

Original Article

Investigation of the effect of chamomile flower powder (CFP) on performance traits, lipid profile and morphology jejunum of Japanese quail from 7 to 35 days of age

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ABSTRACT

This study was conducted to investigate the effect of Chamomile flower powder (CFP) on performance traits, lipid profile and morphology jejunum of Japanese quail from 7 to 35 days of age. A total of 200 7-day-old male Japanese quail were as randomly distributed to 5 treatments, with 4 replicates, and 10 birds in each pen. The experimental treatments included (T1) a control diet (treated with basal diet only), (T2-T5) basal diet supplemented with of 0.5, 1, 1.5 and 2 % of CFP, respectively. Live body weight (LBW) and Feed intake (FI) were determined weekly, and then feed conversion ratio (FCR) was calculated. At the end of the study 5 birds with an average weight close to the same treatment were selected from each experimental treatment and blood and intestine (jejunum section) samples were collected at 35 days of age for further analysis. Blood samples were collected from the axillary vein and poured into test tubes without heparin. Blood serum was centrifuged and stored at -20°C until testing. Then, the birds were slaughtered and after opening the abdominal cavity, a section (3 cm) from the middle part of the jejunum was separated and kept in 10% formalin (pH=7.2) to study the jejunum morphology. The results showed that live body weight, feed intake and feed conversion ratio were significantly improved ($P<0.05$) in the birds CFP-fed compared to the control. The addition of 1% and 1.5% CFP (T3 and T4) to the basal diet showed the best performance results. The level of HDL was significantly ($P<0.05$) decreased in the birds fed CFP (T3 and T4) compared to the control. Other lipid profile parameters were not significantly ($P>0.05$) affected by the CFP used, although the results showed a numerical trend of decrease compared to the control. Villus height and depth crypt of the bird jejunum significantly increased ($P<0.05$) compared to the control. In conclusion, these results suggest that dietary supplementation with 1% and/or 1.5% CFP has beneficial effect on growth performance, lipid profile and jejunum morphometric of quails.

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1. Introduction

The use of medicinal plants as growth promoters and improvers of economic traits became common in poultry feeding after the ban of antibiotics by the European Union. Today, medicinal plants are widely used depending on the purpose of poultry farming. The reasons for the wide use of medicinal plants in poultry nutrition are reported by different researches, the reasons are as follows:

- a. Positive effect on growth performance
- b. Improvement of immune system
- c. Increase in production index and
- d. Other favorable economic traits (1).

Therefore, the deposition of medicinal plant residues in poultry products such as carcasses and consumption of these products by humans has led to healthier nutrition and health of human societies. For this reason, extensive research has been done by researchers to choose a better alternative for antibiotics in poultry nutrition. Therefore, the focus of researchers in their studies has been to identify more species of medicinal plants and their optimal use in poultry nutrition. (2-3). Since the 1950s, antibiotics have been used in poultry nutrition as growth promoters and to promote of economic traits. Because of different reasons such as antibiotic resistance, also their deposition in organs such as the liver and as a result endangering the health of human society. Therefore, in 2005, the European Union banned the use of antibiotics as a growth promoters in animal farm feed (4). Chamomile *Matricaria* can be found in the pharmacopoeias of 26 nations worldwide. In addition, Chamomile is an important traditional medicine that has been used in many parts of the world, showing therapeutic activity against diseases related to the intestines, liver, and stomach; cough; shortness of breath; and asthma (5). After the ban of antibiotics in feeding poultry and other farm animals; researchers have done a lot of research to find alternatives, and one of these alternatives was the use of medicinal plants in the poultry diets, which has been widely used to improve the quantitative and qualitative traits of the carcass, economic traits such as live weight, feed conversion ratio, and also to improve the immune system, etc. reported. Therefore, this research investigates the chamomile plant grown in Kurdistan region; which is one of the conventional and used medicinal plants based on economic traits; Fat profile and morphology of quail jejunum were done from 7 to 35 days of age.

