Research Article

Effects of Chamomile Aqueous Extract on Productive Performance, Egg Quality, and Serum Biochemical Parameters in Laying Japanese Quails

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Abstract | Chamomile, *Matricaria chamomilla* L., is one of the most popular medicinal plants known for its antibacterial, anti-inflammatory, and antioxidant properties. This study was conducted to determine the effect of chamomile aqueous extract on the productivity, egg quality, and serum biochemical parameters of laying Japanese quail. A total of 42 Japanese quail were separated into two groups of 21 birds each, with three replicates per group (7 birds) at random; the control group without any additives and the supplemented group with chamomile aqueous extract (3ml/L) in the drinking water from 2 weeks to 13 week at the end of expermint. The total phenols, flavonoids, , and antioxidant potential of chamomile extract were all assayed. The results showed that the chamomile aqueous extract is rich in phenolic(108.92 µg/ml) and flavonoid (66.41 µmol/ml) compounds. Additionally, chamomile extract is a potent antioxidant(1.77 ng/ml). Laying quails supplemented with chamomile aqueous extract showed increased body weight and egg mass, as well as a lowered feed conversion ratio. Chamomile extract significantly increased serum levels of total protein and albumin while decreasing glucose level compared to the control group. Eggs of Japanese quail supplemented with chamomile extract showed a higher egg weight, egg length, albumen weight, albumen length, and yolk index. It could be concluded that the chamomile aqueous extract can positively affect the egg quality and productivity of laying Japanese quail.

Keywords | Chamomile, Japanese quail, Flavonoid, Antioxidant capacity, Egg traits, Productivity

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INTRODUCTION

Medicinal plants have been appliedas an alternative to antibiotics because of the negative effects that antibiotics have on both the health of humans and birds (El-Galil et al., 2010). Contrary to antibiotics, the majority of medicinal plants' active ingredients are quickly absorbed and have a shorter half-life. Therefore, they have no residues in the animal tissue (Srivastava and Gupta, 2009). Several studies on employing natural growth promoters from medicinal herbs to increase quail productivity have been conducted (Nowaczewski et al., 2010). The Asteraceae family includes the well-known medicinal plant species *Matricaria chamomilla* L. (chamomile), which is frequently referred to as the star among the medicinal species. Anglo-Saxons believed that this herb was one of the nine sacred plants that the Lord had given to humans (Crellin et al., 1990; Ismael and Nasralla, 2013). Ancient Egypt, Greece, and Rome were all familiar with the usage of chamomile in herbal remedies for thousands of years (Issac, 1989). Chamomile is represented by two varieties: German chamomile (*Chamomilla recutita*, synonymous with *Matricaria chamomilla*) and Roman chamomile (*Chamaemelum nobile*). More than 120 different chemical components can be found in chamomile flowers. Azulene, apigenin, chamazulene, bisabolol, bisabololoxide, cis-enynedicycloether, and chamazulene are among the most effective chamomile constituents (Srivastava and Gupta, 2009).

Chamomile has been shown to have anti-inflammatory, antioxidant, sedative, wound-healing, antibacterial, and antimycotic properties (Pirzad et al., 2006; Singh et al., 2011; Srivastava et al., 2010; Khishtan and Beski, 2020). The antibacterial, antifungal, antioxidant, and anti-inflammatory qualities of chamomile may boost body weight and feed conversion (Santurio et al., 2007), the sesquiterpenoid chemicals in chamomile may work like antibiotics by impairing the bacterial cell membrane's normal barrier function, allowing exogenous solutes to permeate the cell (Göger et al., 2018). Chamomile can donate protons and may operate as an antioxidant by scavenging or inhibiting free radicals (Shimada et al., 1992; Osman et al., 2016). Chamomile has anti-inflammatory properties by inhibiting the release of prostaglandin E2 (Srivastava et al., 2009).

