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Original Research Article

Evaluation of thyme and ajwain as antibiotic growth promoter substitutions on growth performance, carcass characteristics and serum biochemistry in Japanese quails (*Coturnix japonica*)

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ABSTRACT

The present study was to evaluate thyme and ajwain as antibiotic growth promoter substitutions on growth performance, carcass characteristics and serum biochemistry in Japanese quails (*Coturnix japonica*). A feeding trial was conducted over 28 d with 4 groups of Japanese quails fed experimental diets formulated to provide no supplementation (control), or control + 55 mg zinc bacitracin/kg, 2 g thyme/kg, and 2 g ajwain/kg. At 35 d of age, 2 quails from each replicate were sacrificed and eviscerated. Liver, empty small intestine and heart were weighed and calculated as a percentage of live body weight. The carcasses were weighed and the cuts were performed to evaluate the yield of legs and breast. At 35 d of age, 2 quails per replicate were chosen and approximately 1-mL blood samples were collected through brachial vein, and concentrations of albumin, total protein, triglyceride, total cholesterol, and high density lipoprotein (HDL) -cholesterol were determined. Final body weight were not affected by the dietary treatment whereas, it tended to increase in quails supplemented with antibiotic. Overall feed conversation ratio values were similar though it tended to improve in quails supplemented with thyme compared with other groups. Daily feed intake, internal organ weights and carcass traits were not influenced by the dietary treatments. Serum total cholesterol contents were lower ($P < 0.05$) in quails supplemented with ajwain compared with control and quails supplemented with antibiotic and thyme. The highest serum HDL-cholesterol was seen in the group supplemented with thyme compared with other groups. It was concluded that the addition of 2 g/kg thyme can improve serum biochemistry in quails, although its effects on performance criteria was negligible.

1. Introduction

Antimicrobial compounds have been commonly included at subtherapeutic doses in poultry diets for promoting growth and protect health of the birds (Ghalamkari et al. (2011, 2012); Landy and Kavyani, 2014; Gheisari et al., 2017; Engberg et al., 2000). Antibiotic growth promoters (AGP) were supposed to improve growth rate, feed conversion and reduce mortality as a result of improved intestinal microbial flora (Miles et al., 2006; Pfaller, 2006). However, there is the fear that the continuous use of feed grade AGP could lead to, not only cross-resistance, but also to the risk of development of antibiotic resistant bacteria in humans (Nasir and Grashorn, 2006; Nanekarani et al., 2012; Goodarzi et al., 2014). Therefore, efforts have been made to reduce the use of chemical additives in poultry diet. Because of the ban on the use of antibiotics in European Union, the poultry producers are exposed to decrease the use of AGP and find alternatives to AGP in poultry feed. Botanical products, also known as phytogenic feed additives, have received enhanced attention recently, because they have been accepted by consumers as non-antibiotic substances (Toghyani et al., 2015; Landy et al., 2011a,b; Fekri Yazdi et al., 2014a,b).

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Thyme (*Thymus vulgaris* L.) an aromatic plant member of the Lamiaceae family is a plant mostly grown in Mediterranean region. Thyme contains compounds with proven antispasmodic, antiseptic, expectorant, carminative, antitussive, antimicrobial and antioxidative properties (Dapkevicius et al., 2002; Nguyen et al., 2000; Vincent, 2002). Thyme possesses thymol, p-cymene, carvacrol, γ-terpinene, linalool and α-pinene (Bagamboula et al., 2004).

Ajwain (*Trachyspermum ammi* L.) belongs to family Apiaceae (Umbelliferae) and is an annual herb, originated in the Middle East, possibly in Egypt and the Indian subcontinent, but also in Iran and Afghanistan (Christie et al., 2005). Ajwain contain compound with proven antiseptic, antifungal, antibacterial and antihelminthic effects (Morsi, 2000). The principle active compounds of ajwain include phenols, mainly thymol and carvacrol which are important pharmacologically active substances (Tsimidou and Boskou, 1994).