2. Materials and Methods

2.1. Birds and Management

The study was carried out based on the experimental protocols in compliance with the Guide to the Care and Use of Laboratory Animals, which was approved by the guidelines of Animal Research Committee of Islamic Azad University, Iran. This research was conducted at the research farm of Kurdistan Azad University in Sanandaj. A total of 160 one-week-old Japanese quails with an average initial live body weight (LBW) of 23.7 ± 2 g were obtained from a commercial farm near the city of Sanandaj and

transferred to the research hall after preparation. Then, in a completely randomized design (CRD) with five treatments, four replications, and 7 birds in each experimental group, the quail were randomly distributed in each experimental cage with dimensions of 100 x 100 x 60 cm. Each of the research cages was equipped with a plastic feeder and nipple water. During the study period (7 to 35 days old), the birds had free access (*ad libitum*) to water and food. The room temperature started from 32 degrees and was reduced by 3 degrees each week to 18-21°C until the end of the study. The lighting system consisted of 23 hours of light and one hour of darkness (23L: 1D).

2.2. Experimental treatments

The experimental treatments included (T1) control (basal diet, without CFP), and (T2-T5) basal diet supplemented with 0.5, 1, 1.5 and 2 % of CFP/basal diet, respectively. The basal diet was formulated on a corn-soybean basis using the NRC (6) tables, and then the required amount of chamomile was added to the basal diet and mixed completely using a mixer and given to the birds in each experimental pen. The composition and nutrient components of the basal diet are shown in **Table 1**.

2.3. Preparation of chamomile flower powder

The required amount of chamomile flower powder in this research was obtained from the research greenhouse of Azad University of Kurdistan, where the colleagues of the agriculture department who study the dominant species of medicinal plants in Kurdistan province. Then the chamomile samples were dried in the laboratory air at a suitable temperature and away from the sunlight completely crushed and mixed using a blender. The chemical composition of chamomile flower was analyzed by GC-MS as described by Mohammed Mohammad et al (7). The chemical composition of chamomile flower powder is shown in **Table 2**.

2.4. Data Collection

2.4.1. Performance traits

Feed intake (FI); body weight gain (BWG) and feed conversion ratio (FCR) were recorded as weekly and finally at the age of 35 days, collected data were used for analysis. Mortality number of birds was recorded daily, and viability (%) was calculated throughout the experimental period. Also, quail mortality was recorded to correct other evaluated growth parameters used.

2.4.2. Blood sample

On the last day of the research (35 days old), after 8 hours of starvation, 4 birds (one bird/each pen) were selected from each treatment with an average live weight close to the same treatment. The blood sample (5ml) was taken from the axillary vein and poured into a test tube without heparin, the Serum was centrifuged ($3000 \times g$ for 15 min at 4°C), stored at -20°C until Serum analysis. Serum samples were sent to a specialized laboratory for measurement of the total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) using an automatic blood chemistry analyzer (AU640, Olympus Corporation, Tokyo, Japan). Immediately after

Table 1. The composition and nutrient components of the basal diet

Ingredients	(%)
Corn	58.20
SBM 44%	33.53
Soy bean oil	1.90
DCP	1.63
Limestone	1.02
NaCl	0.25
Vitamin-mineral premix ¹	0.30
Ca	0.25
Available P	0.46
DL-met	0.12
Met+Cys	0.83
lysine	1.61
Calculated analysis	
ME (kcal/kg diet)	2958
CP	23.12
Ca	0.89
Available P	0.46
Lysine	1.66
Met+Cys	0.89

¹Supplied the following per kilogram of diet: 11,025 IU of vitamin A; 3,528 IU of vitamin D3; 33 IU of vitamin E; 0.91 mg of vitamin K; 2 mg of thiamin; 8 mg of riboflavin; 55 mg of niacin; 18 mg of Ca pantothenate; 5 mg of vitamin B6; 0.221 mg of biotin; 1 mg of folic acid; 478 mg of choline; 28 µg of vitamin B12; 75 mg of zinc; 40 mg of iron; 64 mg of manganese; 10 mg of copper; 2 mg of iodine; and 0.3 mg of selenium.

