

Impact of Varying Dosages of Powdered *Melissa Officinalis* Leaf on Certain Blood and Microbiological Traits in Broiler Intestines

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ABSTRACT: The study used 180 unsexed one-day-old Ross-308 broiler chicks. After that, the chicks were divided into four treatments at random, with three replicates of each treatment (15 chicks per replicate). The initial treatment, T1 (comparison), was an additive-free control diet. For one to four days, the powdered *Melissa* leaves were added to the second treatment (T2), third treatment (T3), and fourth treatment (T4) at the following concentrations: 0, 4, 6, and 8 g/kg feed, respectively. The findings showed that, as compared to the control treatment, adding *Melissa* leaf powder significantly ($P < 0.05$) reduced the levels of low-density lipoproteins, triglycerides, cholesterol, and glucose. Additionally, the results showed that the addition treatments significantly increased the number of red blood cells, packed blood cell volume, and hemoglobin in the blood of the birds. It was also observed that the addition of *Melissa* leaf powder had a positive effect on intestinal bacteria, reducing the number of colon and total bacteria and significantly improving the number of lactobacilli. Based on this, we conclude that broiler chickens' blood and microbial characteristics significantly improved when they were fed crushed *Melissa* leaf powder. The concentration of 8 g/kg feed produced the best results when compared to the other concentrations and for all aspects of the study.

Published Online:
November 27, 2024

KEYWORDS: *Melissa officinalis*, leaf powder, blood and microbial traits, intestines, broiler.

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INTRODUCTION

In terms of producing meat and eggs, the global poultry business has grown and expanded significantly in recent years. The reason for this is that production has increased in comparison to other animal products, and meat output has expanded at a faster rate than eggs (Windhorst, 2006; Al-Jebory et al., 2023 a). In addition to this expansion and advancement, the chicken business witnessed a tendency toward the employment of a number of tools to increase productivity, such as organic acids, enzymes, antibiotics as growth stimulants, and medicinal herbs as feed additives, and to treat and protect poultry from pathogenic microorganisms that have become more prevalent as intensive breeding has increased (Swiatkiewicz et al. 2015; Al-Jebory et al., 2023 c). Antibiotics had adverse health impacts despite their beneficial contribution to the growth of the chicken business. As a result of modern breeds' rapid expansion and broiler breeds' genetic advancements to produce fast-growing flocks with high feed conversion efficiency, these birds' immunity has also declined, leaving them more vulnerable to disease and raising their mortality rates due to the consumption of their products by humans and other animals (Dibner and Richards, 2005). This is due to the observation that there is a negative genetic correlation between immunity and rapid growth (Eid et al. 2004; Al-Jebory et al., 2023 d). Using medicinal plants rather than chemically produced medications has been more popular among scientists and researchers in recent years (2015et al. Dhama). Medicinal herbal plants are one of the sources used as feed additives in poultry feeding due to their importance in improving many characteristics, including productivity, physiology and immunity, whether used directly or after extracting the active compounds they contain using one of the different extraction methods (Akyildiz and Denli, 2016 2011et al. Rahimi). In both cases, the reason for this effect and improvement in these characteristics is due to the presence of active compounds within the chemical content found in all parts of the plant, which are characterized by their effective pharmacological and stimulating activity (2015et al. Dhama). The *Melissa* plant is one of these herbal medicinal plants because its leaves contain volatile oils like citronellal and citrals, which are known for their antioxidant qualities. It has been discovered that these oils help people with Alzheimer's disease and improve memory because of their antioxidant action. This plant is known for having a high percentage of anti-parasitic, anti-bacterial, anti-

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fungal, and anti-tumor compounds (2004 et al. Allahverdev), and it also protects the body's cells from oxidative damage (2017 et al. Miraj). According to studies, its ferulic and rosmarinic acid content, along with its tannin and phenol content, is what gives it its antiviral properties (Boyadzhiev and Dimitrova, 2006 1993 et al. Dimitrova). Based on the aforementioned, the purpose of this study was to determine the impact of adding crushed Melissa leaves as a nutritional supplement to broiler feed, as well as the optimal ratios to use and the degree to which they affected certain microbiological blood parameters of broiler chickens.

