

Original Article

Effect of Chamomile, Wild Mint and Oregano Herbal Extracts on Quality and Quantity of Eggs, Hatchability, and Some Other Parameters in Laying Japanese Quails

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Abstract

According to various reports of beneficial effects of medicinal plants on the performance of broiler chickens and less extensively studies in laying poultries, this study was conducted to find an appropriate and harmless feed additive to enhance the quality and quantity of poultry eggs. The effect of three herbal extracts on quantity and quality of eggs, blood parameters, hatchability, intestinal bacterial population, and intestinal morphology in laying Japanese quail were investigated. The study was applied with 64, ten-week old laying Japanese quails for 8 weeks. The experiment was a completely randomized design with 4 treatments, 4 replications and 4 birds per replicate (the ratio of male to female 1:3). Experimental treatments involved: Control, with no additive in drinking water; chamomile extract; wild mint extract; and oregano extract. Herbal extracts were added 1 mL/L drinking water. The three treatments showed no significant effect on productivity, egg mass, FCR, egg weight, feed intake and qualitative indices of eggs; however, the herbal extracts specially the chamomile extract reduced the cholesterol of eggs ($P<0.05$). Herbal extracts showed no significantly effects on the hatchability of fertile eggs. Oregano extract showed the best effect on reduction of the intestinal bacterial population and increase the villus height in ileum ($P<0.05$). Chamomile extract by reducing the yolk cholesterol can improve the egg market's popularity, and oregano extract by reducing the number of pathogenic bacteria and improving the villus height in ileum can be considered as a beneficial and low-risk additive for laying poultries.

Keywords: Herbal Extract, Yolk Cholesterol, Egg mass, FCR, Egg weight

Introduction

In birds, the digestive tract biochemically converts the feed into an egg mass. Therefore, a healthy digestive system can improve the performance of the laying poultries. To reach this target, manipulation of intestinal microflora to achieve optimal microflora can be affective. A wide range of herbal plants and their product are identified as medicine to exert useful effects within the digestive tract [1]. *In vivo* anticoccidial and antibacterial effects of medicinal plants has been indicated in broiler chickens [2-5]. In addition to the antimicrobial properties, stimulation of digestive secretion, bile, mucus, and increased enzyme activity is identified for herbal feed additives [6-9].

More than 120 constituents have been recognized in chamomile flowers that have potential pharmacological activities [10]. Apigenin, chamazulene, (-)-bisabolol, bisabololoxide, cis & trans-spiroether, cis-enynedicycloether, and azulene are among the most effective compounds in chamomile which was reported for their anti-inflammatory, antioxidative, sedative, wound healing, antibacterial, and antimycotic effects [10-13]. It has been reported that adding 400 mg chamomile/kg diet in broiler chickens improved final live body weight, weight gain and feed conversion [14].

Several pharmacological properties such as antimicrobial activity, effect on gastrointestinal and nervous system have been reported for *Mentha*

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longifolia (L.) Huds. These properties of wild mint are due to the presence of Pulegone, the main compound of this plant, which is responsible for most of its pharmacological effects. Also menthone, isomenthone, menthol, 1, 8-cineole, borneol, and piperitenone have a medicinal properties in wild mint [15-19]. It has been indicated adding wild mint at the rate 15 g/kg of feed improved mean body weight, dressing percentage, gross return, heart weight, and antibodies titer against Infectious Bronchitis in broiler chickens [20].

Diuretic, stimulant, antimicrobial, anti-inflammatory, and anticancer activities of oregano have been attributed to compounds including carvacrol, thymol, rosmarinic acid, borneol, organol, ursolic acid, monoterpene hydrocarbons (limonene, terpinene, ocimene, caryophyllene, -bisabolene and p-cymene) and monoterpene alcohols (linalool, 4-terpineol) [21-24]. Inclusion of 600 and 1200 mg/kg of oregano essential oil in diet increased body weight, improved FCR (Feed Conversion Ratio), decreased populations of cecal *Escherichia coli*, and raised antioxidant activity of serum in broiler chickens [25].

According to various reports of beneficial effects of medicinal plants on the performance of broiler chickens and less extensively studies in laying poultry, in this study the effect of three herbal extracts on quantity and quality of eggs and other productive characteristics in laying Japanese quail were investigated.