Table 2. Chemical composition of chamomile flower (*Matricaria chamomilla* L.)

Compound	Composition (%)
Chamazulene	6.40
Spiroether	5.10
Bisabolol	6.30
β-Farnesene	29.80
α-Farnesene	9.30
Germacrene	6.20

blood sampling, the birds were slaughtered, and then the small intestine was separated. The weight of small intestine (SI) and its relative weights were recorded for each quail as the following equation: $RSIW = (SI \text{ weight, g}) / (LBW \text{ of quail, g}) \times 100$.

2.4.3. Jejunum sample

Immediately after opening the abdominal cavity, the small intestine was separated and a sample (4 samples per each treatment) from the middle part of the jejunum (3cm) was separated and then fixed in phosphate buffered formalin (10%, pH =7.2). The jejunum samples were transported to the pathology laboratory for morphological analysis. The jejunum samples were dehydrated through increasing concentrations of ethyl alcohol (70%, 90%, 96% and 100%), cleared in xylene and then embedded in paraffin. The paraffin blocks were then sectioned with a microtome into four 5µm thick discontinuous paraffin -embedded sections per jejunum sample. The samples were placed on a

microscope slide and stained with standard hematoxylin-eosin solution. Villus height (VH) and crypt depth (CD) were observed in 5 well-oriented intact villi at 40×magnification by using an Olympus BX43 digital microscope (Olympus, Tokyo, Japan). A computer morphometric program (Quick Photo Micro 3.0) was used for morphometric measuring the jejunum villi height and base width of the villi. The same computer program was used to measure the jejunum crypt depth (CD). The Villus height (VL) was measured from the top of the villus to the top of the lamina propria. Surface area was calculated by using the formula = $(2\pi) \times (\text{villi width}/2) \div VL$. Crypt depth was measured from the base upward to the region of the transition between the crypt and villus (8).

2.5. Statistical Analysis

Data were statistically analyzed using SAS ANOVA procedures (9). Differences between means were compared using Duncan's multiple range test (10). Orthogonal polynomial contrasts (linear and quadratic) were also used

to test the significance of the different levels of dietary CFP. The statistical model used was: The result of the analysis of variance according to the model:

$$Y_{ij} = \mu + T_i + e_{ij} \text{ Where;}$$

Y_{ij} = All dependent variables

μ = Overall mean

T_i = the effect of CFP levels; (i = 1, 2, 3, 4)

e_{ij} = Experimental error

3. Results

3.1. Growth performance

The results related to the effect of different levels of CFP on performance traits (FI, LBW, and FCR) in Japanese quail are presented in Tables 3 and Figures 1-3. The highest and lowest live body weight (LBW; $P < 0.041$) and feed conversion ratio (FCR; $P < 0.038$) were observed in treatments T4 and T5 in quails fed with 1.5% and 2% CFP, respectively, which showed a significant effect compared to the control treatment. The obtained data related to FI, although there is no significant difference between the control and other treatments, but numerically, the FI has a decreasing trend ($P > 0.05$). Also, the lowest amount of feed intake compared to the control treatment was observed in the birds fed with the diet containing CFP supplement.

3.2. Lipid profile

The results obtained in this study are presented in Table 4. The data obtained in this research showed that the concentration of TC and HDL in birds fed with 2% CFP (T5) had a significant effect ($P < 0.05$) compared to the control treatment. The amount of HDL and TC in birds fed with 2% CFP had the highest and lowest values, respectively. Also, LDL and TG concentrations, although they did not show significant differences ($P > 0.05$) compared to the control treatment, but they had a decreasing trend.

3.3. Jejunum morphometric

The results related to the effect of different levels of CFP on the morphological traits of the jejunum are shown in Table 5 and Figure 4. The length of the villi; crypt depth and absorption area in birds fed with treatments T4 and T5 showed a significant increase ($P < 0.05$) compared to the control. The ratio of the length of the villi to the crypt depth and the relative small intestine length (RSIW) in quails fed with CFP, although no significant difference ($P > 0.05$) was observed with the control treatment, but they showed an increasing trend numerically.