METHODS

180 unsexed, one-day-old Ross-308 broiler chicks were used in this investigation. The chicks were divided into four treatments at random, with three replicates for each treatment. There were fifteen chicks in each replication. The purpose of this study was to ascertain how adding varying amounts of crushed Melissa leaves to the feed affected the microbiological and physiological traits of broiler chicks. From day one to day twenty-one, the chicks were fed a beginning ration, and from day twenty-two to day forty-two, they were provided a final ration. The chicks were fed crushed Melissa leaves from the time they were one day old until they were forty-two days old. The treatments were given out as follows: Melissa leaves powder was added to the first treatment (T1) (control), second treatment (T2), third treatment (T3), and fourth treatment (T4) at the following concentrations: 0, 4, 6, and 8 g/kg feed, respectively. The features of cholesterol, triglycerides, glucose, hemoglobin, total aerobic bacteria, colon bacteria, and lactic acid bacteria were all studied in this experiment. Six birds from each treatment were killed at 42 days of age, their blood was drawn, and the glucose concentration was determined using the Coles method using a measuring kit (Kit) from the German company Roche. (1986), and the level of triglycerides in the blood serum was measured using the ready-made analysis kit (Kit), and the concentration of cholesterol and low-density lipoproteins (LDL) was estimated using a measuring kit (Kit) from the German company Roche according to the method of Franey and Elias (1969). The samples were then read at a wavelength of (546) nanometers using a spectrophotometer in accordance with the method of (Trinder, 1969; Prencipe, 1982, and Fossati). Regarding blood measurements, the number of red blood cells was estimated using the method suggested by Natt and Herrick (1952), the concentration of blood hemoglobin was computed using the Archer (1965) method, and the hemoglobin concentration was estimated using the method suggested by Varley et al. (1980). In order to make the initial dilution 10⁻¹, 1 g of each bird's small intestine contents (duodenum, ileum, and jejunum) were added to 9 ml of the previously made peptone solution. The solution was then stored in the refrigerator at 4 °C until the microbial test was conducted. Using a sterile pipette to transfer 1 ml of each decimal dilution to empty sterile Petri dishes (Duplicate), the Pour plate count method described in APHA (1978) was utilized to estimate the total number of aerobic, coliform, and lactobacilli bacteria. 15 milliliters of the premade, sterile nutrient culture medium were stored at 4 °C in a water bath. After that, the plate was gently moved in all directions to thoroughly mix the bacterial suspension with the culture media. Following the solidification of the culture media, the dishes were inverted and maintained at 37 °C for 48 hours. Colony growth was examined, and the culture plate with the best decimal dilution in terms of colonies was chosen. The number of bacterial colonies/g of the intestinal sample (colonies/g) was then calculated by multiplying the number of colonies by the reciprocal of the dilution. The effect of several treatments on the attributes under investigation was examined using a completely randomized design. Duncan's multiple range test (1955) was employed to compare the significant differences between the averages, and the pre-made statistical software SAS (2012).

RESULTS AND DISCUSSION

Table 1's statistical analysis revealed the impact of adding powdered lemon balm leaf on the quantity of RBC, the volume of compressed blood cells, and the level of HB. The fourth group increase on the other experimental treatments in terms of the quantity of red blood cells, the volume of compressed blood cells, and the concentration of hemoglobin in the birds' blood ($P < 0.05$).

In comparison to the first treatment (control), which did not include the addition, the blood serum concentrations of glucose, cholesterol, triglycerides, and low-density lipoproteins were significantly lower ($P < 0.05$) in the birds in the second, third, and fourth treatments that added powdered lemon balm leaf to the broiler feed. There was no significant difference in the blood serum glucose concentration between the third and fourth treatments, and there was no significant difference in the blood serum cholesterol concentration between any of the addition treatments. Compared to the other treatments, the fourth therapy had the lowest concentration of low-density lipoproteins.

When lemon balm leaf powder is added, Tables 2 and 3 demonstrate an improvement in blood characteristics. The increased concentration of hemoglobin and compressed blood cell volume may be the result of more red blood cells, which may have been caused by the unique effect of lemon balm leaves, which are antioxidants that shield cells from oxidative stress and work to shield RBC membranes from the effects of free radicals. Furthermore, lemon balm leaves have been shown to be an efficient antidiabetic and hypoglycemic factor. This may be because they boost the liver's absorption and metabolism of glucose, as well as adipose tissue and prevent the liver's production of sugar (2017 et al. Hashemnia). Terpenes are one of the primary substances found in lemon balm leaves that promote the effects of hypolipidemia by preventing the production of cholesterol nuclei in bile and liver biosynthesis (2010 et al. Chung). Changizi-Ashtiyani (2013) reported that lemon balm has demonstrated the capacity to lower fats, cholesterol,

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and low-density lipoprotein (LDL). The three main characteristics of the low-fat Melissa plant are its antioxidant qualities, its ability to raise thyroid hormone, or possibly its ability to speed up fat metabolism, which prevents it from raising blood plasma levels and showing up as a drop in triglycerides and cholesterol.

