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Effect of *in-ovo* injection of lavender oil and dietary supplementation of lavender powder on growth, intestine histomorphology, and serum parameters in broilers

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ABSTRACT

The research was conducted at Animal Production Department, College of Agricultural Engineering Sciences, University of Duhok, Iraq. A total of 400 Ross 308 eggs were incubated, and 348 chicks were hatched. From the hatched chicks, 240 were randomly divided into 5 treatment groups, each with 4 replicates and 12 birds per pen. The treatments included a control group (no additive or injection), eggs injected with 0.05 mL of Lavender essential oil (T_1), eggs injected with 0.1 mL of lavender essential oil (T_2), birds fed with 0.5% lavender powder (T_3), and birds fed with 1% lavender powder (T_4). Results indicated that during days 0-10, birds injected with lavender essential oil (T_1) exhibited significantly higher body weight gain as compared to T_3 and T_4. By day 24, the body weight of birds in T_2 was significantly increased compared to control and T_4. Treatments T_1, and T_3 showed significantly higher body weight gain compared to T_4. Additionally, T_1 and T_2 reported significantly higher body weight and increased feed intake with no significant differences in feed conversion ratio among treatments by day 35. Villi height, crypt depth, tip, basal, and villi/crypt ratio was increased significantly in birds of T_1 group compared to those fed a control diet. All treatments showed a higher villi height/crypt depth ratio over the control. The concentration of AST serum in control group was higher than overall treatments. It was concluded that the *in-ovo* injection and dietary supplementation of lavender essential oil or powder had a positive effect on broiler performance and gut health.

Keywords: Blood biochemistry, Histomorphology, Lavender, Meat chicken

The poultry industry stands out as one of the fastest-growing sectors globally, experiencing widespread expansion across countries of varying economic status due to a robust demand. The efficiency of feed conversion and lower production costs associated with intensive poultry production have led to the raising of large quantities of poultry, particularly chickens (Hasan and M'Sadeq 2020). Pathogens, such as Salmonella and Colibacillosis, pose significant threats, and antibiotic use is common to control infectious diseases (Sarker *et al.* 2019, M'Sadeq 2023). Unfortunately, regular antibiotic use raises concerns about antibiotic resistance, resulting in losses, therapeutic failure, and potential human health risks associated with antibiotic residues in meat (M'sadeq 2019). Therefore, finding viable alternatives to antibiotics is crucial for disease control and reducing antibiotic resistance (Khishtan *et al.* 2024). In the quest for alternatives, medicinal plants have emerged as substitutes for antibiotics in poultry, with previous studies evaluating plant extracts and essential oils for their positive impact on broiler health (Hussein *et al.* 2021). These plant-based alternatives, including phytogenic and herbal products, are favoured for their perceived safety and natural characteristics (Hasan and M'Sadeq 2020, Hossain *et al.* 2023).

Lavender (*Lavandula angustifolia*), a potent aromatic and therapeutic herb, has gained attention as a potential feed additive in the poultry industry. Belonging to the Lamiaceae family, lavender essential oil (LEO) has been extensively used, revealing antiviral, antibacterial, antifungal, and antioxidative properties (Hammer *et al.* 1999, Guidi and Landi 2014, Barbarestani *et al.* 2020). Studies suggest that lavender possesses immune-stimulatory, anxiolytic, sedative, hypnotic, analgesic, and anticonvulsant effects (Adaszyńska-Skwirzyńska and Szczerbińska 2018, Salarmoini *et al.* 2019). Hamid (2023) observed that adding LEO to broiler diets increased body weight gain (BWG) and reduced feed conversion ratio (FCR). Therefore, this study aimed to assess the impact of *in-ovo* injection of lavender essential oil or dietary supplementation as a powder on growth performance at 10, 24, and 35 days, histological changes in the jejunum (including villus height, crypt depth, villi/crypt ratio, and muscle thickness), and serum biochemical parameters.

MATERIALS AND METHODS

The experiment was approved by the Animal Ethics Committee of the College of Agricultural Engineering

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Sciences, animal production department (Approval No: AEC01022022).