Several trials have been performed to evaluate the effect of thyme (Khaksar et al., 2012; Cross et al., 2007; Behnamifar et al., 2015) and ajwain powder (Samadian et al., 2015; Deepika et al., 2013) on quail growth performance and serum biochemical parameters but the results have not been consistent. Thus, present trial was conducted to investigate the potential of dietary supplementation with thyme and ajwain powder as growth promoter agents on performance, carcass traits and serum biochemical parameters in Japanese quails.

2. Materials and methods

2.1. Ethical approval

The birds were handled in compliance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. Also, all procedures complied with the ethical guidelines of the Shahrekord University's Ethical Committee (approval Ref No. 2016-54).

2.2. Animals and dietary treatments

One hundred twenty eight 7-d-old healthy Japanese quails (*Coturnix japonica*) of mixed sexes were weighed and randomly allocated to 1 of 4 treatment groups with 4 replicates of 8 chicks each. The experimental treatments included the control (basal diet), control + 55 mg zinc bacitracin/kg, control + 2 g thyme/kg or 2 g ajwain/kg diet. Table 1 lists the basal diet formulated to meet or exceed the nutritional requirements of Japanese quails (NRC, 1994). All birds were placed on floor pens for 4 wk in a temperature-controlled house and had free access to feed and water daily. The lighting program consisted of a period of 23 h light and 1 h of darkness. The ambient temperature in experimental house was maintained at 37 °C on the first day and gradually decreased to 24 °C in the end of third week and then fixed.

Table 1
The ingredient (as-fed basis) and calculated composition of basal diet.

Item	Content
Ingredients, g/kg	
Corn	508.3
Soybean meal 45% CP	440
Soybean oil	22
Dicalcium phosphate 22 Ca, 17 P	9.8
$CaCO_3$	12.2
NaCl	2.3
$NaHCO_3$	1.3
Trace mineral premix[1]	1
Vitamin premix[2]	1
DL-methionine	0.2
L-threonine	0.9
Choline chloride 60%	1
Calculated composition, g/kg	
Metabolizable energy, kcal/kg	2,900
Crude protein	240
Calcium	8
Available phosphorus	3
Methionine + Cysteine	7.5
Lysine	13.4
Threonine	10.2

[1] Provided the following per kg of diet: Mg, 60 mg; Fe, 120 mg; Cu, 5 mg; Zn, 25 mg; I, 0.3 mg.
[2] Provided the following per kg of diet: vitamin A, 1,650 IU; vitamin D_3, 750 IU; vitamin E, 12 IU; vitamin K, 1 mg; riboflavin, 4 mg; vitamin B_{12}, 0.003 mg; pantothenic acid, 10 mg; nicotinic acid, 40 mg; folic acid, 1 mg.

2.3. Analysis of thyme and ajwain powders components

The dried aerial part of thyme and ajwain were extracted by hydrodistillation in a Clevenger-type apparatus for 2 h according to the method described in the European European Pharmacopoeia (1975). The collected essential oils were analyzed by application of Gas chromatography equipped with mass spectrometry (GC–MS) using the Agilent 6890/5973 GC–MS (Hewlett-Packard, Palo Alto, CA, USA) as described by Vasudeva and Sharma (2012).

2.4. Performance and carcass components

Body weights (BW) of birds were recorded at 7, 14, 28, and 35 d of age. Feed intake (FI) in each pen was recorded weekly to determine feed conversion ratio (FCR; feed:gain). Mortality was recorded as it occurred.

At 35 d posthatch, 2 male quails from each replicate were randomly selected, based on the average weight of the group, individually weighed and sacrificed after a 5-h feed deprivation following standard procedure (Landy et al., 2012). Carcass yield was calculated by dividing eviscerated weight by live body weight. Liver, empty small intestine and heart were removed, weighed and calculated as a percentage of live body weight. The carcasses were weighed and the cuts were performed to evaluate the yield of legs and breast.