Table 4 displays the effects of using Melissa leaf powder on the logarithmic numbers of total aerobic bacteria, colon bacteria, and lactic acid bacteria of the duodenum, ileum, and jejunum contents in the broiler intestines. It also demonstrates that the logarithmic numbers of total aerobic bacteria and colon bacteria of the duodenum, ileum, and jejunum contents in the broiler intestines significantly decreased, while there were no significant differences between the addition treatments. In addition, the quantity of lactobacilli bacteria in the duodenum, ileum, and jejunum region decreased significantly in favor of the third and fourth treatments, whereas there were no discernible variations between the first and second treatments. The significant decrease in the logarithmic numbers of harmful bacteria with the increase in beneficial bacteria in the treatments of lemon balm leaf powder may be due to the fact that these leaves contain active compounds with antibacterial properties such as euquinol by increasing the permeability of the plasma membrane of these bacteria, which causes the plasma membrane to lose its ability to protect the cell cytoplasm, which causes the cell organelles and part of the cytoplasm to leak from the bacterial cell, thus causing the death of the bacterial cell (2014et al. Abdellatif). Flavonoid compounds work to reduce harmful bacteria at the expense of beneficial ones by supporting the microbial balance within the digestive tract, as they stimulate beneficial bacteria to secrete lactic acid, which reduces the pH, which does not allow harmful bacteria to live and reproduce, which improves general health (2017et al. Klūga). The fourth treatment (8 g/kg feed) achieved the best results in terms of the productive characteristics included in the current study, so we recommend using the concentration (8 g/kg feed) in broiler rations.

Table 1. Impact of adding lemon balm leaf powder to the feed in some blood traits

Treatments	RBC (10 ⁶ /ml ³)	p.c.v.%	Hb (g/100ml)
T1	2.14± 0.12 b	27.25± 0.56 b	9.08± 0.19b
T2	2.27 ± 0.05 b	29.25± 1. 19ab	9.75± 0.40 ab
T3	2.90 ±0.06 a	30.03 ± 0.89 ab	10.01 ± 0.30 ab
T4	2.93 ± 0.06a	32.25 ± 0.88a	10.75 ± 0.29 a
Significance	*	*	*

a,b mean*(P<0.05).

Table 2. Effect of adding lemon balm leaf powder to the diet some biochemical traits

Treatments	Glucose (mg/100ml)	Triglycerides (mg/100ml)	Cholesterol (mg/100ml)	LDL (mg/100ml)
T1	184.01 ± 2.09 a	144.53 ± 3.15a	138.96 ± 1.50 a	68.04± 4.62a
T2	172.07 ±1.16b	138.67 ± 7.22ab	128.50 ± 1.58b	58.24± 3.65 ab
T3	151.10 ± 2.02c	135.50 ± 8.19b	123.42±1.78 b	46.73 ± 2.47 bc
T4	152.00 ± 1.73c	128.66 ± 3.84c	123.83 ± 1.95b	39.63 ± 4.92 c
Significance	*	*	*	*

a,b mean*(P<0.05).

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Table 3. Effect of adding lemon balm leaf powder to the diet in intestinal bacteria

Treatments	Duodenum			Jejunum			Ileum		
	Aerobic bacteria	Colon bacteria	<i>Lactobacilli</i> bacteria	Aerobic bacteria	Colon bacteria	<i>Lactobacilli</i> bacteria	Aerobic bacteria	Colon bacteria	<i>Lactobacilli</i> bacteria
T1	11.31±0.05 a	6.12 ±0.06a	4.16±0.04 c	11.21±0.02a	5.88 ±0.04a	3.96 ±0.04b	10.78 ±0.06a	5.65 ±0.06a	3.92 ±0.07 c
T2	11.13±0.06 b	5.68 ±0.04b	4.41 ±0.02b	10.78±0.04 b	5.73 ±0.03b	4.01 ±0.05b	10.65±0.04b	5.46±0.07b	3.98 ±0.04 c
T3	11.08±0.04 b	5.52±0.05c	4.50 ±0.05 b	10.57 ±0.05 c	5.57 ±0.05 c	4.35 ±0.02a	10.44±0.05 c	5.34 ±0.08c	4.46±0.06 a b
T4	10.55±0.04 b	5.27 ±0.03d	4.85 ±0.08a	10.48 ±0.04 c	5.45 ±0.02 c	4.40 ±0.02a	10.35±0.03 c	5.16 ±0.10d	4.78 ±0.09a
significance	*	*	*	*	*	*	*	*	*

CONCLUSION

It is concluded from the current study that *Melissa officinalis* leaf powder improved the blood and microbial characteristics of the intestines in broilers, and the concentration of 8 g/kg feed had the best effect.

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