This study was conducted at Animal Production Department, College of Agricultural Engineering Sciences, University of Duhok, Iraq. A total of 400 eggs of the Ross 308 breed were acquired from Qandil Hatching Egg Company in Erbil city. Upon arrival, the eggs underwent candling on the 18th day of incubation. For the *in-ovo* injection treatments (0.05 mL and 0.1 mL), 140 eggs were injected with lavender oil (70 eggs received 0.05 mL and remaining 70 eggs received 0.1 mL) into the amnion fluid using a 25 mm long needle. The injection process occurred within 30 min after removing the eggs from the incubator, maintaining a temperature of approximately 35°C during injection, in accordance with the protocol outlined by Barry *et al.* (2019). Throughout the incubation period, the temperature was maintained at approximately 37.7°C, with humidity set at 57% during setting and 87% at hatching, and turning was conducted four times a day at 6 h interval.

Out of the 400 eggs, a total of 348 chicks successfully hatched. From the hatched chicks, 240 chicks were randomly assigned to five treatment, with each treatment comprising four replicates and 12 birds per pen. Temperature and light programs followed the guidelines provided for Ross 308 broilers (Aviagen 2012). Feed and water were provided *ad lib*. and the feeding program included three periods: starter from 0 to 10 days, grower from 10 to 24 days, and finisher from 24 to 35 days. Key performance indicators, such as body weight gain, feed intake (FI), and feed conversion ratio (FCR), were measured on days 10, 24, and 35.

Dietary treatments: The components and proportions of the fundamental diet are explicitly outlined in Table 1. The formulation of these diets employed the Concept 5 feed formulation software developed by Creative Formulation Concepts, LLC, headquartered in Annapolis, MD, USA. The dietary interventions were structured as: control group wherein eggs were not subjected to injection, and birds did not receive supplementation with lavender powder; eggs were subjected to injection with 0.05 mL of lavender essential oil on the 18th day of incubation (T_1); eggs underwent injection with 0.1 mL of lavender essential oil on the 18th day of incubation (T_2); the control diet was enriched with 0.5% lavender powder (T_3); and the control diet was enriched with 1% lavender powder (T_4).

Sample collection: On the 24th day of the study, a random selection of two birds from each pen was made, and they were subsequently weighed and humanely euthanized through cervical dislocation. A volume of 5 mL of blood was then drawn from the jugular vein of each bird, and the serum was prepared by subjecting 5 mL of clotted blood to centrifugation at 3000 rpm for 5 min. The resulting serum was carefully stored at -20°C for subsequent measurements of serum biochemicals. Simultaneously, for morphometric analysis, intestinal tissue, specifically from the jejunum, was meticulously collected. Approximately 1 cm of the jejunum was procured from each sample, ensuring precision and consistency in the collection process.

Table 1. Composition and ingredients of the basal starter, grower and finisher diets

Ingredient	Starter diet	Grower diet	Finisher diet
Corn	55.165	57.147	61.713
Soybean meal	38.012	34.935	29.872
Sunflower oil	1	2.25	3.063
Limestone	1.34	1.158	1.074
Monocalcium phosphate	0.73	0.851	0.68
Phytase	0.02	0.02	0.02
Salt	0.121	0.361	0.365
Na-bicarbonate	0.2	-	-
Vitamins and menials’ premix	2.5	2.5	2.5
Choline Cl 70%	0.102	0.094	0.093
L-lysine HCl 78.4	0.325	0.245	0.23
DL-methionine	0.341	0.316	0.287
L-threonine	0.144	0.122	0.102
Calculated composition			
AME Kcal	3000	3100	3200
ME poultry MJ/kg	12.552	12.971	13.389
Crude protein %	23	21.500	19.536
Crude fat %	2.965	4.198	5.018
Crude fiber %	2.126	2.056	1.961
Lysine %	1.37	1.230	1.100
methionine%	0.641	0.605	0.553
Calcium %	0.96	0.870	0.800
Phosphorous avail. %	0.480	0.440	0.400
Sodium %	0.16	0.16	0.16

Measurements and analyses: The histomorphology of the jejunum was assessed through a series of standardized procedures. The fixed tissue samples underwent dehydration, clearing, and embedding in paraffin wax to facilitate subsequent histological analysis. Longitudinal sections, each measuring 7 µm, were precisely cut using a microtome (Thermo Scientific, Rockville, MD, USA). These sections were individually placed on Superfrost® slides and subjected to staining with Haematoxylin and Eosin. Visual examination of the stained sections was conducted using light microscopy, specifically an Olympus CX41 microscope equipped with a 10× objective. Colour images were captured utilizing a colour video camera (DinoCapture 2.0, ANMO Electronics Corporation, Taiwan). Quantitative analysis of various morphometric parameters was performed on 10 villi per chick. The parameters included villous height (VH), crypt depth (CD), villous height to crypt depth ratio (VH:CD), villous apical, villus basal, villus surface area, as well as the area, length, and width of bursal follicles.

