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Studying the effect of adding chamomile flower powder to diet on some physiological, microbial and egg traits in quail

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Abstract
A field experiment was conducted in the poultry field of the Veterinary Public Health Branch / College of Veterinary Medicine / University of Basra to study the effect of adding chamomile flower powder to the diet on some physiological, microbial and egg traits in quail. 270 *Cotrinux Japonica* quails, 6 weeks old, were used in this study. The birds were randomly distributed into 3 treatments, 90 birds per treatment, with three replicates (30 birds/replicate). Chamomile flower was added in powdered form to the bird feed at a concentration of 0.1% and 0.3% compared with the control group from the age of (6-10) weeks. Upon completion of the experiment, some physiological characteristics were measured, such as the concentration of total protein, Globulin, Albumin glucose, cholesterol, Triglyceride, uric acid and Creatinie in the serum, the internal characteristics of the egg, and the total number of bacteria in the intestine. The results of the study indicated to: It was that there was a significant increase ($p<$ 0.05) in the concentration of total protein, the percentage of egg shell weight, the albumin weight and yolk weight in the blood serum, while A significant decrease ($p<$ 0.05) was observed in the total number of Fungi, *Escherichia coli* and *Staphylococcus* bacteria treated with 0.3 mg chamomile flower compared to the control group at 10 weeks of age.

Keywords: Chamomile flower, physiological, microbial and egg

1. Introduction
As a result of the negative effect of antibiotics, medicinal plants such as chamomile have been used as an alternative to antibiotics, because these plants do not leave a negative effect or toxins in animal tissues, which reflects positively on animal and human health (Srivastava and Gupta, 2009) [2]. (There are several research on increasing the activity and productive performance of quail using natural growth stimulants such as natural plants (Nowaczewski *et al*., 2010) [3]. The chamomile plant is an annual herbaceous plant that is characterized by rapid growth and has many branches. The average height of the herb is about 50-60 cm. It has light green leaves that are carried on an upright stem that carries small, feathery, lobed leaves. At the end of that stem are spherical-shaped flowers consisting of disc florets that are yellow in colour. They are many in number, and the diameter of these florets ranges between 0.4 - 1.5 cm. The flower contains plant dyes or yellow-colored substances known as Apigenin, and anti-septic substances that expel intestinal gases, such as Saponin and Artemisin, as the first substance is used to expel nematodes and roundworms (Paula, 1999) [4]. Chamomile contains the most effective and active ingredients such as cis-enynedicycloether, and chamazulene (Srivastava and Gupta, 2009) [5]. Research has proven that chamomile is a good antioxidant, antibacterial and antifungal (Pirzad *et al*., 2006; Singh *et al*., 2011; Srivastava *et al*., 2010; Khishtan and Pesky, 2020) [6, 7, 8, 9]. These chamomile properties may enhance weight gain, growth, and the bird's feed conversion rate. The presence of sesquiterpenoids in chamomile weakens the bacterial cell membrane this allows external solutes to penetrate the cell (Göger *et al*., 2018) [11]. Chamomile also acts as an antioxidant through free radical inhibition (Shimada *et al*., 1992) [12]. The presence of camisole, essential oils and flavonoids in chamomile, improves the work of the intestines because these substances work similarly to the work of probiotics, which leads to improving the work of the digestive system by facilitating the digestion of food elements, which reflects positively on the health of birds and improves the growth performance of birds. Therefore, the purpose of this study is to study the effect of adding chamomile flower powder to the

diet on some physiological, microbial and egg traits in quail.

2. Materials & Methods

2.1 Product Extract: Dry chamomile flowers weighing 7 grams were brought from a local herbal shop in Basra Governorate, Iraq. To make the extract, 95 ml of distilled boiled water was added to 7 g of chamomile flowers in a glass bottle. Water was used to make the extract in a vial. After waiting for 12 hours, the suspension was filtered and ready for use (Ibrahim and Boutros, 2008; Towson, 2019) [15].