In laying birds, the digestive system biochemically converts the diet into an egg mass. Therefore, having a healthy digestive tract may help laying birds produce better. The flavonoids, essential oils, and chamazulene found in chamomile flowers operate in the same way as probiotics in the small intestine (McCrea et al., 2005). Their effects may help maintain the intestine's normal microbiota and improve the digestibility of nutrients. Therefore, the purpose of this study was to determine how chamomile affects the Japanese quail's productivity, egg quality, and serum biochemical parameters.

MATERIALS AND METHODS

Extract preparation

Dry chamomile flowers (*M. chamomilla*) were purchased from local herb store in Moscow, Russia. Chamomile flowers weighing 5 grams plus 95 ml of boiled distilled water were used to make the extract in a flask. After 12 h, the suspension was filtered through filter paperand was freshly used (Ibrahim and Butris, 2008; Tousson, 2019).

Estimation of total flavonoids, total phenols, and antioxidant capacity of the chamomile extract

Total phenolic content

Phenolic compounds colorimetric assay kit (Sigma-Aldrich) was used to determine the total phenols of the chamomile extract using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Folin-Ciocalteu phenol reagents (1.0 mL) and deionized water (10 mL) were combined with 1 mL of extract or standard solution to determine the total phenolic content. Sodium carbonate 20% (2.0 mL) was added to the mixture after 5 minutes and kept in total darkness for 1 hour. At 750 nm, the absorbance was measured against gallic acid as a standard (Slinkard and Singleton, 1977).

Total flavonoid concentration

Plant flavonoids colorimetric assay kit (Assaygenie) was used to determine the total flavonoids in the chamomile extract using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Using a technique developed by Park et al. (2008), the total flavonoid content was calculated by combining 0.3 ml of the extract with 0.15 ml of $NaNO_2$, 0.15 ml of $AlCl_3.6H_2O$, and 3.4 ml of 30% methanol. NaOH (1 ml) was added after 5 minutes. At 506 nm, the absorbance was measured against a known catechin concentration as a standard.

Total antioxidant capacity

Total Antioxidant Capacity (TAC) Assay Kit (OxiSelect) was used to determine the total antioxidant capacity of the extract using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). The cupric ion reducing capacity assay (CUPRAC) was used to determine the total antioxidant capacity. 1 ml neocuproine, 1 ml cupric chloride, and 1 ml ammonium acetate buffer were added to test tubes containing 2 ml of distilled water. The extract was added to the mixture, incubated for half an hour at room temperature, and measured at 450 nm (Apak et al., 2004).

Birds

A total of forty two (21days-old) unsexed Japanese quail chicks (Coturnix japonica) were obtained from Animal Research Farm, RUDN University, with initial body weight of 128.56±1.75g were used in this study. Birds were randomly divided into two groups (n= 21), and each group included three replicates (n= 7) and were placed into galvanized wire cages (95×65×160 cm) and reared up to day 91 of age.

Experimental design

The experimental groups were as follow: (1) control group; fed basal diet and without any additive in drinking water (2) supplemented group; fed basal diet and received chamomile aqueous extract, (3ml/L) in drinking water at 2 to 8 weeks of age. Quail chicks were housed in battery cages rather than litter floor. Water and the commercial pelleted feed were provided without restriction throughout the experiment (2-13 weeks of age). During the laying period, a lighting plan of 16 hours per day was used. The temperature and relative humidity were roughly 24 °C and 70%, respectively. Table 1 displays the composition of the feed. The current experiment was conducted at the RUDN University's Animal Research Farm in Moscow, Russia). During the experiment, body weight (BW) and feed intake (FI) were recorded at aged (8 weeks and 13 weeks). All experimental procedures of this study were conducted according the guidelines of the Local Experimental Animal Care Committee and the ethics of the institutional committee of Department of Pharmacology, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

Table 1: Composition of the diets provided to laying Japanese quails.