Serum biochemistry: On the 24th day of the study, blood specimens were obtained from the jugular vein, and subsequent serum isolation was carried out for the quantification of various biochemical parameters, including Total Protein (TP), albumin, cholesterol, glucose, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride, and globulin. The assessments were performed using an automatic COBAS INTEGRA 400 plus analyzer (Cedex Bio HT Analyzer).

Statistical analysis: The statistical analysis was conducted using the SAS statistical package, specifically employing Proc GLM (SAS 2013). In instances where interactions between challenges and feed additives were observed, the separation of means was achieved through Duncan's multiple range test ($P<0.05$). Statistical significance was determined at $P<0.05$, while highly significant differences were denoted at $P<0.01$ or $P<0.001$.

RESULTS AND DISCUSSION

Broiler performance: The performance outcomes on days 10, 24, and 35 are detailed in Table 2. The findings of this investigation reveal a substantial impact of lavender essential oil and lavender powder on the performance of broiler chickens. On day 10, birds subjected to lavender essential oil injections in treatments T_1 and T_2 exhibited higher body weight gain ($P<0.05$) compared to those in treatments T_3 and T_4. No significant differences were observed among the control, T_3 and T_4 treatments. Additionally, no notable differences in feed conversion ratio and feed intake were observed between the treatments during the initial 10 days.

The influence of lavender essential oil and lavender powder became evident in the performance outcomes from day 0 to 24. Birds in treatments T_1, T_2, and T_3 demonstrated higher body weight gain ($P<0.05$) than those in treatment T_4. In comparison to the control group, birds in group T_2 exhibited a significant increase in body weight gain. Nevertheless, no significant differences between treatments were noted for feed intake and feed conversion ratio during this period.

From day 0 to 35, T_1 and T_2 exhibited significantly higher body weight gain compared to the control and T_4 treatments ($P < 0.05$). No significant differences were found between the control, T_3 and T_4 groups. Birds in T_1 and T_2 treatments also displayed higher feed intake compared to T_4 and control treatments ($P < 0.05$).

The findings align with those of Nasiri-Moghaddam *et al.* (2012), who reported that supplementing the broiler diet with 350 ppm lavender essential oil led to increased body weight gain (BWG) and decreased feed conversion ratio (FCR) from 22 to 42 days of age. The improvement in FCR associated with the inclusion of plant-derived compounds in diets has been noted in previous studies (Brenes and Roura 2010). The active constituents present in herbal plant oils are thought to function as solubility enhancers, modulating the intestinal microbiota ecosystem and enhancing the release of intracellular enzymes, thereby contributing to enhanced poultry growth performance (Alagawany *et al.* 2021, Beski *et al.* 2021). Medicinal plants, such as lavender, have been demonstrated to possess appetizing attributes and stimulate gut bacterial processes in birds (Salajegheh *et al.* 2018). In this study, increased FI with lavender diets was accompanied by a rise in BWG but not by an improvement in FCR, Amad *et al.* (2013) noted that adding essential oils derived from natural plants in the Labiate family led to progress in broiler birds. Additionally, the quantity of lavender oil (LO) dispersed (1 g/kg feed) could impact growth performance. Barbarestani *et al.* (2020) reported that Lavender had a significant positive impact on growth parameters in broilers compared to control birds. Present experimental results demonstrated an increase in BWG and FI with no significant impact on FCR over the experiment period. These outcomes are in line with those of Kiyma *et al.* (2017), who observed a significant increase in final body weight by adding 24 or 48 mg/kg lavender to broiler chicken diets at 39 days old. The enhancement in the performance of the birds observed with lavender supplementation may be attributed to improved gastrointestinal health, as evident in current study.