2.2 Birds

A total of two hundred and seventy (6 weeks old) unsexed Japanese quail chicks (*Coturnix japonica*) were obtained from Veterinary Public Health Farm, Veterinary Medicine College, University of Basrah, with an initial body weight of 225.24 g used in this study. Birds were randomly divided into three groups (n= 90), and each the group included three replicates (n = 30) and were placed into galvanized wire cages (75 x 70 x 45 cm) and reared up to week 10 of age, according the following groups: T_1: There is no treated group (control), T_2: a group of treated with chamomile flowers powder 0.1% in diet. T_3: a group treated with chamomile flower powder 0.3% in diet. And the food and water provided to the birds were free.

2.3 Nutrition

The birds were fed on a growth and production diet. These diet were prepared from one of the feed factories in northern Iraq. Table 1: Feed Ingredient and Chemical Analysis:

Table 1: Feed ingredient and chemical analysis:

Ingredients	Growth diet (%)
Yellow corn	52
Soybean meal	29
Protein concentrated (50% protein)	9
Wheat bran	3
Limestone	3.2
Salt	0.3
Vegetable oil	2.7
Mixture of vitamins and antibiotic	0.8
Total	100
Metabolic energy (kcal/kg)	3095,8
Protein (%)	21,69
Calcium (%)	1.04
methionine%	0.48
Phosphorus (%)	0.37

*NRC 1994

2.4 The traits studied in the experiment

2.4.1 Biochemical Traits

2.4.1.1 Total Protein concentration

Using a spectrophotometer at a wavelength of (550 nm), the absorbance of the samples was measured according to the following equation:

Total Protein (g/100 ml) = Sample Absorbance/ Absorbance of standard solution x 6. (Henry *et al*., 1974) [18].

2.4.1.2 Albumin concentration

The concentration of albumin in blood serum was measured by measuring the absorbance of the samples and the standard solution with a spectrophotometer at a wavelength of 630 nm, according to the following equation:

Total Albumin (g/100 ml) = Sample Absorbance/ Absorbance of Standard Solution x 5. (Henry *et al*., 1974) [18].

2.4.1.3 Globulin concentration

The concentration of globulin in blood serum was measured by calculating the difference between the concentration of total protein and albumin, according to the following equation:

Globulin Concentration g/100 ml = Total Protein Concentration - Albumin Concentration (Henry *et al*., 1974) [18].

2.4.1.4 Glucose Concentration

The glucose concentration was measured by calculating the absorbance of the samples using a spectrophotometer at a wavelength of (505 nm) according to the following equation:

Glucose Concentration mg/100 ml = Sample Absorbance / Absorbance of Standard Solution x 100. (Henry *et al*., 1974) [18].

2.4.1.5 Cholesterol concentration

Cholesterol concentration was measured by measuring the absorbance of the samples with a spectrophotometer at a wavelength of (500 nm) according to the following equation:

Cholesterol Cconcentration mg/100 ml = Sample Absorbance / Absorbance of Standard Solution x 200. (Henry *et al*., 1974) [18].

2.4.1.6 Triglyceride concentration

The concentration of triglycerides was measured by measuring the absorbance of the samples with a spectrophotometer at a wavelength of (540 nm) according to the following equation:

Triglyceride Cconcentration mg/100 ml = Sample Absorbance / Absorbance of Standard Solution x 200 (Henry *et al*., 1974) [18].

2.4.1.7 Uric acid Concentration

Uric acid concentration was measured by measuring the absorbance of samples with a spectrophotometer at a wavelength of (600 nm) and using the method of Tietz, (1986) [19] according to the following equation:

Uric acid Concentration mg/100 ml = Sample Absorbance / Absorbance of Standard Solution x 5.

2.4.1.8 Creatinine Concentration

Creatinine concentration was measured by reacting it with picric acid in a basic solution to form a coloured complex. Based on the colorimetric method with protein precipitation of Tietz, (1986) [19].