Nutrient	Unit	Value
Metabolic energy	kcal/100g	285
Crude protein	%	20
Fat	%	3.5
Fibers	%	5.1
Linoleic acid	%	1.8
Lysine	%	1.05
Methionine	%	0.50
Methionine + cystine	%	0.72
Tryptophan	%	0.2
Threonine	%	0.55
Calcium	%	3.3
Sodium	%	0.16
P	%	0.8
Available P	%	0.75
NaCl	%	0.33

(p) phosphorus

Productive performance parameters

Egg mass was estimated at 13 weeks aged by multiplying average egg weight by hen-day egg production: Hen day egg production = (total number of eggs produced during the period/ total number of hen-days in the same period) x 100.

Egg quality parameters

External egg quality parameters

The shape index (%) was calculated by using the external egg dimensions as SI=d/D*100, where d represents the small diameter of the egg (mm) and D represents the large diameter of the egg.The shell surface area (cm^2) was obtained using the formula: $S=4.835*W^{0.662}$, where W represents egg weight. The egg yolks were removed and weighed, and the egg shells were cleaned and let dry for 24 hours at room temperature. The shells were then weighed. Egg weight - (yolk weight + shell weight) was used to compute albumen weight. A digital balance (HR 200, Japan), a digital caliper (Xiaomi DUKA CA2, China), and a micrometer (ACCUD, China) were used to assess the egg quality parameters.

Internal egg quality parameters

The heights of the albumen and yolk were determined using a micrometer. The formula used to calculate the Haugh unit is as follows: $HU=Log [H+7.57 - (1.7\times W^{0.37})]$ where H is the albumen's height and W is the weight of the quail egg. The internal quality unit (IQU) of the albumen was determined using the following formula: $IQU =100*\log(h+4.18-0.8989*W^{0.6674})$, where h is the thickness of the albumen and W is the weight of the egg (Kondaiah et al., 1983). For albumen quality evaluation, the following traits were calculated: Albumen index: AI = h/ (0.5*(d+D))*100, where h is the thick albumen's height, d is its small diameter, and D is its large diameter. Egg yolk quality was determined using the yolk index: YI = h/D*100, where h stands for yolk height and D for yolk diameter (Kondaiah et al., 1983).

Serum biochemical parameters

At the end of the experiment (13th week), blood samples were freshly collected in test tubes and left for clotting before being centrifuged at 2000×G for 10 min to separate serum for the biochemical assay of alanine aminotransferase (ALT), urea,creatinine, protein, glucose, total bilirubin, and direct bilirubin. These parameters were spectrophotometrically assayed by using an automatic biochemical analyzer Miura, Italy at the veterinary clinic, RUDN Universityusing reagents from Vector-Best company. The calculation is made by the photometric method based on El-Deen et al. (2009) and Inaotombi et al. (2018).

Statistical analysis

The obtained digital data were subjected to statistical processing using the Excel computer program (Microsoft ®, USA). The significance of differences between control and extract-supplemented quails was evaluated by the Student's *t*-test. The differences were considered significant if the probability value did not exceed 5% ($p\leq0.05$).

RESULTS AND DISCUSSION

Total flavonoids, phenols, and antioxidant capacity of chamomile extract

Regarding to this findings, chamomile aqueous extract is rich in phenolic and flavonoid compounds. Additionally, chamomile extract is a potent antioxidant. The results showed that the chamomile aqueous extract total phenolic content, total flavonoid content and the total antioxidant capacity were found to be 108.92 µg/ml, 66.41 µmol/ml, and the 1.77 ng/ml, respectively (Table 2).

Links Researchers

Table 2: Total flavonoids, phenols, and antioxidant activity of chamomile extract.

Type of extract	Part of plant	Total phenols µg/ml	Total flavonoids µmol/ml	Antioxidant activity µg/ml
Aqueous	Flowers	108.92	66.41	1.77

Table 3: Effect of chamomile extract on the productive performance indices of laying Japanese quails.