Table 3. Effect of experimental treatments on growth performance of *Japanese quail* at 35 days age

Experimental treatments	Growth performance		
	feed intake (g)	Live Body weight (g)	Feed conversion ratio (g/g)
T1) Control (BD ¹ , without CFP)	509.4	164.2 ^b	3.07 ^a
T2) BD+0.5% CFP	491.2	179.1 ^b	2.81 ^a
T3) BD+1% CFP	481.2	192.3 ^a	2.51 ^a
T4) BD+1.5% CFP	476.4	201.4 ^a	2.37 ^b
T5) BD+ 2% CFP	475.1	204.3 ^a	2.33 ^b
SEM ²	7.21	3.68	0.04
<i>P-value</i>	0.073	0.041	0.038

^{a-b} The means in a column with different superscript letters are significantly different ($P < 0.05$). ¹Basal diet ²Standard error means.

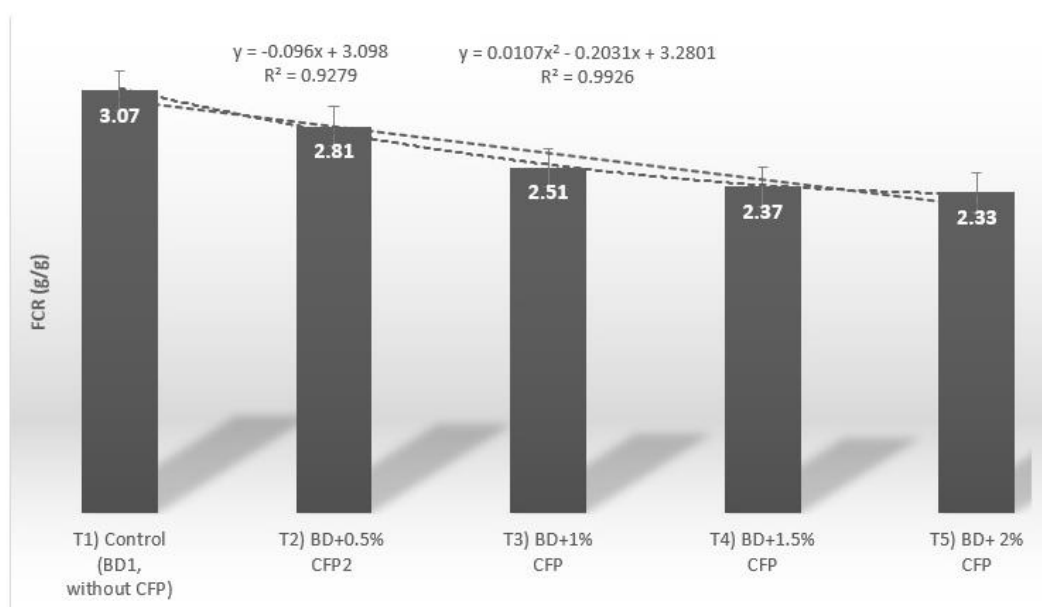


Figure 1. The effect of experimental treatment on the FCR Japanese quail

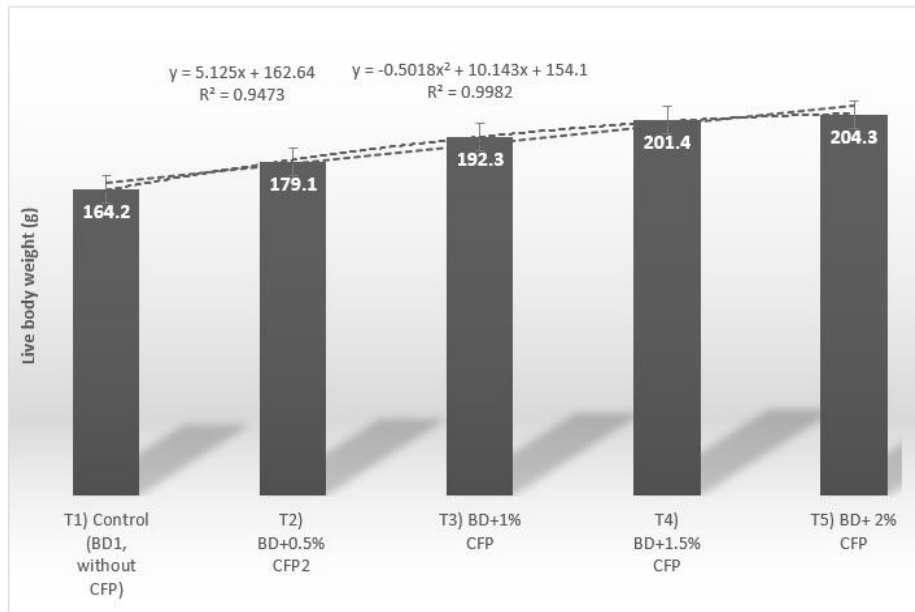


Figure 2. The effect of experimental treatment on the LBW Japanese quail

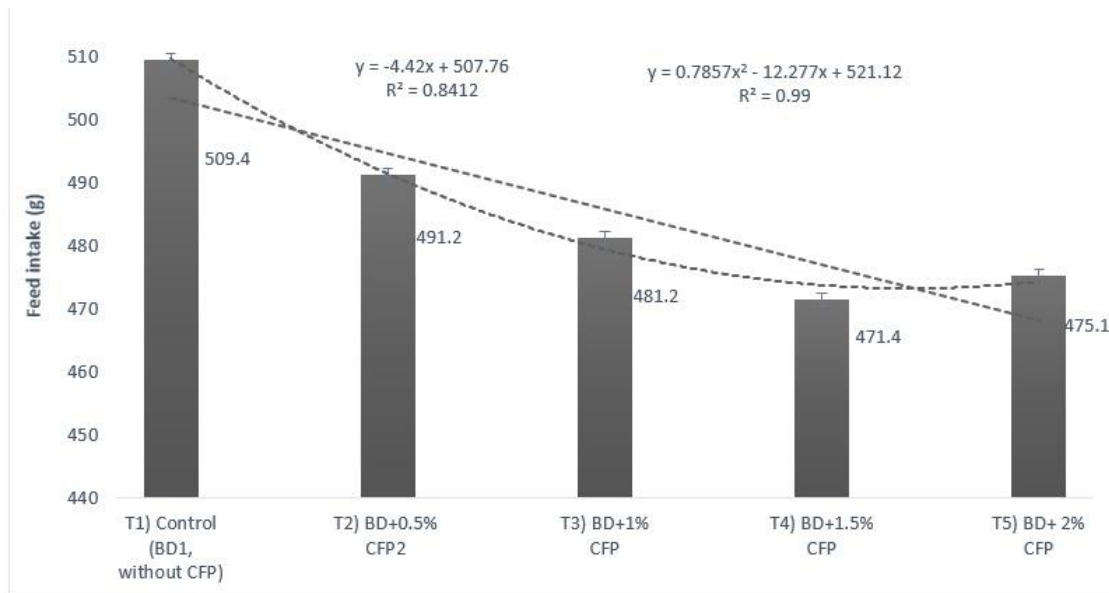


Figure 3. The effect of experimental treatment on the FI Japanese quail

Table 4. Effect of experimental treatments (CML, % basal diet) on lipid profile of *Japanese quail* at 35 days age*

