

The effect of adding farnesol oil to broiler diet on the production index, some physiological indicators, and intestinal microbial content

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Abstract

This experiment was conducted in the poultry field belonging to the Animal Production Department / College of Agriculture / University of Diyala, for a period of 35 days from 23/10/2025 to 26/11/2025, to study the effect of adding Farnesol Oil to the diet of broiler chickens (Ross 308) on the production index value, some physiological indicators, and the microbial content of the intestine. The experiment used 225 unsexed hybrid broiler chickens (Ross 308) aged one day with an average weight of 38.5 g, randomly distributed into five treatments with three replicates per treatment (15 birds/replicate).

The treatments were as follows: T1 = (control treatment) standard diet without additives, T2 = standard diet with 250 mg/kg of farnesol oil added, T3 = standard diet with 500 mg/kg of farnesol oil added, T4 = standard diet with 750 mg/kg of farnesol oil added, T5 = standard diet with 1 g/kg of farnesol oil added .

The results of the experiment were as follows:

The results of the experiment showed significant differences ($P \leq 0.05$) between the experimental treatments in favor of T5 in terms of the production index value. It should be noted that treatment T5 recorded the highest production index value of 503.27, followed by treatment T2 with 500.33. and the control treatment T1 recorded the lowest value for the production index at 415.92 .

The results of the experiment recorded a highly significant effect ($P \leq 0.01$), with T5 and T4 , followed by T3 , followed by T2 , compared to T1, . As for the number of coliform bacteria, T1 significantly outperformed the others, followed by T2 and T3, , respectively, followed by T4, , and T5,. T5 significantly exceeded ($P \leq 0.01$) the number of lactic acid bacteria, followed by T4,, then T3, then T2, , compared to T1, .

Introduction

Farnesol is a non-cyclic alcohol with a pleasant aroma from important natural and synthetic compounds of the natural sesquiterpene type. It is an organic compound consisting of 15 carbon atoms

with the molecular formula (C₁₅H₂₆O). It is found in the essential oils of ambrette, citronella, sunflower, rose, jasmine, and orange blossom [13].

Bioactive plant materials represent a new generation of natural supplements that include natural plants, spices, plant extracts, and essential oils and are characterized by numerous biological properties [9]. The medicinal and beneficial properties of essential oils in the diet of broiler chickens are reflected in improved production characteristics [5]. In addition to the ability to increase the body's immune response [8].

This has a positive effect on maximizing the genetic potential of chickens and reducing mortality, thereby increasing economic profitability. In addition, bioactive plant compounds and essential oils have been shown to lower blood cholesterol by inhibiting the key enzymes involved in cholesterol and fat synthesis, which significantly reduces cholesterol in the blood and edible tissues and the percentage of abdominal fat in broiler chickens [15].

Some plant species, such as the acacia tree (*Acacia caffeine*), contain large amounts of farnesol, which is a type of essential oil [19].

Farnesol is a non-cyclic alcohol of the sesquiterpene type that is synthesized internally via the ergosterol pathway. This compound is one of the main components of many plant essential oils, such as lily of the valley, citrus, and nutmeg. Farnesol is a key intermediate in the biosynthesis of cholesterol. Farnesol, which is non-toxic to the natural environment, could be a very interesting alternative to antibiotics, which

are often ineffective and lead to increased microbial resistance [14].

The use of antibiotics plays an important role in promoting growth and improving gut health in poultry, despite the ban on their use in some countries due to their residues in poultry products and the significant increase in the emergence of bacteria resistant to these antibiotics. Therefore, it is important to develop safe and highly effective alternatives to antibiotics to maintain overall animal health [11]. Plant essential oils are a class of volatile aromatic compounds that contribute to the regulation of intestinal bacteria by reducing the number of pathogenic bacteria, improving gut health, and enhancing the growth performance of broiler chickens, which is noteworthy for improving the economic benefits of broiler chickens [1].