Age	Parameter	Control	Supplemented	p-value
8 weeks	body weight (g)	175.57±11.95	189.33±10.45*	0.031
13 weeks	Final body weight (g)	244.66±7.83	255.5±6.5	0.120
13 weeks	Feed Intake (g/h/d)	35.98±1.1	34.35±.7	0.233
13 weeks	Egg mass (g/h/d)	8.72±0.5	9.19±0.57	0.142
13 weeks	FCR (g feed/g egg)	4.12±0.07	3.73±0.06*	0.041

Values are represented as the mean ± SE (n = 21 per group). Mean values within a column with (*) were significantly different (P < 0.05). (FCR) feed conversion ratio.

Effect of chamomile extract on productive performance:

The impact of chamomile on quail performance differs according to the chamomile composition and the extract processing methods. Our findings demonstrated that laying quails supplemented with chamomile extract showed increased body weight at 8 weeks of age in comparison to the control group. Additionally, compared to the control group, chamomile-supplemented quails displayed increased egg mass and a lower feed conversion ratio (Table 3).

Effect of chamomile extract on egg quality of laying Japanese quail

According to our research, supplementing Japanese quail with chamomile extract had an impact on both external and internal egg quality indices. Eggs from quail supplemented with chamomile extract had a higher egg weight and egg length than eggs from the control group (Table 4). Moreover, the eggs of chamomile-treated quails had greater albumen weight, albumen length, and yolk index (Table 5).

Table 4: Effect of chamomile extract on the external egg quality traits in laying Japanese quails.

Parameter	Control	Supplemented	p-value
Egg weight (g)	11.64±0.211	12.02 ±0.210	0.434
Egg length (mm)	32.18±0.317	33.22 ± 0.247*	0.011*
Egg Width (mm)	25.33±0.190	25.41 ± 0.153	0.322
Shell weight (g)	1.02±0.02	0.98±0.03	0.156
Shape Index%	78.94±0.52	76.48±0.26*	0.043*
Shell surface area (cm^2)	24.53±0.29	25.08±0.28	0.411

(*) denotes significant differences between the control and Supplemented group at p≤0.05.

Effect of chamomile extract on serum biochemical parameters

By providing details on the physical health of the bird, serum biochemistry values can assist in differentiating between healthy, normal birds and those who are abnormal or diseased. The chamomile aqueous extract group had significantly increases in total protein and albumin levels while lowering glucose levels as compared with a control group.The level of cytoplasmic enzyme alanine aminotransferase (ALT), showed no changes between the two groups. Total bilirubin, direct bilirubin and creatinine did not exhibit any variations (Table 6).

Table 5: Effect of chamomile extract on the internal egg quality traits in laying Japanese quails.

Parameter	Control	Supplemented	p-value
Albumen weight (g)	7.02±0.16	7.35±0.12*	0.013*
Yolk weight (g)	3.54±0.07	3.46±0.11	0.244
Albumen length (mm)	48.49 ± 0.686	52.36 ± 0.791*	0.033*
Albumen Width (mm)	36.00 ± 0.662	36.65 ± 0.244	0.521
Yolk Width (mm)	23.99 ± 0.158	23.64 ± 0.221	0.462
Albumen height (mm)	4.54 ± 0.183	4.67 ± 0.146	0.356
Yolk height (mm)	10.92 ± 0.086	11.17 ± 0.163	0.125
Albumen Index	10.61±0.46	10.52±0.36	0.371
Yolk Index	45.62±0.42	47.36±0.96*	0.023*
Haugh Unit	89.33±0.92	90.07±0.89	0.490
IQU	60.33±1.71	61.02±1.99	0.112

IQU: Internal quality units of the albumen. * denotes significant differences between the control and supplemented group at p≤0.05.

Table 6: Effect of chamomile extract on the biochemical parameters of laying Japanese quails.

