Mentha pulegium L.(pennyroyal) on performance, carcass traits, hematological and blood biochemical parameters of broilers. Journal of Medicinal Plants Research. 2011;5:3763-8. [DOI]
13. Bölükbaşı Ş, Ürüşan H, Prakasita VC, Asmara W, Widyarini S, Wahyuni AE. Biological activities of pennyroyal (Mentha pulegium L.) in broilers
Combinations of herbs and probiotics as an alternative growth promoter: an in vitro study. Livestock Science. 2012;146(2-3):189-92. [PMID: 31190720] [PMCID: PMC6515817] [DOI]
14. Prakasita VC, Asmara W, Widyarini S, Wahyuni AETH. Combinations of herbs and probiotics as an alternative growth promoter: an in vitro study. Veterinary World. 2019;12(4):614. [PMID: 31190720] [PMCID: PMC6515817] [DOI]
15. Kim DW, Kim SH, Yu DJ, Kang GH, Kim JH, Kang HG, et al. Effects of single or mixed supplements of plant extract, fermented medicinal plants, and Lactobacillus on growth performance in broilers. Korean Journal of Poultry Science. 2007;34(3):187-96. [DOI]
16. Khajeh Bami M, Afsharmanesh M, Ebrahimnejad H. Effect of dietary Bacillus coagulans and different forms of zinc on performance, intestinal microbiota, carcass and meat quality of broiler chickens. Probiotics and Antimicrobial Proteins. 2020;12:461-72. [PMID: 31134523] [DOI]
17. Amiri N, Afsharmanesh M, Salarmoini M, Meimandipour A, Hosseini SA, Ebrahimnejad H. Effects of nanoencapsulated cumin essential oil as an alternative to the antibiotic growth promoter in broiler diets. Journal of Applied Poultry Research. 2020;29(4):875-85. [DOI]
18. Hossain ME, Ko SY, Park KW, Firman JD, Yang CJ. Evaluation of green tea by-product and green tea plus probiotics on the growth performance, meat quality and immunity of growing-finishing pigs. Animal Production Science. 2012;52(9):857-66. [DOI]
19. Zhang ZF, Kim IH. Effects of multi-strain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. Poultry science. 2014;93(2):364-70. [PMID: 24570458] [DOI]
20. Wealleans AL, Sirukhi M, Egorov IA. Performance, gut morphology and microbiology effects of a Bacillus probiotic, avilamycin and their combination in mixed grain broiler diets. British Poultry Science. 2017;58(5):523-9. [PMID: 28682111] [DOI]
21. Hung AT, Lin SY, Yang TY, Chou CK, Liu HC, Lu JJ, et al. Effects of Bacillus coagulans ATCC 7050 on growth performance, intestinal morphology, and microflora composition in broiler chickens. Animal production science. 2012;52IS - 9:874-9. [DOI]
22. Wang C, Wang MQ, Ye SS, Tao WJ, Du YJ. Effects of copper-loaded chitosan nanoparticles on growth and immunity in broilers. Poultry science. 2011;90(10):2223-8. [PMID: 21934004] [DOI]
23. Abdel-Moneim AM, Selim DA, Basuony HA, Sabic EM, Saleh AA, Ebeid TA. Effect of dietary supplementation of Bacillus subtilis spores on growth performance, oxidative status, and digestive enzyme activities in Japanese quail birds. Tropical animal health and production. 2020;52:671-80. [PMID: 31485898] [DOI]
24. Ghalamkari G, Toghyani M, Landy N, Tavalaeian E. Investigation the effects using different levels of Mentha pulegium L.(pennyroyal) in comparison with an antibiotic growth promoter on performance, carcass traits and immune responses in broiler chickens. Asian Pacific Journal of Tropical Biomedicine. 2012;2(3):S1396-S9. [DOI]

25. Hernández-Coronado AC, Silva-Vázquez R, Rangel-Nava ZE, Hernández-Martínez CA, Kawas-Garza JR, Hume ME, et al. Mexican oregano essential oils given in drinking water on performance, carcass traits, and meat quality of broilers. Poultry science. 2019;98(7):3050-8. [PMID: 30839090] [PMCID: PMC6591684] [DOI]
26. Abedini A, Hassanabadi A, Afzali N, Kermanshahi HTI. The effect of different dietary levels of Pennyroyal probiotic antibiotic on performance, carcass characteristics and sellected nutrients digestibility in broiler chickens. Archives of Medical Laboratory Sciences. 2017;3(2):15-22. [DOI]
27. Al-Khalaifa H, Al-Nasser A, Al-Surayee T, Al-Kandari S, Al-Enzi N, Al-Sharrah T, et al. Effect of dietary probiotics and prebiotics on the performance of broiler chickens. Poultry science. 2019;98(10):4465-79. [PMID: 31180128] [DOI]
28. Park JH, Kim IH. Supplemental effect of probioticBacillus subtilis B2A on productivity, organ weight, intestinalSalmonella microflora, and breast meat quality of growing broiler chicks. Poultry Science. 2014;93(8):2054-9. [PMID: 24902699] [DOI]
29. Oumzil H, Ghoulami S, Rhajaoui M, Ilidrissi A, Fkih-Tetouani S, Faid M, et al. Antibacterial and antifungal activity of essential oils of Mentha suaveolens. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2002;16(8):727-31. [PMID: 12458474] [DOI]
30. Beski SS, Al-Sardary SY. Effects of dietary supplementation of probiotic and synbiotic on broiler chickens hematology and intestinal integrity. International Journal of Poultry Science. 2015;14:31-6. [DOI]
31. Kabir SL, Rahman MM, Rahman MB, Rahman MM, Ahmed SU. The dynamics of probiotics on growth performance and immune response in broilers. Int J Poult Sci. 2004;3(5):361-4. [DOI]