Material and Methods

The study was conducted for 8 weeks through a completely randomized design, a total of sixty-four, 10-week old laying Japanese quails were divided into 4 treatments, 4 replication groups with 4 quails in each replication (male to female ratio of 1:3). Treatments included chamomile (*Matricaria Chamomilla* L.), wild mint [*Mentha longifolia* (L.) Huds.] and oregano (*Origanum vulgare* L.) extracts. The herbal extracts were purchased from Exir-e Gol-e Sorkh Co. (Mashhad, Iran). Brix of wild mint, chamomile and oregano extracts were 6, 9 and 9, respectively which as recommended by the manufacturer were added to the birds' drinking water in the ratio of 1:1000. The control group had no additive in their drinking water. All the groups had the same feed formulation based on NRC (National Research Council) 1994. The birds had

free access to water and feed throughout the experiment. The ingredients and their amounts in this ration are presented in Table 1. The lighting program was kept at 16 Light/8 Darkness during the experiment.

Egg production was recorded daily, and the feed intake was measured at the end of each week by subtracting the amount of the remaining feed from the amount of feed distribution. The weight of eggs was measured on a daily basis using a digital scale with error of ± 0.01 g. Egg mass was calculated by multiplying hen average egg weight and day egg production.

Qualitative properties of eggs including weight, shell thickness, yolk color, shell strength, Haugh unit and yolk weight were measured in the last week. Yolk of the eggs was separated and weighed, and the egg shells were washed, cleared and incubated at room temperature for 24 hours to dry. Then, the shells were weighed using the digital scale with ± 0.01 g error. Shell thickness was gauged using a micrometer at three points in the center of shells, and the average of the measured values was considered as the thicknesses of the shell [26]. The height of albumen was measured using the micrometer and the Haugh unit was calculated using the following formula:

$$HU = \text{Log} [H + 7.57 - (1.7 \times W^{0.37})]$$

Where H is the height of albumen and W is weight of the quail egg. The yolk color was assessed using the DSM (Royal DSM is a purpose-led global science-based company in Nutrition, Health, and Sustainable Living.) index of the yolk color. The shell strength was evaluated using the so-called Eggshell Force Gauge through the method of Er *et al.* (2007) [27].

For determination of egg yolk cholesterol concentration, 1 g of pooled yolks of each replication was added to 9 mL of 2% NaCl solution. Samples were shaken for 2 hours by an electrical shaker. Then, 1 mL of the diluted yolk was re-diluted 10 times. In this study, 10 μL of this sample was mixed with 100 μL of salt solution and 1 mL of the enzymatic reagent. The same procedure was also implemented for the standard of cholesterol. As the blank sample, 10 μL of deionized water was used instead of sample or standard of cholesterol. The samples were incubated in a water bath at 37 °C for 15 minutes and then the light absorbance at wavelength of 500 nm was read [28].

At the end of the course of experiment, a quail was selected from each experimental unit and 1 mL blood was taken from its wing vein. Blood samples were centrifuged in the lab at 4000 RPM (Revolutions Per Minute) for 5 min. Concentrations of total protein, albumin, cholesterol, triglycerides and HDL (High Density Lipoprotein) in blood serum samples and egg yolk cholesterol were measured using commercial diagnostic kits (Zistshimi Co.) and a spectrophotometer (Jenway Genova MK3, UK).

The eggs were collected, ranked and those inappropriate for hatching were removed from the collection. Then the eggs were transferred to clean room, disinfected for 20 min by adding 25 g potassium permanganate crystals to 35 mL of 40% formaldehyde per cubic meter, and kept at 10-15 °C. At the end of the experiment, 30 eggs from each cage, i.e. 120 eggs per treatment were put into incubator and the hatchability was calculated.

To assess the microbial population, a bird from each experimental unit was selected and sacrificed at the end of experiment. The contents of cecum were collected in sterile dishes. Collected samples were immediately put on ice, transferred to the lab and prepared for microbial culture. To measure the microbial population, one gram of cecal contents were serially diluted and 10 µL of each dilution was spot on each plate containing plate count agar, KF Streptococcus agar and MacConkey agar media to count total aerobes, Streptococci and *Escherichia coli* respectively. After incubation for 24 hours at 37 °C, the bacteria were counted in petri dishes and the number of bacteria in the initial volume was calculated using the following formula:

Number of bacteria=Number of colonies×(1/Dilution factor)×Cultured volume. Then, the logarithms to base 10 of the obtained values were used in CFU/g (Colony Forming Unit) for later analyses.