Table 2. Lavender essential oil and lavender powder have an impact on broiler performance at day 10, 24 and 35

Days	Control	T_1	T_2	T_3	T_4	P-value	SEM
Body weight gain (g)							
0-10	210.75^{bc}	242.75^{ab}	257.75^{a}	192.25^{c}	190^{c}	0.002	7.6
0-24	936.25^{cb}	1022.00^{ab}	1067^{a}	1021.75^{ab}	891.75^{c}	0.03	20.99
0-35	1904.75^{b}	2123.25^{a}	2122.25^{a}	2016^{ab}	1937.75^{b}	0.01	27.82
Feed intake (g)							
0-10	233.50	255.75	272	211.75	200.50	0.06	9.26
0-24	1143	1172.25	1264.75	1157.25	1045.25	0.14	27.14
0-35	2792^{b}	3064^{a}	3067.25^{a}	2932.75^{ab}	2815.50^{b}	0.02	37.97
Feed Conversion Ratio (kg feed/ kg weight gain)							
0-10	1.099	1.056	1.058	1.102	1.051	0.90	0.02
0-24	1.218	1.146	1.093	1.131	1.174	0.16	0.016
0-35	1.466	1.443	1.445	1.454	1.453	0.16	0.003

$^{a-c}$, Means the different column was superscript are significantly different ($P>0.05$). T_1, Eggs injected with 0.05 mL of lavender essential oil; T_2, Eggs injected with 0.1 mL of lavender essential oil; T_3, Birds fed with 0.5% lavender powder; T_4, Birds will be fed with 1% lavender powder.

Jejunum morphology: Table 3 presents the results of jejunum histomorphology. In comparison to birds fed the control diet, all treatments demonstrated a significant increase in villous height. Particularly, birds in treatments T_1 and T_4 exhibited significantly higher villous height than those in treatments T_3 and T_2. The crypt depth of birds in the control group was significantly lower than that observed across all treatments. Furthermore, the jejunum crypt depth of birds in T_2, T_3, and T_4 significantly decreased compared to birds in T_1. In terms of villous width, both tip and basal measurements for birds in T_1 were significantly higher than those in other treatments. Birds in T_2 displayed significantly higher muscle thickness compared to the other groups. The muscle thickness of birds in T_1, T_3, and T_4 was lower than that of the control group ($P < 0.001$). All treatment groups significantly increased the villous to crypt depth ratio when compared to the control group, with no significant differences observed among the treatment groups.

Essential oils have been shown to enhance the performance of broiler chickens by improving nutritional availability in the digestive tract, as demonstrated by Murugesan *et al.* (2015). Current findings align with those of Abd El-Hack *et al.* (2024), who observed an increase in villous height by a plant extract containing a combination of oregano, cinnamon, and pepper essential oils. Phytogenic feed additives in the small intestine have the potential to augment villous height and the villous height/crypt depth ratio, thereby enhancing the absorptive contact area and the reliability of nutrient absorption. However, present results contradict those of Oladokun *et al.* (2021), whose study revealed no consistent differences in villous length or crypt depth in the jejunum of broilers received lavender as feed additives.

The primary mechanism by which active compounds in herbs influence broiler chicken performance is believed to be through the improvement of digestive systems, nutrient accumulation, and immune system regulation, as suggested by Mathlouthi *et al.* (2012). This study demonstrated a significant impact on villus length and crypt depths when lavender oil was injected, or lavender powder was added to the diet. According to Salajegheh *et al.* (2018) suggested that broilers received lavender decreased crypt depth and increased villous height to crypt depth ratio.

Serum biochemistry: The impact of lavender essential oil and lavender powder on serum biochemical parameters is detailed in Fig. 1. No significant differences were observed between treatments for glucose, creatinine, cholesterol, triglycerides, total protein, albumin, globulin, and ALT ($P > 0.05$). However, the control group exhibited a significantly higher AST value than the other treatments

Table 3. Effect of lavender essential oil and lavender powder on jejunum at day 24

Period	Control	T_1	T_2	T_3	T_4	P-value	SEM
Villi height (µm)	875.78^c	1321.95^a	1250.59^b	1252.91^b	1297.98^a	0.001	10.19
Crypt depth (µm)	155.33^c	186.46^a	175.13^b	176.48^b	177.20^b	0.001	0.89
Tip width (µm)	136.96^b	187.16^a	160.60^b	156.94^b	141.70^b	0.002	3.78
Basal width (µm)	141.69^b	205.85^a	166.41^b	162.91^b	145.83^b	0.001	4.55
Muscle thickness (µm)	181.98^c	250.67^b	280.31^a	247.27^b	242.06^b	0.001	2.18
Villi/crypt ratio	5.66^b	7.16^a	7.17^a	7.13^a	7.37^a	0.001	0.05

$^{a-c}$, Means the different column was superscript are significantly different (P>0.05). SEM, standard error of the mean; FCR, feed conversation ratio; T_1, Eggs injected with 0.05 ml of lavender essential oil; T_2, Eggs injected with 0.1 ml of lavender essential oil; T_3, Birds fed with 0.5% lavender powder; T_4, Birds will be fed with 1% lavender powder.