[21] reported that farnesol acts as a potent antioxidant that contributes to the elimination of free radicals and the accumulation of reactive oxygen species (ROS) within the cell, which contribute to programmed cell death and mitochondrial degradation. Furthermore, farnesol and its derivatives have shown fungicidal properties.

Therefore, this study aimed to improve the productive, immune, and intestinal performance of Ross308 broiler chickens by adding different levels of farnesol oil to their feed.

Materials and Methods

3-1-Date of the experiment

This experiment was conducted at the poultry farm affiliated with the Animal

Production Department, College of Agriculture - University of Diyala, for a

period of 35 days from 23/10/2025 to 26/11/2025, to study the effect of adding farnesol oil to the diet of broiler chickens (Ross 308) on their productive and

physiological performance and some microbial and histological characteristics of the intestine.

3-2- Chick management

The chicks were obtained from Diyala Governorate, from the Jadida al-Shat area, with an average weight of 38.5 gm. They were one day old and not yet fledged. They were raised in a semi-closed hall, and the floor of their cages was covered with sawdust about 5 cm thick and paper towels until the end of the first week. The chicks were placed in floor cages with an area of 1.5 m² per cage, with each cage holding 15 birds. During the first week, plastic dish-shaped feeders and inverted plastic drinkers were used to provide water. The feeders were then replaced with circular hanging feeders with a diameter of 45 cm, and the inverted drinkers were replaced with automatic plastic hanging drinkers until the

birds reached marketing age (five weeks). With regard to the lighting program, lighting was applied for 23 hours with one hour of darkness during the first three days, followed by lighting for 20 hours with four hours of darkness until marketing age (five weeks), based on the [4]. The chicks were received at a temperature of 33-34°C on the first day, and then the temperature was reduced by 0.5°C per day until reaching 21°C at the end of the third week, where it remained until marketing age. At night, incubators with electric heaters were used to provide adequate heat from the start of the rearing period until the end of the experiment.

3-3- Preparation of diets

The birds were fed ready-made diet produced by Fedco Feed Factory in Erbil. The starter diet for the first 1-14 days contained 21% crude protein and 3050 kcal/kg of energy, The grower diet for 15-28 days contained 19% crude protein and 3150 kcal/kg of energy, and the finisher diet for days 35-29 contained 18% protein and 3200 kcal/kg of energy. Farnesol oil was added to

the feed according to the amount added for each treatment by mixing it manually with a small amount of feed, gradually increasing the amount and mixing well until the desired homogeneity was achieved for the weekly amounts allocated for each treatment. After mixing, it was packed in sealed bags and labeled according to each treatment to preserve the effectiveness of the additives.

3-4- Experimental Design

The experiment used 225 one-day-old hybrid broiler chickens (Ross 308) that were not vaccinated. They were randomly distributed into five treatments with three

replicates per treatment (15 birds/replicate), as shown in the experimental design in The treatments were as follows:

1-First treatment T0: (control treatment) Standard diet without additives.

2-Second treatment T1: Standard diet with 250 mg of farnesol oil/kg of diet.

3-Third treatment T2: Standard diet supplemented with 500 mg of farnesol oil/kg of diet.

4-Fourth treatment T3: Standard diet supplemented with 750 mg of farnesol oil/kg of diet.

5-Fifth treatment T4: Standard diet supplemented with 1 g of farnesol oil / kg of diet.

3-5- Characteristics of the studied

3-5-1-Production index

Good breeding and efficient management depend on several factors, including average live weight at marketing, mortality rate, feed conversion efficiency, and rearing period. To achieve optimal performance, birds must have a high market weight, low mortality rate, shorter rearing period, and high feed conversion efficiency to achieve a high production coefficient. To evaluate the productive performance of a broiler flock, a Note that vitality ratio = 100 - mortality ratio

measure that combines all these factors is used, and a low or good rating is given to the flock based on the Production Index (PI) value according to the equation in the [4].