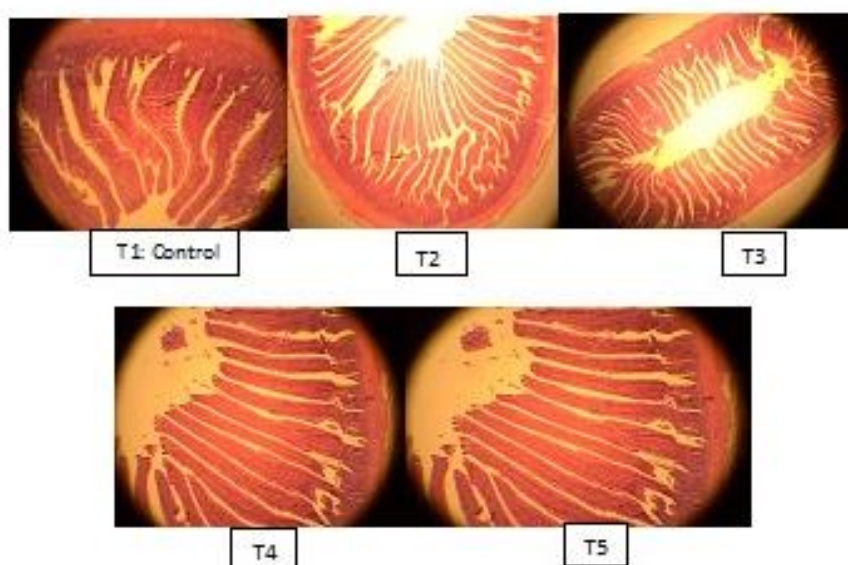
Experimental treatments	Lipid parameters ³			
	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
T1) Control (BD ¹ , without CFP)	206.3a	221.2	57.8a	103.6
T2) BD+0.5% CFP	201.8a	224.5	56.3a	106.4
T3) BD+1% CFP	194.1a	211.8	52.9a	95.1
T4) BD+1.5% CFP	168.3b	197.4	43.1b	97.6
T5) BD+ 2% CFP	170.5b	195.1	40.3b	96.5
SEM ²	3.7	4.6	1.2	2.6
<i>P</i> -value	0.023	NS	0.411	NS ⁴

^{a-b} The means in a column with different superscript letters are significantly different ($P < 0.05$). 1-basal diet. 2-Standard error means. 3-TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein. 4-Not significant

Table 5. The effect of experimental treatments on the jejunum morphometric traits of *Japanese quail* at 35 days age.

jejunum morphology						
Experimental treatments	Villus width (μm)	Villus height (μm)	Crypt depth (μm)	VH:CD ratio	Villus Surface Area (mm ²)	RSIW ⁴ (%LBW)
T1) Control (BD ¹ , without CFP)	238.3	813.3b	161.9bc	4.91	478.9b	2.06
T2) BD+0.5% CFP	241.9	824.1b	174.6b	5.16	515.7b	2.09
T3) BD+1% CFP	239.2	863.6a	181.2a	5.45	503.4b	2.14
T4) BD+1.5% CFP	241.5	971.3a	186.4a	5.84	554.8a	2.16
T5) BD+ 2% CFP	242.1	984.4a	195.1a	5.92	571.1a	2.21
SEM ³	1.12	11.2	4.2	0.19	10.7	0.02
<i>P</i> -value	NS ⁵	*	*	NS	*	NS

^{a-c} The means in a column with different superscript letters are significantly different ($P < 0.05$). 1-Basal diet. 3-Standard error mean. 4-Relative small intestine weight. 5-Not significant

**Figure 4.** The effect of experimental treatments on the jejunum morphometric traits of *Japanese quail* at 35 days age (H&H; 100x)

4. Discussion

4.1. Growth performance

The results reported by most researchers regarding the use of chamomile plant and its extracts indicate its positive effects on the economic and performance traits of poultry; although there are differences in the reported results among researchers, but most of them confirm the improvement of yield traits. On the contrary, Dada and Tabeidian (11) indicated that feeding basal diets supplemented with chamomile extract or chamomile powder extract had no significant effect on performance traits of broilers. Therefore, the reason for these beneficial effects can probably be attributed to the presence of active compounds in the chamomile plant. Windisch et al (12) reported that chamomile oil is a beneficial dietary supplement because it increases the body weight of chickens and improves feed conversion. The bioactive components, which act on the gastrointestinal ecosphere primarily mainly by inhibiting the growth harmful microbes in the digestive tract, constantly improving digestive enzymes, improving utilization of the digestive process of intestinal products, and improving liver enzymes, may be responsible for the beneficial development in average live body weight, weight gain, and feed conversion ratio in the treated groups (13). Therefore, this result is favorable from the economic point of view. Abdul et al (14) reported that the active constituents of chamomile (flavonoids, kamasolen and bisaboldaxid essential oils) have the same role as probiotic and thus improve the natural intestinal microflora and may be help in the absorption of nutrients that enhance growth. In addition, the improvement in feed utilization may be attributed to the properties of these materials, which could act not only as antibacterial, anti-protozoa and antifungal but also as antioxidants (11). The bioactive components, which act on the gastrointestinal ecosphere primarily by inhibiting the growth of harmful microbes in the digestive tract, steadily improving digestive enzymes, improving utilization of the digestive process of intestinal products, and improving liver enzymes, may be responsible for the beneficial development in average live body weight, weight gain, and ratio of feed conversions in the treated groups (12).