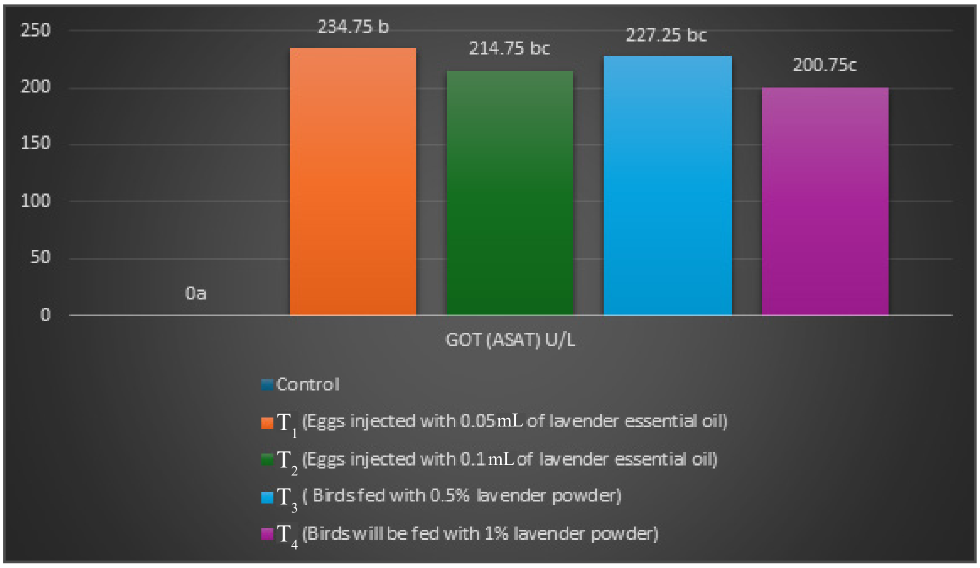

Fig. 1. Effect of lavender essential oil and lavender powder on serum biochemistry on day 24.

(P < 0.05).

Limited studies have investigated the influence of lavender on physiological blood parameters. The experimental data from present study aligns with those of Barbarestani *et al.* (2020) who observed a decrease in cholesterol level and LDL type and concentration by increasing the daily intake of 600 mg/kg of lavender essential oil. They attributed this effect to the inhibition of 3-hydroxy-3-methyl glutaryl-CoA (HMG-CoA) cytochrome activity in the liver, leading to a decrease in cholesterol and an increase in bacillus. The present findings are consistent with those of Torki (2021), who noted no influence on blood biochemical markers when supplementing laying hens with lavender essential oil (LEO). Similarly, Mokhtari *et al.* (2018) found no variations in protein content, lipids, or serum triglycerides when employing various doses of LEO as a feed supplement. In contrast, Barbarestani *et al.* (2020) observed no alterations in glucose concentrations, protein content, cholesterol, HDL, or VLDL in their experiments with LEO in broiler chicken feeds.

In contrast to the present study, Bölükbaşı *et al.* (2008) reported reduced blood cholesterol levels and triglyceride levels in broiler chicks fed a mixture of essential oils from oregano, sage, and lavender when compared to the control treatments.

Cost economics: Incorporating lavender oil and lavender powder into broiler production represents a cost-effective strategy with potential health and performance benefits. Lavender oil, priced at US$2.8 for 100 mL, costs only US$0.0028 per egg when injected with 0.1 mL of oil. For the lavender powder, priced at US$3 per kg, it is added at a rate of 0.1% to the broiler's feed. Assuming a broiler consumes 3.5 kg of feed during 35 days, the amount of lavender powder added is 0.1% of 3.5 kg, which equals 3.5 g. Thus, the cost of lavender powder for each broiler is US$0.0105.

It can be concluded that the inclusion of lavender in the diet of Ross 308 broilers led to improved body weight gain and feed conversion ratio in the treated birds. The supplementation of lavender oil and powder had a significant positive impact on villous height and the villous-to-crypt depth ratio. Remarkably, the birds treated with lavender exhibited a substantial reduction in serum AST levels, indicating potential benefits for liver health. Lavender supplementation can serve as an effective tool to enhance broiler performance.

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