Effect of thyme, garlic and caraway herbal extracts on blood parameters, productivity, egg quality, hatchability and intestinal bacterial population of laying Japanese quail

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Abstract:

BACKGROUND: In the current Iranian poultry industry, antibiotics are the most frequently used additive in poultry feeds to increase productivity. The negative effects on human health, resulting from the consumption of chicken whose feeds contain antibiotics, makes finding an appropriate alternative of great importance. As a result of their nature, herbal extracts could be specifically considered for this purpose. **OBJECTIVES:** This study aimed to find an appropriate and harmless feed additive to increase the quality and quantity of poultry eggs. **METHODS:** A completely randomized design including 4 treatments, 4 replications and 4 birds in each experimental unit was applied. Herbal extracts were added to drinking water of treatments in ratio of 1:1000, with the control group containing no additives in their water. The number and weight of produced eggs were measured on a daily basis, feed consumption on weekly basis and the egg quality, yolk cholesterol, hatchability and intestinal bacterial population were measured at the end of experiment after 8 weeks. **RESULTS:** Herbal extract treatments showed no overall effect on quality and quantity of produced eggs in comparison with control; however, the thyme and garlic extracts reduced the cholesterol of serum and yolk relative to the control. Herbal extracts did not significantly affect the hatchability of fertile eggs. The herbal extracts caused a significant decrease in the intestinal bacterial population of laying quails. **CONCLUSIONS:** The garlic extract had the most decreasing effect on the cholesterol of serum and egg yolk. Furthermore, thyme extract had the most decreasing effect on the intestinal bacterial population.

Introduction

Currently, the use of antibiotics is a common way of preventing diseases and increasing meat and egg production. It serves as an essential tool for achieving higher yields in poultry systems. Nevertheless, the continuous use of antibiotics in feeds leads to problems such as increasing drug resistance, remaining drug in the chicken's body and loss of the natural balance of intestinal microflora (Awad et al., 2009). Although antibiotics are less frequently used in egg-laying chickens' husbandry systems compared to the meat type ones,

the increase of pathogenic bacterial resistance to antibiotics in poultry and its transfer to humans is a major concern. For these reasons, researchers have focused on the use of additives which preserve the desired features and do not have negative effects on health and environment. Among the additives, herbal extracts and their products are of great importance. These products are harmless to humans and animals with no undesired side effects. In addition, there is no complication of drug resistance due to their consumption. Previous research has shown that herbal plants and their extracts have different biological activities in poultry, ranging from antibacterial properties, anti-parasitic, anti-viral and antioxidant (Kamel et al., 2001; Botsoglou et al., 2002; Papageorgiou et al., 2003; Youn and Noh, 2001; Lee et al., 2004). It has been reported that they have the ability to stimulate the immune system and endocrine glands (Lee et al., 2004). Several clinical reports, including meta-analyses, have revealed the cholesterol lowering effect of garlic in humans (Warshafsky et al., 1993). Allicin (the active compound produced by garlic) may reduce the level of serum cholesterol, triglyceride and LDL (Alder and Holub, 1997). Mottaghitalab and Taraz (2002) concluded that diets containing garlic powder has potential as feed additives, which may be beneficial in reducing serum and egg cholesterol in hens. Khan et al. (2008) also reported that feed consumption, feed efficiency, egg weight and egg mass were not affected over 6 weeks, when laying hens were fed with 0, 2, 6 and 8% dietary garlic powder. Serum and egg yolk cholesterol concentrations decreased with increasing levels of dietary garlic. Thymus vulgaris L. is a herbaceous perennial plant belonging to the Lamiaceae family. Thymol, a major component of thyme-essential oils, has been widely studied for its antimicrobial properties (Dorman and Deans, 2000). Carvacrol, an isomer of thymol, is found in essential oils isolated from oregano and thyme. Like thymol, carvacrol also displays antimicrobial activity (Helander et al., 1998). Given their antimicrobial activity, it would be expected that thymol and carvacrol could have positive effects on growth performance in broilers. Such studies showed that thyme plant could be considered as an alternative natural growth promoter for poultry instead of antibiotics (Cross et al., 2007). Studies on Carum carvi L. against the pathogenesis of gastric lesions became important with the discovery of its antioxidant activity. Some reports found that it inhibits superoxide radicals, lipid peroxides and hydroxyl radicals. In addition terpenes including carvones and limonene are known to induce the detoxifying enzyme glutathione S-transferase in some mice target tissues (Crowell, 1999). Furthermore, studies have reported an increase in BW and decrease in FCR, when using cumin in diets (Al-Kassi, 2010). As mentioned above, it has become clear that there is quite a lot of benefits of caraway as a medical and nutritional resource to be used for poultry. The aim of the present study was to investigate the effects of thyme, garlic and caraway extracts as herbal additives on egg production and quality of layer quails.