Production Guide(PI)=

$$\frac{\text{Body weight average(kg)} \times \text{Vitality Ratio}}{\text{Education Days Number} \times \text{Nutritional conversion efficiency}} \times 100$$

3-5-2-Physiological characteristics

3-5-2-1- Blood biochemistry characteristics

Blood samples were collected at 35 days of age after cutting the jugular vein during slaughter, with six birds from each treatment randomly selected from both sexes. Blood samples were collected immediately after slaughter from the jugular vein into test tubes containing Gel Clot, and the tubes were placed at an angle. Sample collection took 15 minutes, after which the samples were transported at room temperature to the laboratory for another 30 minutes. The serum was then separated using a centrifuge at 4000 rpm for 10 minutes. The average time between sample collection and separation was 37.5 minutes, with a range of

30 to 45 minutes between the first and last bleeding, according to the method of [10] The biochemical properties of the blood were measured using a German COBAS INTEGRA 400 Plus device, which operates automatically and can process 400 samples per hour with high accuracy. Several ready-made kits from the Spanish company SPINREACT were used.

Cholesterol, Glucose, Uric Acid, Total Protein, ALT , Albumin, Globulin, HDL, LDL,AST, ALP

3-5-3- Microbial characteristics

3-5-3-1-Preparation of culture media

The culture media were obtained from medical supplies and were of Indian origin. 28 and 51.53 g of Nutrient Agar and MacConkey Agar, respectively, were weighed, while M.R.S Agar, of Italian origin, weighed 68.3 g. These quantities were dissolved in 1 litre of distilled water, and the solution was

mixed well until the culture medium was completely dissolved, then left to cool slightly. The medium was then distributed into Petri dishes in quantities ranging from 15-20 ml per dish, then the dishes were left to solidify and stored upside down in the refrigerator.

3-5-3-2- Estimation of Microbial Populations

Tests were conducted to estimate bacterial populations in the Microbiology and Nutrition Laboratories of the Animal Production Department, Faculty of Agriculture, University of Diyala. At the end of the experiment, two birds were slaughtered per replicate and samples were taken from the jejunum of the carcasses, then placed in tightly sealed sterile plastic bottles. One gram of the sample was added to a bottle containing 9 ml of normal saline solution. After mixing thoroughly, decimal dilutions were prepared by transferring 1 ml from the first bottle to another bottle containing 9 ml of saline solution, and the process was repeated until a dilution of 10⁻⁵ was reached.

nutrient media were used to identify each bacterial species: Nutrient Agar for total bacteria, MacConkey Agar for coliform bacteria, and M.R.S Agar for lactic acid bacteria. 0.1 ml of each decimal dilution was transferred using a sterile pipette (Micropipette) to two Petri dishes prepared in advance with the specific culture medium for each, and the samples were spread on the surface of the solid medium using a sterile L-shaped glass rod. The plates for total aerobic bacteria and coliform bacteria were then incubated upside down at 37 °C for 24 hours, while the plates for lactic acid bacteria (M.R.S Agar medium) isolated in an anaerobic jar upside down at the same temperature for 48 hours. The growing colonies in each medium were counted to estimate the number of colony-forming units (cfu). The number was then multiplied by the dilution factor to obtain the final number of bacterial colonies per gram of sample, and the bacterial concentrations were converted to logarithmic numbers to base 10, expressed as log₁₀ cfu/g.

The tests aimed to estimate the numbers of the following types of bacteria:

- 1- Total Bacteria.
- 2- Coliform Bacteria.
- 3- Lactic Acid Bacteria.

The numbers of these bacteria were calculated using the spread plating method according to [17]. Different solidified

3-6-statistical Analysis

Statistical analysis was performed using Complete Randomize Design (CRD), and significant differences between transaction averages were determined using Duncan's multiple range test [6]. at a significance level of 0.05, regardless of the significance of the F test in the analysis of variance table

[2]. The SPSS statistical software [18]. was used to analyses the data according to the mathematical model mentioned below.

The mathematical model of complete random design:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Whereas:

Y_{ij} = The viewing value(j)that took the transaction (i).