Creatinine Concentration mg/ deciliter = Sample Absorbance / Absorbance of Standard Solution x 2

2.4.2 Microbiology calculation
Swabs were taken from the duodenum and colon of two quails for each treatment, and they were placed in sterile test tubes. These tubes were numbered according to the treatments, and they were placed in ice and transported on the same day to the radioisotope laboratory (Ahli laboratory) to calculate the numbers of *E. coli*, Staphylococci, and fungi bacteria. For the duodenum and colon according to the method of Harrigan and Mecance (1976) [20].

2.4.3 Qualitative Traits of eggs
10 eggs were taken for each replicate and kept in the refrigerator for 24 hours at the end of the experiment at 72 days of age to measure the qualitative characteristics of the eggs.

2.4.3.1 Egg Shell Weight (g)
After weighing the yolk and white of the egg, the shell was left to dry for a week, and after removing the shell membrane, the inner shell was weighed using a sensitive balance.

2.4.3.2 Egg Shell Thickness (mm)
The egg was measured from its pointy end and the other wide end, after removing the membranes, using a thickness measuring machine (Vernier).

Average thickness Shell Tapered Shell thickness (mm) + Broad Shell thickness (mm) /2).

2.4.3.3 Albumin Weight (g)
Regarding the weight of the white (in grams) after it is separated from the yolk.

2.4.3.4 Albumen & Yolk Height
Two albumen readings were recorded for each egg using a metal ruler, for the area extending from the yolk to the outer edge of the albumen.

2.4.3.5Albumin & Yolk Diameter
Using an electronic calliper, the diameter of the yolk and the diameter of the white were measured

2.4.3.6 Yolk Weight
The yolk is weighed by breaking the egg carefully, then the yolk is isolated from the white using filter paper and a strainer with large holes, and the weight of the yolk (g) is measured using a sensitive balance.

2.4.3.7 Yolk Index
Based on the equation of Rose (1997) [23] and to calculate the yolk index, which represents the product of the height of the yolk (mm) divided by its diameter (mm), the diameter of the yolk was measured using an electronic Vernier calliper.

Yolk Index = Height of Yolk (mm)/Diameter of Yolk (mm)

2.4.3.8 Huagh Unit
The Hoff unit was calculated according to the following equation:
Haugh unit = 100 Log (H+7.57-1.7W0.37)
H: represents the height of whiteness (mm)
W: Represents the weight of the egg (g)
7.57: (A fixed number)

2.5 Statistical analysis
The study results were analyzed using a completely randomized design (CRD) using SPSS, (2019) [24]. To test the significance of the differences between the studied means, the Duncan (1955) [25] multinomial test was used, at a significance level (p<0.05), and a mathematical model was used to analyze the data.

3. Results and Discussion
3.1 Effect of chamomile flower powder to the diets on some bio chemicals traits of blood during (6-10) weeks
Table 2 showed the effect of adding chamomile flower powder to the feed on some biochemical traits of Japanese quail after 6 weeks of the experiment (total protein, albumin, globulin and glucose). The results of the experiment showed that there were significant differences in the total protein, albumin, globulin concentrations in the blood serum reached 6.14, 2.87, 3.41 mg/100 ml blood serum respectively in the T_3 significantly (p< 0.05) compared to the T_1 reached 4.81, 2.07, 3.01 mg/100 ml blood serum respectively. It may be due to the effective effect of chamomile components through their effect of reducing heat stress in the birds that were studied. Thaxton and Puvadolpirod (2000) [26] and Boutros (2007) indicated that adding chamomile flower powder It works to reduce the harmful effect of heat stress by increasing the secretion of the hormone thyroxine, thus increasing the speed of food metabolism, increasing vital reactions in the body, and then building muscle tissue, which results in maintaining a high rate of dietary protein in the birds' blood. The results of the experiment showed a significant decrease in the average glucose concentration in the blood serum for the T_3 treatment (p< 0.05) compared to the control treatment T_1, where the glucose concentration decreased to 147.02 mg/100 ml blood serum, while in the control treatment T_1 it was 181.00 mg/100 ml blood serum. The reason for the decrease in the percentage of glucose in the blood serum for the T_2 and T_3 treatments compared to the control T_1 treatment due to the increase in adrenal gland hormones in the blood of birds exposed to heat stress, which leads to an increase in the level of glucose in the blood through the decomposition of glycogen, and this is what happened in his treatment. Control (free from chamomile flower powder), in addition to chamomile containing blood sugar-lowering agents similar to the hormone insulin (Mustafa, 2003) [27].