4.2. Lipid profile

The results of this research are consistent with the report of Abdul et al (14). These researchers reported that by increasing the amount of chamomile powder in the basal diet, the level of serum cholesterol decreased significantly compared to the control. Also, Imai and Nakachi, (13) reported that serum cholesterol was significantly decreased compared to the control in mice that fed with chamomile extract. Research results have shown that chamomile contains various compounds that probably have an inhibitory effect on the absorption of fats in the blood, such as catechin. Therefore, they prevent the storage of lipids in the liver and other body tissues. The reduction of tissue cholesterol may also be seen as a negative effect of catechin. Chamomile in micellar form has also been

reported to be effective in the reabsorption of bile acids, so increased secretion of nonabsorbable bile acids may reduce blood cholesterol in birds fed chamomile leaf powder (15). Riffraff et al. (16) reported that chamomile reduces blood cholesterol for two reasons, which are: **a)** the flavonoids in chamomile increase the indirect phosphorylation of Hydroxy-methylglutaryl-CoA (HMG CoA) reductase enzyme and **b)** the synthesis and production of endogenous cholesterol in the body. Also, the conversion of cholesterol into bile acids is basically done in the liver, and in fact, it is the main way to remove cholesterol from the body, and ultimately, these factors reduce the concentration of cholesterol in the blood.

4.3. Jejunum morphometric

The data obtained in this study are consistent with the reports of other researchers. Sherzad et al. (17) reported that the addition of chamomile leaves alone and its combination with other medicinal plants significantly increased the length of the villi and the depth of the crypts in broiler chickens compared to the control treatment. Also, the relative length of the small intestine increased significantly with chamomile consumption compared to the control treatment. Natural feed additives such as chamomile flower have also been shown to have beneficial effects on the stimulation and activity of the digestive system by improving the palatability of the diet and increasing the appetite of poultry (18). The active compounds in chamomile reduce the growth of harmful microbes in the digestive system; also the increase the beneficial microorganism's jejunum, the result of these changes is a positive effect on the performance and functional characteristics of the birds and improves digestive enzymes, improved utilization of the digestive process of intestinal products (17). These results may be related to the presence of bioactive compounds in the medicinal plants that could improve the gut health and intestinal morphology. Improvement of the jejuna morphology of birds including villus height or villus height-to-crypt depth ratio, may indicate the efficient digestion, better absorptive capacity which may lead to the better nutrient uptake and utilization and ultimately better performance (17, 18, 19). In conclusion, based on the analysis of the data obtained in this research, the results showed that chamomile flower powder, which is one of the dominant medicinal plants in the Kurdistan region of Iran, yield traits, serum lipid profile and morphological characteristics of the jejunum of Japanese quail during the growth period. Thus improve the health and economic condition status of Japanese quail.

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Authors' Contribution

Research project proposal: A.F.
 Review of research sources and background: B.M.
 The practical part of the research: K.P.KH.
 Analysis of raw research data: A.F.
 Data interpretation: A.F.
 Article drafting and submission: K.P.KH.
 Study concept and design: and B.M.
 Study supervision: A.F.

Ethics

This research was conducted based on the protocols approved by Islamic Azad University and the research ethics charter.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

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

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