For the morphological examination after slaughtering the bird, small intestine was removed immediately and from the middle part of three sections (duodenum, jejunum, ileum) the fragments were separated by 1 to 2 cm. The separated segments were washed with PBS (Phosphate Buffered Saline) and put on the plastic dishes involved with 10% neutral buffered formalin. Paraffin wax technique was used for preparing the

thin tissue slides. A rotary type microtome (Erma, Japan) was used for cutting the paraffin sections. The sections cut at a thickness of 6 µm and their wrinkles were smoothed out with warm water (45 °C) and put on slides. The slides after paraffin removal and dewatering were kept in a solution containing 5 g/L Periodic Acid-Schiff for 15 min and stained with hematoxylin-eosin [29]. The morphometric variables measured included villi height, crypt depth, and villi width at the top and the base. To measure the villus height and villus width, microscope (Carl ZEISS standard 20, Oberkochen, Germany) of 40× magnification and for crypt depth 100× magnifications were used and one of the ocular lenses of microscope was equipped with a graticule [30]. Finally, note values based on calibration were converted to mm by using millimeter slides. The mean from 15 villi per sample was used as the average value for further analysis.

The data obtained through the experiment were analyzed using the GLM procedure in SAS 9.1 software [31] and means of experimental groups were compared using Duncan's multiple-range test at 5% level of significance. The following statistical model was used:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} is the mean of the j th observation of the i th treatment; μ is the sample mean; T_i is the effect of the i th treatment; and e_{ij} is the effect of error.

Table 1 Nutrient content of diets of laying Japanese quails

| Calculated nutrient contents | |
|------------------------------|------|
| ME (kcal/kg) | 2900 |
| Crude protein (%) | 18 |
| Fat (%) | 7 |
| Fiber (%) | 5 |
| DL-Methionine (%) | 0.47 |
| L-Lysine (%) | 1.08 |
| Methionine+Cysteine (%) | 0.8 |
| Calcium (%) | 2.5 |
| Available phosphorus (%) | 0.06 |

ME: Metabolizable Energy

Table 2 Effect of three herbal extracts on the quantitative performance indices of laying Japanese quails

| Treatments | Egg weight (g) | Egg mass (g) | FCR | Feed intake (g hen ⁻¹ day ⁻¹) | Hen day egg production (%) |
|------------|----------------|--------------|------|--|----------------------------|
| Chamomile | 11.47 | 7.90 | 4.45 | 34.35 | 67.76 |
| Wild Mint | 11.83 | 8.19 | 4.67 | 34.96 | 69.81 |
| Oregano | 12.16 | 8.53 | 4.71 | 32.95 | 70.05 |
| Control | 11.47 | 7.93 | 4.29 | 33.35 | 68.81 |
| P Value | 0.11 | 0.42 | 0.28 | 0.39 | 0.89 |
| SEM | 0.26 | 0.21 | 0.17 | 0.25 | 1.12 |

SEM= Standard Error of the Means

FCR: Feed Conversion Ratio

Table 3 Effect of three herbal extracts on the qualitative indices of eggs and hatchability in laying Japanese quails

| Treatments | Shell weight (g) | Shell thickness (mL) | Shell strength (kg/cm ²) | Haugh unit | Yolk color (DSM) | Yolk weight (g) | Hatchability (%) |
|------------|------------------|----------------------|--------------------------------------|------------|------------------|-----------------|------------------|
| Chamomile | 0.84 | 0.213 | 0.89 | 86.32 | 5.82 | 3.84 | 74.74 |
| Wild Mint | 0.85 | 0.214 | 0.98 | 84.47 | 6.2 | 3.51 | 70.61 |
| Oregano | 0.82 | 0.233 | 1.01 | 83.83 | 6.5 | 3.64 | 71.85 |
| Control | 0.83 | 0.205 | 0.92 | 87.45 | 5.16 | 3.46 | 73.16 |
| P Value | 0.583 | 0.295 | 0.24 | 0.705 | 0.241 | 0.523 | 0.923 |
| SEM | 0.013 | 0.052 | 0.056 | 0.553 | 0.238 | 0.227 | 1.402 |

SEM= Standard Error of the Means

Table 4 Effect of three herbal extracts on egg yolk cholesterol and blood parameters of laying Japanese quails

| Treatments | Cholesterol (mg/dl) | Triglyceride (mg/dl) | HDL (mg/dl) | Albumin (g/dl) | Total protein (g/dl) | Yolk cholesterol (mg/g) |
|------------|---------------------|----------------------|-------------|----------------|----------------------|-------------------------|
| Chamomile | 151.28 c | 147.08 c | 34.07 | 2.84 | 3.35 | 11.45 c |
| Wild Mint | 170.65 bc | 186.32 bc | 34.71 | 3.12 | 3.56 | 12.22 b |
| Oregano | 185.47 ab | 222.71 ab | 38.83 | 3.18 | 3.95 | 12.77 ab |
| Control | 199.16 a | 260.33 a | 32.47 | 2.53 | 3.31 | 13.34 a |
| P Value | 0.002 | 0.017 | 0.405 | 0.298 | 0.455 | 0.001 |
| SEM | 5.51 | 14.55 | 14.55 | 0.131 | 0.152 | 0.44 |