μ = Overall average of the experience.

τ_i = Treatment effect(i).

ξ_{ij} = The experimental error of observation, which is normally distributed, random, and independent with a mean of zero and a variance equal to $\epsilon\sigma^2$.

Results and Discussion

4-1-Productivity index

Table 1 shows a significant difference ($P \leq 0.05$) between treatments T2 and T5, which recorded the highest production index values of 500.33 and 503.27, respectively, compared to T1, T3, and T4, which recorded 415.92, 488.95, and 491.54, respectively.

Table 1 of the statistical results shows that there are significant differences ($P \leq 0.05$)

between all experimental treatments T1, T2, T3, T4, and T5 in terms of the production index value. It is noted that treatment T5 recorded the highest production index value of 503.27, followed by treatment T2 with 500.33, while the control treatment T1 recorded the lowest numerical value for the production index at 415.92.

Table 1 Effect of adding farnesol oil to Ross 308 broiler feed on production index (g/bird) (mean \pm standard error).

IP Productivity Index Value	Treatment
415.92 \pm 34.11 c	T1
500.33 \pm 36.70 a	T2
488.95 \pm 43.31 b	T3
491.54 \pm 51.94 b	T4
503.27 \pm 22.63 a	T5
*	Significance level in the analysis of variance table

T1: Control (no addition), T2: addition of 250 mg of farnesol oil/kg feed, T3: addition of 500 mg of farnesol oil/kg feed, T4: addition of 750 mg of farnesol oil/kg feed, T5: addition of 1 g. Farnesol oil/kg feed.

Different letters within the same row indicate significant differences between means at the ($P \leq 0.05$) level according to Duncan's multiple range test.

The results of the productive traits showed that adding different levels of farnesol to the feed of broiler chickens (Ross308) for a period of 35-1 days resulted in significant differences in the production traits of broilers in the third, fourth and fifth weeks of the experiment. The improvement in production traits in terms of weight is attributed to the farnesol content of the active substances present in it, which are divided into two groups: first, hydrocarbon compounds, and second, oxidized compounds. However, the effect of farnesol, a non-cyclic sesquiterpene alcohol found in plant essential oils, on broiler production has not been fully determined. Our results showed that the addition of farnesol to the

4-2-Physiological characteristics

4-2-1-Biochemical blood characteristics

Table 2 shows that there are no significant differences between all T1, T2, T3, T4 and T5 experiment parameters when adding different levels of farnesol oil to broiler feed (Ross 308) in terms of glucose, total protein, cholesterol, uric acid, albumin, globulin, HDL, LDL, AST, ALT and ALP concentrations.

A study conducted by [11]. on the effect of adding farnesol oil (96% purity) on the biochemical characteristics in the blood serum of broiler chickens exposed to lipopolysaccharide (LPS) challenge showed a decrease in TB, TC, and LDH levels and an increase in TG and LDH levels in the serum. (LPS) Lipopolysaccharide challenge.

diet inhibits the decrease in mRNA levels of myosin-2 in the ileum of birds exposed to lipopolysaccharide (LPS),

suggesting that farnesol may enhance intestinal barrier function by regulating the expression of myosin-2. The study showed that the TLR4/NF- κ B signaling pathway was activated by lipopolysaccharide (LPS), and some inflammatory cytokines were overexpressed, leading to exacerbated tissue injury [11].

The results of our study are consistent with those of [3] .on the effect of essential oils added to broiler feed and their impact on performance data, meat quality, and increased carcass yield and tenderness due to their high content of unsaturated fatty acids, and extending shelf life. Studies have found positive effects of these oils on production performance.