Parameter	Control	Supplemented	p-value
ALT (U/L)	7.25 ±1.35	7.5 ±0.8	0.266
Urea (mmol/L)	5.6±0.1	5.25±0.25	0.155
Creatinine (mg/dl)	0.91±0.13	0.93±0.02	0.344
Total protein (g/dl)	3.56±0.14	4.66±0.24*	0.022*
Albumin (g/dl)	1.41±0.05	1.70±0.06*	0.027*
Globulin (g/dl)	2.77±0.39	2.90±0.18	0.254
A/G ratio	0.50±0.05	0.58±0.05*	0.023*
Glucose (mg/dl)	286±11.6	241±9.27*	0.037*
Total bilirubin (µmol/L)	1.1±0.1	1.6±0.2	0.314
Direct bilirubin(µmol/L)	0.2±0.02	0.35±0.05	0.215

* denotes significant differences between the control and Supplemented group at p≤0.05. ALT: alanine aminotransferase (A/G ratio) albumin/globulin ratio.

Chamomile is one of the nine sacred plants that the Lord had given to humans (Crellin et al., 1990; Ismael and Nasralla, 2013). A total of 120 secondary metabolites, including 36 flavonoids and 28 terpenoids, have been found in chamomile, contributing to its therapeutic benefits (Velíšek et al., 2009). Free radicals such as peroxide radical (ROO), hydroxyl radical (OH), superoxide radical (O_2^-), and nitric oxide radical (·NO), are the primary cause of many clinical diseases (Aruoma, 1998). Chamomile is well-known for its antioxidant properties (Franke and Schilcher, 2005; Srivastava et al., 2010). The phenolic and flavonoid compounds found in medicinal plants have potent antioxidant activity. These natural antioxidants play an important role in preventing free radical damage. Al Chlabi and Abdul-Rahman (2018) found that chamomile treatment improved the antioxidant status of quails. The antioxidant qualities of chamomile can be credited with improvements in reproductive parameters (McKay and Blumberg, 2006). Sotiropoulou et al. (2020) found a significant correlation between chamomile's total phenolic content and antioxidant activity. According to our findings, aqueous chamomile extract is rich in phenolic and flavonoid compounds. Additionally, chamomile extract is a potent antioxidant. The extract has a total phenolic content of 108.92 μg/ml, a flavonoid content of 66.41 μmol/ml, and an antioxidant capacity of 1.77 ng/ml.

The impact of chamomile on quail performance differs according to the chamomile composition and the extract processing methods.

Due to its antibacterial, antifungal, and anti-inflammatory effects, chamomile extract may enhance performance (Abaza et al., 2003), or depending on the tannins concentration, it may reduce feed intake and conversion (Dada et al., 2015). In this study's findings demonstrated the increased egg mass and a lower feed conversion ratio in a chamomile-supplemented group as compared to the control group. These findings support the findings of El-Galil et al. (2010), who found that increasing the levels of chamomile flower in the experimental meals for laying Japanese quail boosted live body weight and egg production. Abu Taleb et al. (2008) stated that chamomile supplementation enhanced ovarian relative weight, carcass weight, and growth rate. The increase in feed consumption, the improvement in nutritional digestibility of diets, and the earlier sexual maturation of birds receiving chamomile may be responsible for the beneficial effect of chamomile on quail productivity. McCrea et al. (2005), the flavonoids, essential oils, and chamazulene present in chamomile flowers operate in the same way as probiotics in the small intestine by acting as antibacterial, antifungal, and anti-inflammatory agents. Their effects might enhance nutrient absorption and preserve the intestine's normal microbiota. The active compounds in chamomile flowers may inhibit the excessive growth of a harmful intestinal microorganism (Kolacz et al., 1997). Chamomile's antibacterial, antifungal, and anti-inflammatory properties may boost productive performance (Abaza et al., 2003). Additionally, chamomile increases the thyroxin hormone's activity, which speeds up the metabolism of nutrients and increases body weight (Al-hamo, 2003). However, Tenório et al. (2017) reported that, the chamomile extract had no impact on the birds' performance.