(a,b,c) Means within a column with no common superscript differ significantly (P<0.05)

SEM= Standard Error of the Means

HDL: High Density Lipoprotein

Table 5 Effects of three herbal extracts on intestinal bacteria population of laying Japanese quails (log CFU/g)

| Treatments | Total count of aerobic bacteria | <i>Escherichia coli</i> | Streptococcus |
|------------|---------------------------------|-------------------------|---------------|
| Chamomile | 6.32 bc | 6.28 a | 5.32 ab |
| Wild Mint | 6.90 ab | 6.22 a | 5.15 ab |
| Oregano | 5.84 c | 5.30 b | 5.00 b |
| Control | 7.43 a | 6.34 a | 5.57 a |
| P Value | 0.018 | 0.044 | 0.042 |
| SEM | 0.207 | 0.159 | 0.088 |

SEM= Standard Error of the Means

(a,b,c) Means within a column with no common superscript differ significantly (P<0.05)

Table 6 Effect of three herbal extracts on intestinal morphology in laying Japanese quails (cm)

| Treatments | Duodenum | | | Jejunum | | | Ileum | | |
|------------|---------------|--------------|-------------|---------------|--------------|-------------|---------------|--------------|-------------|
| | Villus Height | Villus Width | Crypt Depth | Villus Height | Villus Width | Crypt Depth | Villus Height | Villus Width | Crypt Depth |
| Chamomile | 1.180 | 0.155 | 0.041 | 0.580 | 0.140 | 0.038 | 0.467b | 0.0131 | 0.039 |
| Wild Mint | 1.177 | 0.132 | 0.048 | 0.592 | 0.142 | 0.034 | 0.442b | 0.118 | 0.035 |
| Oregano | 1.150 | 0.143 | 0.040 | 0.562 | 0.127 | 0.042 | 0.521a | 0.119 | 0.036 |
| Control | 1.120 | 0.133 | 0.047 | 0.555 | 0.122 | 0.039 | 0.437b | 0.114 | 0.040 |
| P Value | 0.523 | 0.554 | 0.155 | 0.924 | 0.644 | 0.617 | 0.042 | 0.421 | 0.626 |
| SEM | 0.016 | 0.006 | 0.001 | 0.021 | 0.006 | 0.003 | 0.017 | 0.004 | 0.002 |

SEM= Standard Error of the Means

(a,b) Means within a column with no common superscript differ significantly (P<0.05)

Result

The effect of herbal extracts on quantitative performance of quails; including productivity, daily feed intake (g), egg mass, feed conversion ratio, and weight of eggs (g) has been reported in Table 2. The extracts showed no significant effect on quantitative indices in this experiment ($p>0.05$).

As observed in Table 3, the herbal extracts had no significant effect on shell thickness, Haugh unit, yolk weight and shell strength and also hatchability ($p>0.05$).

Effect of herbal extracts on blood parameters and the yolk cholesterol have been presented in Table 4. All treatments showed no overall effect on total protein, albumin and HDL in quails' serum ($P>0.05$). As can be seen, the herbal extracts significantly decreased the triglyceride and cholesterol of quails' serum and egg yolk cholesterol, but the effect of chamomile was more than the others ($P<0.05$).

In Table 5, the effect of herbal extracts on populations of total aerobic bacteria, Streptococci and *Escherichia coli* is reported. All three experimental groups reduced population of aerobic bacteria and streptococcus, but the effect of oregano extract was more than the others ($P<0.05$). Oregano extract reduced the *Escherichia coli* population ($P<0.05$).

In Table 6, the effect of herbal extracts on intestine morphology in laying quails has been presented. The experimental treatments did not effect on the measured parameters ($P>0.05$) in three parts of the intestine, but oregano extract increased the villus height in the ileum ($P<0.05$).