Materials and Methods

The study was performed in the poultry farming unit of the Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University. Through a completely randomized design, a total of sixty-four (64), 10-week old laying Japanese quails were divided into 4 treatments and 4 replication groups with 4 quails in each replication (male to female ratio of 1:3). The experiment was carried out in eight weeks. Treatments included thyme, caraway and garlic extracts which were added to the birds' drinking water in the ratio of 1:1000, and the control group had no additive in their drinking water. All the groups had the same feed formulation based on NRC 1994. The ingredients and their amounts in this ration are

presented in Table 1. The herbal extracts were purchased from Exir-e Gol-e Sorkh Co. (Mashhad, Iran). Brix of thyme, garlic and caraway extracts were 11, 13 and 8, respectively. The lighting program was 16L/8D, and the temperature of the nests was kept at 20°C during the experiment. The birds had free access to water and feed throughout the experiment. Egg production and wastes were recorded daily, and the feed intake was measured at the end of each week by subtracting the quantity of the remaining feed from the quantity of feed given. 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 day egg production and average egg weight.

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 h 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 (Van Den Brand et al., 2004). The height of albumen was measured using the micrometer and the Haugh unit was calculated using the following formula:

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

where H is the height of albumen and W is weight of the quail egg. The yolk color was assessed using the DSM 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). 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 for 5 min. Concentrations of total protein, albumin, cholesterol, triglycerides and HDL in blood serum samples and egg yolk cholesterol were measured using commercial diagnostic kits (Zistshimi Co.) and a spectrophotometer (Jenway Genova MK3, UK).

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 h by a 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 min and then the light absorbance at wavelength of 500 nm was read (Pasin et al., 1998).

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 10-15ºC. 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 Mac Conkey agar media to count total aerobes, Streptococci and *E. coli*, respectively.

After incubation, 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 for later analyses.

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

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

where Y_{ij} is the mean of the jth observation of the ith treatment; μ is the sample mean; T_i is the effect of the ith treatment; and e_{ij} is the effect of error.

Results

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 measured quantitative indices in this experiment ($p>0.05$).

Effect of herbal extracts on blood parameters and the yolk cholesterol have been presented in Table 3. As can be seen, the caraway extract significantly increased the total protein, albumin and triglycerides of the blood serum ($p<0.05$), while the garlic extract led to a significant decrease in cholesterol of quails' serum. The cholesterol decreased in yolks of the eggs fed with garlic extract ($p<0.01$).

As is observed in Table 4, the herbal extracts have no significant effect on shell thickness, Haugh unit, yolk weight and shell strength and also hatchability ($p>0.05$).

In Table 5, the effect of extracts on populations of total aerobic bacteria, Streptococci and coli bacilli is reported. The thyme group reduced three enumeration of coli bacilli ($p<0.05$), and aerobic bacteria ($p<0.01$). Thyme and garlic extracts reduced the streptococci population ($p<0.05$).

Table 1. Nutrient content of diets of laying quail.