A decrease in TB, TC and LDH levels and an increase in TG and LDH levels in serum were observed with . Dietary supplementation with farnesol alleviated the downregulation of HDL in the serum of birds exposed to the challenge and reduced serum LDH levels. Farnesol reduced abdominal fat in birds, which may be related to the regulation of HDL levels by farnesol. The intake of LEO supplements increased the activity of superoxide dismutase in serum and liver and the activity of glutathione peroxidase in serum, but reduced the concentrations of malondialdehyde in serum ($P \leq 0.05$). We conclude that adding 600 mg/kg of LEO to broiler feed has the

potential to improve antioxidant activity [22].

The results of our study are consistent with those of [12] who studied the feeding of birds on feed supplemented with 200 g/kg of fennel essential oil. The dietary treatments had no effect on glucose, triglyceride, cholesterol, low-density lipoprotein cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) concentrations. triglycerides, cholesterol,

low-density lipoprotein cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and malondialdehyde in the blood ($P \leq 0.05$).

Different letters within the same row indicate significant differences between means at the $P \leq 0.05$ level according to Duncan's multiple range test. N.S. means no significant differences between means.

Table 2 Effect of adding farnesol oil to Ross 308 broiler feed on the biochemical blood characteristics of broilers at 35 days of age (mean \pm standard error)

Morale level in the analysis table	Treatment					Qualities
	T5	T4	T3	T2	T1	
N.S	266.00 \pm 8.14	261.75 \pm 9.64	270.00 \pm 9.60	260.75 \pm 8.72	265.00 \pm 7.46	Glucose (mg/100ml)
N.S	4.23 \pm 0.12	4.17 \pm 0.12	4.22 \pm 0.13	4.22 \pm 0.11	4.20 \pm 0.07	Total protein)mg/100ml(
N.S	130.66 \pm 2.40	129.25 \pm 2.78	130.50 \pm 3.01	130.50 \pm 3.01	131.00 \pm 1.41	Cholesterol)mg/100ml(
N.S	5.60 \pm 0.17	5.52 \pm 0.21	5.57 \pm 0.22	5.57 \pm 0.21	5.56 \pm 0.15	Uric Acid)mg/100ml(
N.S	1.58 \pm 0.03	1.57 \pm 0.04	1.58 \pm 0.04	1.56 \pm 0.04	1.60 \pm 0.01	Albumin)100ml/mg(
N.S	2.65 \pm 0.08	2.60 \pm 0.08	2.64 \pm 0.09	2.65 \pm 0.07	2.60 \pm 0.05	Globulin)mg/100ml(

N.S	62.33±1.20	61.75±1.79	62.00±1.47	61.75±1.37	62.00±0.70	HDL
N.S	45.66±1.45	44.25±1.88	46.00±2.16	44.25±2.01	45.20±1.28	LDL
N.S	188.00±3.46	187.25±3.96	189.00±4.22	187.00±3.93	190.00±1.70	AST
N.S	7.76±0.26	7.70±0.27	7.72±0.31	7.77±0.25	7.94±0.13	ALT
N.S	155.00±3.78	155.25±4.58	157.50±4.94	154.25±4.66	158.00±2.62	ALP

T1: Control (no addition), T2: addition of 250 mg farnesol oil/kg feed, T3: addition of 500 mg farnesol oil/kg feed, T4: addition of 750 mg farnesol oil/kg feed, T5: addition of 1 g farnesol oil/kg feed.

4-3-Microbial characteristics

Table 3 indicates a highly significant effect ($P \leq 0.01$) on the total bacterial count in treatments T4 and T5, where bacterial counts of 1292.50 and 1321.66 cfu/gm, respectively, compared to T2 and T3, where bacterial counts of 1018.00, 1201.25, and 1240.00 cfu/gm were recorded, respectively, surpassing T1, which recorded bacterial counts of 1018.00 cfu/gm. As for the number of colon bacteria, there was a highly significant effect ($P \leq 0.01$) that improved significantly, as treatment T5 recorded the lowest number of E. coli, significantly exceeding ($P \leq 0.01$) T5 over all addition and control treatments. As we can see from the table, all addition treatments had lower E. coli counts than the control treatment, with T2, T3, T4, and T5 recording 423.75, 386.25, 368.75, and 350.00 cfu/gm, respectively, compared to T1, which recorded 555.00 cfu/gm. In the number of lactic acid bacteria, we observe a highly significant effect ($P \leq 0.01$) for T4 and T5, which recorded counts of 923.75 971.66

cfu/gm compared to T1, T2, and T3, which recorded 463.00, 777.50, and 878.75 cfu/gm, respectively.