Table 2: Effect of chamomile flower powder to the diets of quails on Total Protein, Albumin, Globulin and Glucose serum of blood during (6-10) weeks (Mean ± Standard Error)

Group	Some bio chemicals traits			
	Total Protein serum mg/100 ml serum	Albumin serum mg/100 ml serum	Globulin serum mg/100 ml serum	Glucose serum mg/100 ml serum
T_1	4.81±0.01^c	2.07±0.02^c	3.01±0.05^c	181.00±19.06^a
T_2	5.62±0.02^b	2.62±0.07^b	3.14±0.8^b	169.87±8.31^b
T_3	6.14±0.05^a	2.87±0.00^a	3.41±0.03^a	147.02±11.61^c
Sig. 0.05	*	*	*	*

Small letters referred to significant difference among groups at (p≤0.05). N.S referred to no significant difference. * referred to significant difference, Treatments: T_1, the control treatment without the addition of chamomile flower powder, T_2 and T_3, the treatments with the addition of chamomile flower powder at levels of 0.1 and 0.3%, respectively.

It was noted from Table 3 that the concentration of cholesterol in the blood serum decreased significantly ($p< 0.05$) as the percentage of chamomile flower powder added to the diet increased. The percentage of cholesterol for the three treatments reached 187.58, 162.48 and 145.22 (mg/100 blood serum) for the treatments T_1, T_2 and T_3 respectively. The results of this study were similar to the results of Mustafa (2003) [27], who showed that chamomile affects the metabolic activities of male rats, as it reduces the activity of the enzyme acetylcholin esterase in the blood serum. These results are consistent with what was found by Yasseen and colleagues (2003) that giving chamomile flower powder to mice orally at concentrations of 100-500 mg/kg live weight led to a decrease in the concentration of blood glucose and total fats, as well as a decrease in the effectiveness of the enzyme Acetylcholin Estrase in the blood serum, which is responsible for About the formation of cholesterol in the liver. As for uric acid for the experimental treatments, it was noted that its percentage was mathematically lower for the two treatments T_2 and T_3 compared to the control treatment T_1, as this percentage for the treatments T_1, T_2 and T_3 reached (4.10, 3.87 and 3.12) mg/100 blood serum. It is noted that the percentage of uric acid in blood serum decreases as the percentage of chamomile flower powder added to the diet increases. This may be attributed to the effect of chamomile oil, as it was found to activate the work of the liver and gallbladder to improve digestion and absorption, which led to a mathematical decrease in uric acid whenever the levels of chamomile flower powder in the feed increased.

Table 3: Effect of chamomile flower powder to the diets of quails on Cholesterol, Triglyceride, Uric acid and Creatinine serum of blood during (6-10) weeks (Mean ± Standard Error)

Group	Some bio chemicals traits			
	Cholesterol serum mg/100 ml serum	**Triglyceride serum mg/100 ml serum**	**Uric acid serum mg/100 ml serum**	**Creatinine serum mg/100 ml serum**
T_1	187.58±10.05[a]	120.44±2.09	4.10±0.12[a]	2022.1±0.02
T_2	162.48±15.19[b]	122.89± 2.16	3.87±0.02[b]	2023.3±0.00
T_3	145.22±16.17[c]	123.33± 0.49	3.12±0.23[c]	2023.1±0.01
Sig. 0.05	*	N.S	*	N.S

Small letters referred to significant difference among groups at ($p\leq0.05$). N.S referred to no significant difference. * Referred to significant difference, Treatments: T_1, the control treatment without the addition of chamomile flower powder, T_2 and T_3, the treatments with the addition of chamomile flower powder at levels of 0.1 and 0.3%, respectively.