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

Discussion

As reported in Table 2, consuming garlic extract does not influence the performance of egg-laying quails. This is consistent with the findings of Reddy et al., (1991) where feeding hens of strain Babkuk B-300 with 0.02% garlic essence for 8 weeks, did not affect their egg production, egg mass, body weight and feed intake.

In addition, Yalçin et al. (2006) did not observe significant changes in body weight, feed intake and feeding yield when they added 0.5 and 10 g trade garlic powder per kg of feed ration of laying hens.

The herbal essences are thus expected to decrease the level of cholesterol. It has been shown that such herbal essence compounds as broneol, citral, graniol, menthone, fenchone, fenchyl alcohol and beta-ionone reduced the activity of liver HMG-CoA reductase (Fitch et al., 1989; Middleton and Kok-pheng, 1982; Yu et al., 1994).

Several studies have revealed the reduction of cholesterol level by garlic (Silagy and Neil, 1994; Warshafsky et al., 1993). This is in agreement with the observations in this study (Table 3). Some studies, on the other hand, have reported that trade garlic essences, the garlic powder and trade garlic extract may not

Table 2. Effects of herbal extracts on the performance of Japanese quail including production, daily feed intake (g), egg mass production (g), feed conversion and egg weight (g). SEM= Standard Error of the Means. NS= non significant.

Treatment	Egg weight (g)	Egg mass	FCR (feed intake/ kg mass)	Feed intake (g hen^{-1} day^{-1})	Hen day egg production (%)
Control	11.47	7.73	4.31	33.35	68.81
Thyme	11.33	7.25	4.83	34.71	67.51
Caraway	12.61	8.11	4.00	32.45	66.10
Garlic	10.72	7.98	4.53	35.10	69.25
P value	NS	NS	NS	NS	NS
SEM	0.284	0.188	0.143	0.402	1.061

Table 3. Effects of herbal extracts on blood parameters and egg yolk cholesterol of Japanese quail. (a,b,c) Means within a column with no common superscript differ significantly (*p<0.05) (**p<0.01). SEM= Standard Error of the Means. NS= non significant.

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	Albumin (g/dl)	Total protein (g/dl)	Yolk cholesterol (mg/g)
Control	185.47^{ab}	222.71^{ab}	32.47	2.53^{b}	3.303^{b}	13.34^{ab}
Thyme	168.00^{b}	191.32^{ab}	34.59	2.72^{b}	3.370^{b}	12.55^{b}
Caraway	202.41^{a}	256.45^{a}	38.53	3.17^{a}	4.306^{a}	13.62^{a}
Garlic	142.23^{c}	143.08^{b}	33.47	2.61^{b}	3.450^{b}	11.15^{c}
P value	**	*	NS	*	*	**
SEM	6.319	14.168	1.380	0.086	0.147	0.287

Table 4. Effect of Herbal extracts on shell thickness, HU, shell strength, shell weight, yolk weight, yolk color and hatchability percentage in Japanese quail. SEM= Standard Error of the Means. NS= non significant.

Treatment	Shell weight (g)	Shell thickness (ml)	Shell strength (kg/Cm^2)	Haugh unit	Yolk color (DSM)	Yolk weight (g)	Hatchability (%)
Control	0.83	0.205	0.86	87.45	5.16	3.46	73.16
Thyme	0.85	0.223	1.06	87.66	5.83	3.85	77.58
Caraway	0.82	0.210	0.84	86.22	6.00	3.89	76.58
Garlic	0.84	0.201	0.88	85.95	5.16	3.76	71.50
P value	NS	NS	NS	NS	NS	NS	NS
SEM	0.006	0.004	0.040	0.600	0.186	0.129	1.735

Table 5. Effect of three herbal extracts (Garlic, Thyme and Caraway) on intestinal microbial flora in Japanese quail. (a,b) Means within a column with no common superscript differ significantly (*p<0.05) (**p<0.01). SEM= Standard Error of the Means. NS= non significant.