Discussion

As shown in Tables 2 and 3, the treatments have no significant effect on performance and quantitative and qualitative indices of laying Japanese quails. Abaza (2007) reported that adding 0.5% dried chamomile flower to laying hens diet did not have any effect on quality of produced eggs such as shell thickness, yolk weight, albumin weight, shell weight, and Haugh unit. Between the functional indices only feed intake was lower than control group [32] which is somewhat in line with our results. It has been reported that a basal diet supplemented with dried wild mint 15 g/kg improved performance parameters in broiler chickens due to the improvement of the enzymatic activities [20]. However, in another study the use

of mint powder did not have any significant effect on the performance indexes of broiler chickens [33]. It was observed that values of body weight, daily weight gain, and feed conversion ratio showed that oregano given as single supplement at the level of 5 g/kg or in combination with α -tocopheryl acetate could serve as an alternative to the approved feed additives flavomycin and lasalocid [34]. In another experiment performance of the broilers was unaffected by the dietary oregano essential oil at levels of 50 and 100 mg/kg of feed [35]. In a study on laying hens, results showed that there were no significant differences in egg production, feed consumption, FCR, egg weight and shape, yolk diameter, height and color, Haugh unit, and egg shell thickness among control group and diet supplemented with oregano essential oil at ranges of 50 and 100 mg/kg [36]. Conflicting reports were presented about the effectiveness of chamomile, wild mint, and oregano plants. Although the reason for these contradictory results is unclear, it may be related to variety of herbal product, chemicals' stability, and the period and type of experiments.

As indicated in Table 3, three extracts showed no significant effect on hatch percentage. In a study, the diets were supplemented with 10, 20, 30, 40, and 50 g/kg of *Oregano Onites*, it was reported that the inclusion level of 20 g/kg increased fertility and above that level fertility was reduced and the hatchability was not impressed generally [37]. In another study, different levels of black cumin and parsley were added to the laying quail diets that the hatchability percentage not only did not increased, but also decreased with high levels of each or combination of two plants [38].

In Table 4, the effect of treatment on total protein, albumin, and HDL levels in laying quails were not significant, but chamomile extract more affected cholesterol and triglyceride of quails' serum and yolk cholesterol in comparison with the other groups. Following the reduction of serum cholesterol, yolk cholesterol was decreased. According to AL-Bayati (2012) treating 5% orally aqueous extract of chamomile (5 mL/kg) for 15 days reduced significantly the serum cholesterol level in the hyperlipidemic quinea pigs, due to the effect on the synthesis or absorption or catabolism of sterol [39]. In another study, serum cholesterol and triglyceride of hyperlipidemia rats were decreased significantly with using of chamomile extract, but the level of serum HDL did not change [40]. Using 0.5% chamomile flower powder in the

diet of laying hens reduced the serum cholesterol level [29]. Medicinal plants with different mechanisms such as changes in lipid oxidation, induce of inhibiting lipid accumulation by lipid catabolism, inhibition of lipid production, inhibition of adipocyte differentiation and lipogenesis, reducing lipid peroxidation, activation of lipase enzymes, up-regulation of adiponectin expression in adipocyte cell, and decrease in hepatic HMG-CoA reductase activity can reduce level of serum lipids. However, few studies have been implemented to clear these interactions [41].

In table 5, oregano has the most antibacterial activity among experimental treatments. It has been indicated that *Origanum vulgare* L. showed action mainly against the Gram-positive pathogens and among Gram-negative bacteria, *Escherichia coli* was affected [42]. Waldenstedt (2003) reported that Oregano supplement decreased number of *Clostridium perfringens* in cecal contents of broilers at 31 days [43]. Roofchae et al. (2011) illustrated populations of cecal *Escherichia coli* were significantly lower in 300 and 600 mg/kg Oregano supplemented groups in comparison with the control [25]. Two phenolic compounds, carvacrol and thymol are responsible for the antibacterial properties of Oregano and other constituents such as monoterpene hydrocarbons g-terpinene and pcymentene also contribute to the antibacterial activity [44].

As shown in Table 6, treatments had not significant effect on morphologic parameters in different parts of the intestine, just oregano extract improved villus height in the ileum. Antimicrobial compounds reduced the number of intestinal bacteria. As a result, with the reduction of bacteria population, the amount of toxins reduced in the intestine. The presence of toxins decreased the height of villi and depths of the crypts [45]. Due to the effect of oregano on reducing the bacterial population of the intestine as shown in Table 5, it can be concluded that oregano decreased the production of microbial toxins and the destruction of epithelial cells, consequently increased the height of villi and the surface area for nutrient absorption [46] that can be improved growth performance. In the present study, the oregano had a positive effect on the performance (egg weight, egg mass, and egg production) of laying Japanese quails but it was not significant.

The result of this study indicated that chamomile extract had an effective role in reduction of cholesterol in serum and yolk. Low cholesterol

level of serum can improve the animal health and durability; also low level cholesterol of eggs can improve the egg market's popularity. Oregano extract reduced the intestinal population bacteria and increased the villus height in the ileum. Considering the effect of rearing conditions on the efficacy of feed additives and the positive effects of applied treatments on some of the measured indices, using of these feed additives from the beginning of the rearing period and after hatch may have a better effect.

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