Eggs from quail supplemented with chamomile extract had a higher egg weight and egg length than eggs from the control group. Moreover, the eggs of chamomile-treated quails had greater albumen weight, albumen length, and yolk index as compared with eggs of a control group. Since eggs are a plentiful and cheap source of animal protein, chamomile can boost the egg's nutritional value by increasing the weight of the albumen and the egg. The increased feed intake, improved nutritional digestibility of diets, and increased nutrient absorption in birds receiving chamomile may be responsible for the beneficial effect of chamomile on egg quality indices. So, this study was in line with the findings of Haddad (2012), who found that adding chamomile flower powder to the diets of commercial layers resulted in a significant increase in Haugh unit, albumen index, yolk index, albumen height, and yolk height. Also, Giannenas et al. (2021) found that the chamomile-supplemented birds eggs showed increased shell thickness and strength. Furthermore, eggs had higher tyrosine and phenylalanine content, as well as higher yolk oxidation resistance. According to many studies, albumen length and egg length increased as egg weight did (Alkan et al., 2015; Ukwu, 2017).

By providing details on the physical health of the bird, serum biochemistry values can assist in differentiating between healthy, normal birds and those who are abnormal or diseased (Fudge, 1997). Abnormal levels of blood biochemical parameters; ALT, urea, creatinine, albumin, and bilirubin maybe seen with liver or kidney disease, metabolic disorders, or nutritional problems. The chamomie- supplemented group was observed a significant increase in total protein and albumin serum levels while lowering glucose levels. Total bilirubin, direct bilirubin, creatinine, and ALT did not exhibit any variations as compared to the control group. These results supported those of El-Galil et al. (2010), who investigated how serum biochemical parameters in laying Japanese quail responded to the addition of chamomile powder to the feed. The levels of glucose, albumin, creatinine, ALT, and aspartate aminotransferase (AST) were not significantly different. However, chamomile increased total protein and globulin concentrations while decreasing cholesterol concentration. Abu Taleb et al. (2008), observed that, Japanese quail's total protein and globulin levels were significantly increased and their cholesterol levels were decreased when chamomile was added to their meal at a 0.3% concentration.Chamomile improves the nutritional value of poultry meat (as chamomile significantly increases protein levels while loweringglucose levels). Cemek et al. (2008) showed that chamomile had the a protective effect on pancreatic cells because it reduces the oxidative stress caused by hyperglycemia.

CONCLUSIONS AND RECOMMENDATIONS

Chamomile extract is rich in phenolic and flavonoid compounds with a potent antioxidant capacity. Furthermore, aqueous chamomile extract can positively affect the productive performance and egg quality (egg mass, egg weight, egg length, albumen weight, albumen length, and yolk index) of laying Japanese quail. Chamomile improves the nutritional value of quail meat by increasing protein levels while lowering glucose levels. Chamomile-supplemented quail meat will be more beneficial for those who suffer from diabetes and atherosclerotic cardiovascular disease.

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NOVELTY STATEMENT

The findings of this study provide novel insights into the potential of chamomile extract as a natural feed additive for improving the productivity and egg quality of laying Japanese quail. The study highlights the antioxidant properties of chamomile extract, as well as its ability to increase protein levels and lower glucose levels in quail meat. These results suggest that chamomile supplementation could be a valuable strategy for producing more nutritious and healthier quail meat, particularly for individuals with diabetes and atherosclerotic cardiovascular disease. Overall, this study emphasizes the importance of exploring natural compounds as a means to improve the productivity and nutritional value of poultry.

AUTHOR'S CONTRIBUTION

Sahar Ezeldien and Foromo Dramou contributed equally to the conceptualization, design, and execution of the experiment, as well as the analysis and interpretation of the data. Fatma M. Youssef supervised the study and provided critical guidance and feedback throughout the research process. AA Nikishov and SB Seleznev provided technical support and assistance in conducting the experiment. All authors contributed to the writing and revision of the manuscript and approved the final version for submission.

Conflict of interest

The authors have declared no conflict of interest.

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