3.2 The effect of chamomile flower powder in quail diet on the number of microorganisms in the duodenum and colon within (6-10) weeks

Table 4 showed the effect of adding different percentages of chamomile flower powder on the microbial of the duodenum (Fungi, *Staphylococcus* & *E. coli*) of quail. The results of the study indicated a decrease in the number of fungi in T_3 treatment, which amounted to 2.4 x 10^6 compared to T_2 & T_1 treatments, which they recorded 4.25 x 10^6 and 0.8 x 10^7 respectively. The same table shows a significant decrease ($p<0.05$) in the number of bacteria for treatments T_2 & T_3 compared to control treatment T_1. The number of *Staphylococcus* bacteria for three treatments was T_1, T_2 & T_3 1.2 x 10^7, 4.15 x 10^6, & 2.9 x 10^6 bacterial cells/g, respectively. As for the numbers of *E. coli* bacteria, they were significantly lower ($p< 0.05$) for treatments T_2 & T_3 compared to control T_1, as their numbers reached 0.6 x 10^7, 3.45 x 10^6, & 2.65 x 10^6 For the three transactions T_1, T_2 & T_3 respectively. Thus, we conclude the positive role of the chamomile plant in reducing the number of fungi & harmful bacteria in the duodenum. Perhaps the reason is due to the role of the active compounds in the chamomile plant, including bisabolol - α, which is effective against bacteria (*E. coli*, *Staphylococcus*) & against fungi (Dermarderasion & Liberti, 1988) [28]. Or the reason may be because the chamomile flower contains another active compound, Chamazulene, which has strong effectiveness against harmful microbes (Kedzia, 1991) [29].

Table 4: Effect of chamomile flower powder in the diets of quails on the number of microorganisms in the duodenum within (6) weeks (Mean ± Standard Error)

Group	Numbers of bacteria and fungi/bacterial cell/1 gm		
	Fungi	***Staphylococcus***	***E. coli***
T_1	0.8[a] ±2.66 × 10^7	1.2[a] ±23.09 × 10^7	0.6 a ±1.12 × 10^7
T_2	4.25[a] ± 2.66× 10^6	4.15[b]± 1.16 × 10^6	3.45 b ±2.66 × 10^6
T_3	2.4[b]±4.61 × 10^6	2.9[b]±2.66 × 10^6	2.65 b ±5.33 × 10^6
Sig. 0.05	*	*	*

Small letters referred to significant difference among groups at ($p\leq0.05$). N.S referred to no significant difference. * referred to significant difference, Treatments: T_1, the control treatment without the addition of chamomile flower powder, T_2 and T_3, the treatments with the addition of chamomile flower powder at levels of 0.1 and 0.3%, respectively.

The results of Table 5 indicated the numbers of fungi and bacteria in the colon. As for the numbers of fungi in the colon, the two treatments T_2 and T_3 differed significantly ($p< 0.05$) from the control T_1, as the general average of the numbers of colon fungi for the treatments T_1, T_2 and T_3, respectively, was 0.64 × 10^7, 4.83 × 10^6 and 2.13 × 10^6. The results of the same table with the T_2 and T_3 treatments showed a significant decrease in the numbers of *Staphylococcus* bacteria, which were recorded at 4.80 × 10^6 and 2.66 × 10^6, respectively, compared to the control treatment T_1, which recorded 2.26 × 10^7. The two treatments using chamomile flower powder as T_2 and T_3 showed a significant decrease in *E. coli* bacteria and recorded 4.8 × 10^5 and 3.01 × 10^5, respectively, compared to the control treatment T_1, which was 1.89 × 10^6. These results were consistent with the results of Aggag and Yousef (1972) [30], who emphasized the role of compounds, including α-bisabolol, against *Staphylococcus*, in addition to what many

researchers found, Szalonti and colleagues (1976) [31] and Szalonti and colleagues (1977) [32], about the effectiveness of the chamomile plant against fungi. Studies conducted by Der Marderasion and Liberti, 1988 [28] also indicated the role of α-bisabolol as an anti-bacterial and anti-fungal. The role of another effective compound in the chamomile plant is Chamazulene against pathogenic microbes (Kedzia, 1991) [29] and perhaps Because of everything mentioned above, the role of chamomile was clear in its effect on harmful microorganisms in the digestive tract, which was reflected in reducing the chance of contracting the disease and the absence of deaths during the experiment, which improved the productive characteristics and qualitative characteristics of Japanese quail eggs.