Treatment	Bacillus** (log CFU/g)	Total count of aerobic bacteria** (log CFU/g)	Streptococcus** (log CFU/g)
Control	6.35a	7.43a	5.57a
Thyme	5.70b	5.70c	5.05b
Caraway	5.93ab	6.65b	5.27ab
Garlic	5.90ab	6.02bc	5.12b
P value	*	**	*
SEM	0.086	0.199	0.064

have decreasing effects on the level of cholesterol (Berthold et al., 1998; Isaacsohn et al., 1998). Although the reason for these contradictory results is unknown, it may be related to complementation methods, chemicals' stability and the period of experiments (Amagase et al., 2001). According to Sklan et al., (1992), 2% garlic feeding for 14 days decreases the hepatic cholesterol level. Reduction of cholester-

ol level in plasma by the garlic is mediated by organosulfur compounds affecting the metabolism of cholesterol. These compounds block the secretion of hepatic enzymes involved in converting acetate to cholesterol, and thereby reduce the biosynthesis of cholesterol in the liver which in turn decreases the cholesterol concentration in the blood plasma (Liu and Yeh, 2000).

Case et al. (1995) showed that 150 ppm concentration of thymol and carvacrol (ingredients of thyme essence) decreases serum cholesterol in leghorn hens. This decreasing effect of garlic and thyme on serum and yolk cholesterol levels is consistent with reports in Table 3, with garlic treatment showing stronger effect than thyme.

Feeding caraway has led to an increasing level of triglycerides in blood serum. This result is inconsistent with Khajeali et al. (2013) who observed the decrease of triglycerides in serum of broiler fed with 1, 1.5 and 2% caraway concentrations. This contradiction may be due to the biodiversity of medicinal herbs, difference in the effect of herbal alcoholic extracts and the direct use of caraway grains in that study. Use of concentrated extracts allows adding low amounts of them to the feed rations, while less-concentrated substances (such as the whole plant or the dried plants) should be added in higher amounts. To ensure the continuous quality of such products, posing stringent standards for active compounds is necessary, but this is a difficult task due to the diversity in origins of plants or their extracts.

Reports in Table 3 indicate the significant increase of triglycerides, total protein and albumin of the blood serum in quails by the caraway extract. The same effect was observed in the cholesterol level of serum, but it was not statistically significant. The increase of serum fats has generally led to an increase in yolk weight and consequently the egg weight, although this effect was not significant. This necessitates further studies.

Table 4 reports the effect of herbal extracts on hatch percentage. Statistical analysis of the data shows no significant difference between the samples in comparison with the control group. The high variation of hatchability values within groups compared to variation between groups is the reason for insignificant differences, despite the obvious differences in means.

An experiment was conducted to compare the effects of various amounts of oregano and thyme in hatchability of eggs of Japanese quail stored for various time intervals, had reported eggs from the group with 1.5% thyme that was stored for one week produced the highest hatching, and among the eggs that stored for two and three weeks, higher hatchability resulted for the thyme group with 3% thyme (Gholipoor et al., 2013). This is not in agreement with the observation of this experiment.

Table 5 shows the effect of herbal extracts on intestinal bacterial populations in quails. These results indicate the decreasing population of microflora in the birds. Numerous in vitro studies have shown that essential oils including thymol, carvacrol, etc., have antimicrobial activity against intestinal microbes such as C. perfringens, S. typhimurium and E. coli (Helander et al., 1998; Hammer et al., 1999). The antimicrobial action of essential oil is mediated by the lipophilic property to perforate the bacterial membrane, which releases membrane components from the cells to the external environment (Helander et al., 1998). Studies carried out with broilers seem to confirm the in vitro findings. In a trial reviewed by Losa (2001), it was observed that the inclusion of a mixture of plant extracts reduced by 70%, the number of broilers infected with C. perfringens. A significant reduction of C. perfringens colonization was observed in the intestine of broilers fed diets containing mixtures of thymol, eugenol, curcumin and piperin, or thymol, carvacrol, eugenol, curcumin and piperin (Mitsch et al., 2004).