Different letters within the same row indicate significant differences between means at the ($P \leq 0.01$) level according to Duncan's multiple range test.

[11] suggested that farnesol may contribute to promoting gut health in broiler chickens, helping birds to improve growth performance. To explore this concept further, we investigated the gut physiology of birds challenged with (LPS), which are components of the cell wall of pathogenic bacteria, injected intraperitoneally into experimental birds. The study demonstrated that LPS challenge caused immune imbalance and physiological dysfunction, and that farnesol helped maintain physiological balance. Under pressure, it helped repair intestinal damage and improve

oxidative stress and antioxidant imbalance caused by lipopolysaccharide (LPS) in birds. Dietary supplementation with farnesol increased the surface area of villi, and the use of farnesol as a dietary supplement had a protective effect on the intestines of broiler chickens by reshaping the intestinal bacteria, especially *Candidatus Arthromitus*.

Many scientists have reported an increase in villi height due to essential oil supplements. The increase in villi height resulting from essential oils is associated with their antioxidant properties, but in cinnamon essential oils, the phenolic group acts to increase hydrogen [7]. The results of our study are consistent with those of [20] in evaluating the effect of basil, thyme and sage essential oils in the diets of Cobb broiler chickens.

Table 3 Effect of adding farnesol oil to Ross 308 broiler feed on total bacteria, coliform bacteria, and lactic acid bacteria counts in the jejunum (mean \pm standard error).

Significance level in the analysis of variance table	Treatment					Qualities
	T5	T4	T3	T2	T1	
**	1321.66 \pm 17.40 a	1292.50 \pm 7.77 a	1240.00 \pm 17.91 Ab	1201.25 \pm 23.75 b	1018.00 \pm 46.22 c	Total bacteria)cfu/gm(
**	350.00 \pm 14.43 B	368.75 \pm 11.96 B	386.25 \pm 24.78 B	423.75 \pm 31.31 b	555.00 \pm 39.84 a	Coliform bacteria cfu/gm)(
**	971.66 \pm 14.81 a	923.75 \pm 18.41 a	878.75 \pm 30.16 Ab	777.50 \pm 22.03 b	463.00 \pm 84.96 c	Lactic acid bacteria)cfu/gm(

T1: Control (no addition), T2: addition of 250 mg farnesol oil/kg feed, T3: addition of 500 mg farnesol oil / kg feed, T4: Add 750 mg of farnesol oil / kg feed, T5: Add 1 g of farnesol oil / kg feed.

The diets were prepared as follows: a diet containing 0.05% basil essential oil (EOB), a diet containing 0.05% thyme essential oil (EOT), and a diet containing 0.05% sage essential oil (EOS). The results showed a significant change in the intestinal microbiota, with a decrease in the numbers of *Escherichia coli*, coliforms, and staphylococci in the small intestine and caecum, while the number of Lactobacilli bacteria increased in the experimental groups compared to the control group. However, oregano oil was more effective in

Conclusions

The addition of farnesol oil at a concentration of 75 mg/kg and 1 g/kg feed improved production performance and improved the microbial characteristics of broiler intestines by increasing the number of lactic acid bacteria and reducing the number of coliform bacteria for all addition treatments compared to the control treatment.

changing the intestinal microbiota of chickens in the small intestine and caecum.

The results of our study are consistent with those of [22]. Feeding chickens LEO supplements Feeding chickens with LEO600 feed reduced the number of *Escherichia coli* bacteria in the contents of the caecum and cecum and increased the length of the villi and the ratio of villi height to crypt depth in the jejunum compared to standard feed ($P \leq 0.05$).

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