Table 5: Effect of chamomile flower powder in the diets of quails on the number of microorganisms in the colon within (6-10) weeks (Mean ± Standard Error)

Group	Numbers of bacteria and fungi/bacterial cell/1 gm		
	Fungi	***Staphylococcus***	***E. coli***
T_1	0.64a ±2.11 × 10^7	2.26 a ±87.43 × 10^7	1.89 a ±0.56 × 10^6
T_2	4.83 a ± 2.66× 10^6	4.80 b± 2.44 × 10^6	4.8 b ±4.61 × 10^5
T_3	2.13b ±5.33 × 10^6	2.66 b ±7.06 × 10^6	3.01 b ±7.26 × 10^5
Sig. 0.05	*	*	*

Small letters referred to significant difference among groups at (p≤0.05). N.S referred to no significant difference. * referred to significant difference, Treatments: T_1, the control treatment without the addition of chamomile flower powder, T_2 and T_3, the treatments with the addition of chamomile flower powder at levels of 0.1 and 0.3%, respectively.

3.3 Effect of chamomile flower powder on Quality traits of eggs within (6-10) weeks: The results of Table 6 indicated a significant increase in the following traits: egg shell weight, Albumin weight, and Yolk weight for treatments T_2 and T_3, which recorded a significant increase in the egg shell weight trait of 1.42 and 1.54, respectively, compared to the control treatment T_1, which recorded the lowest rate of 1.30. As for the Albumin weight traits, it was 6.90 and 7.88 for the T_2 and T_3 treatments compared to the control treatment T_1, which recorded 5.50, while the yolk weight traits for the T_2 and T_3 treatments was 4.40 and 4.97, while the control treatment T_1 was 3.70, and the rest of the traits did not record any significant differences for all treatments. The reason may be due to what Al-Hammu (2003) indicated that chamomile has a role in enhancing the hormone thyroxine in increasing rates of nutritional metabolism and vital reactions in the body, and this was reflected in the improvement in the weight of the egg shell for the T_2 and T_3 treatments compared to the control treatment T_1 through increased Ca^{+2} representation by the chicken, thus providing the necessary calcium for the formation of the egg shell.

Table 6: Effect of chamomile flower powder in the diets of quails to the quality traits of eggs within (10) weeks (Mean ± Standard Error)

Group	T_1	T_2	T_3	Sig. 0.05
Egg Shell Weight	1.30±0.01^c	1.42±0.03^b	1.54±0.04^a	*
Egg Shell Thickness (mm)	2.30±0.01	2.52±0.25	2.49±0.02	N.S
Albumin Weight	5.50±0.14^c	6.90±0.15^b	7.88±0.20^a	*
Albumin Height	3.82±0.18	3.90±0.17	3.90±0.37	N.S
Albumin Diameter	53.00±5.00	54.66±5.00	58.00±5.00	N.S
Yolk Height	13.13±0.54	13.16±0.01	13.25±0.50	N.S
Yolk Weight	3.70±0.04^c	4.40±0.06^b	4.97±0.05^a	*
Yolk Diameter	24.12±1.70	24.00±1.74	25.20±1.70	N.S
Yolk Index	0.51±0.00	0.50±0.03	52.21±0.04	N.S
Hough Unit	85.13±0.51	85.19±1.12	85.30±1.30	N.S

Small letters referred to significant difference among groups at (p≤0.05). N.S referred to no significant difference. * referred to significant difference, Treatments: T_1, the control treatment without the addition of chamomile flower powder, T_2 and T_3, the treatments with the addition of chamomile flower powder at levels of 0.1 and 0.3%, respectively.

Conclusion

Chamomile extract can improve growth performance and Egg quality of Japanese quail.

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