The antibacterial effect of caraway essence against gram-negative bacteria such as E. coli and gram-positives such as S. aureus has been reported. This effect has been attributed to such compounds as carvene, limonene, carvacrol and linalool (Gachkar et al., 2007). The same effect can be seen in Table 5. Another study investigated the antibacterial effect of some medicinal herbal essences against antibiotic-resistant microorganisms such as methicillin-resistant *S. aureus* and vancomycin-resistant enterococci, and revealed a stronger effect of thyme in comparison with other herbal essences (Nelson et al., 1997). These findings are in thorough consistency with this study as it recorded decreasing intestinal microflora of quails. It is important to remember that the in vivo antimicrobial property of essential oil in birds can be influenced by a basal diet and environmental conditions.

Garlic consumption seems to improve the quality of produced eggs, especially as it reduces the yolk cholesterol. The herbal extracts used in this study decreased the intestinal population of bacteria (the bacteria population competes with birds for feed nutrients). This can also to some extent improve the performance of egg-laying birds in terms of quantitative traits. Since medicinal herbs possess overlapping effects, essences of several plants are recommended to be simultaneously used in feeds of birds, with regard to the specific properties of their biological activities, in order to improve their effects in increasing yield, quality and productivity.

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چکیده

زمینه مطالعه: در حال حاضر در صنعت دام و طیور کشور، آنتی‌بیوتیک‌ها به عنوان پرکاربردترین افزودنی به منظور افزایش تولید در خوراک دام و طیور به کار می‌روند که با توجه به اثرات سوء ایجادشده در انسان از مصرف محصولات دامی که در تغذیه آنها از آنتی‌بیوتیک استفاده شده است، یافتن یک جایگزین مناسب برای آنها از اهمیت ویژه‌ای برخوردار بوده که در این میان گیاهان دارویی به دلیل ماهیتشان می‌توانند به طور ویژه‌ای مورد توجه قرار گیرند. **هدف:** هدف از این مطالعه یافتن یک افزودنی غذایی مناسب و بی‌ضرر برای افزایش کمیت و کیفیت تخم‌های تولیدی در طیور تخم‌گذار است. **روش کار:** از طرحی کاملاً تصادفی با ۴ تیمار، ۴ تکرار و ۴ پرنده در هر واحد آزمایشی استفاده شد. عصاره‌های گیاهان دارویی به نسبت ۱ به ۱۰۰۰ به آب آشامیدنی تیمارهای مربوطه اضافه گشت و آب آشامیدنی گروه شاهد بدون هیچ افزودنی در اختیار پرندگان قرار گرفت. مقدار و وزن تخم‌های تولیدی به صورت روزانه، مصرف خوراک به صورت هفتگی و کیفیت تخم‌های تولیدی و کلسترول زرده و قدرت جوجه درآوری و جمعیت باکتریایی روده در انتهای دوره آزمایشی بعد از هشت هفته اندازه‌گیری شد. **نتایج:** تیمارهای گیاهان دارویی به طور کلی تأثیری بر کمیت و کیفیت تخم‌های تولیدی نسبت به تیمار شاهد نداشتند، ولی عصاره آویشن و سیر باعث کاهش کلسترول سرم و زرده در مقایسه با تیمار شاهد شدند. همچنین عصاره‌های گیاهان دارویی تأثیر معنی‌داری بر درصد جوجه درآوری بلدرچین‌های مولد نداشتند. گیاهان دارویی به طور معنی‌داری باعث کاهش جمعیت باکتریایی روده‌های بلدرچین‌های مولد شدند. **نتیجه گیری نهایی:** عصاره گیاه دارویی سیر باعث بیشترین اثر کاهشی بر کلسترول سرم بلدرچین‌ها و زرده تخم‌های تولیدی و عصاره گیاه دارویی آویشن باعث بیشترین اثر کاهشی جمعیت باکتریایی شدند.

واژه های کلیدی: فراسنجه‌های خونی، کمیت و کیفیت تخم‌های تولیدی، عصاره گیاهان دارویی، بلدرچین ژاپنی

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