2.5. Serum biochemistry

At 35 d posthatch, after 12 h of fasting, approximately 2 mL of blood from 2 male quails in each replicate was collected from the brachial vein into non-heparinised tubes for serum biochemistry and incubated at 37 °C for 2 h, centrifuged at 2,000 × *g* for 10 min, and serum was separated (SIGMA 4e15 Lab Centrifuge, Germany). Serum samples were analyzed for albumin, total protein, triglyceride, total cholesterol and high density lipoprotein (HDL)-cholesterol using an auto-analyzer (Kodak Ektachem Analyzer, Eastman Kodak Co., Rochester, NY).

2.6. Statistical analysis

All data were analyzed using the General Linear Model procedures of SAS (SAS Inst. Inc., Cary, NC, USA) for a completely randomized design. Means were compared using Duncan test at 5% probability.

3. Results and discussion

3.1. Thyme and ajwain powders components

The analysis of thyme revealed that the major essential oils were thymol (47.98%), γ-terpinene (0.92%), P-cymene (9.77%) and carvacrol (4.30%), and the main essential oils in ajwain were thymol

(81.3%), γ-terpinene (2.49%), P-cymene (9.77%) and carvacrol (2.45%).

3.2. Performance and carcass traits

Data on performance indices are summarized in Table 2. There were no marked effects of dietary treatments in FI during different growth periods as well as for the whole experiment. At 14 d of age, quails receiving basal diet or dietary containing thyme had higher BW compared with quails receiving antibiotic or dietary containing ajwain that were not different between them ($P < 0.05$). At 21 and 28 d of age, quails fed antibiotic had higher BW compared with quails receiving ajwain, but did not differ from the quails fed basal diet or basal diet containing thyme ($P < 0.05$). Quails BW did not differ between the experimental treatments on d 35, though it tended to increase in quails supplemented with antibiotic. Significant differences between treatments were noted in FCR form 29 to 35 d. Dietary containing thyme or ajwain had better FCR values compared with control group, whereas did not differ from the antibiotic that was intermediate. From 7 to 35 d, the FCR value was not affected by dietary treatments, though it tended to improve in quails supplemented with thyme. No treatment effects were observed on mortality. Antibiotics incorporation into poultry feed may reduce the growth and colonization of a variety of intestinal pathogens in the gut of birds (Ferket, 2004). According to Bedford (2000), improvement in performance of the birds might be attributed to the more balanced microflora provided by the antibiotics in the diet. In the present study, improvement in BW of quails obtained could be attributed to affirmative effects of antibiotic on digestibility and utilization of feed, as reported by Kavyani et al. (2012). Factors which could have contributed to the positive effects of the medicinal plants on the performance of birds are their antibacterial and antioxidant effects of natural phenolic compounds in the intestine (Nascimento et al., 2000). Addition of quercetin to laying hen diets had affirmative effects on performance criteria by modulation of antioxidant enzymes such as hepatic superoxide dismutase activity and more balance biota (Liu et al., 2013; Rupasinghe et al., 2010). Khaksar et al. (2012) reported that supplementing quails' diet with 1 g/kg thyme essential oil could improve performance, some blood parameters and gut microflora. The lack of significant effect of the additives on BW obtained at 35 d of age can be attributed to the highly digestible ingredients in the basal diet and the ideal conditions of experimentation. As reported by Toghyani et al. (2010) addition of growth promoters to poultry diets may have more impact when the diet used is less digestible. Similarly Ghalamkari et al. (2011, 2012) showed that flavophospholipol did not improve performance of well-nourished, healthy chicks raised in an ideal condition of experimentation. Furthermore, possibly the medicinal plants dosages applied in the present trial has not been such a level that would cause a positive influence on performance criteria, since there are reports of significant increased productive traits in broilers receiving diets supplemented with 5 g/kg thyme (Toghyani et al., 2010) and 1% ajwain seeds (Habibi et al., 2016) which are considerably higher levels compared with the dosage used in our trial.

Table 3 shows carcass, cut yields and relative organ weights as a percentage of live body weight of 35-day-old quails fed dietary treatments. No pathological lesions were noticed for heart, liver and small intestine. Carcass traits evaluated including liver, heart, small intestine weights, carcass and cut yields were not markedly affected by dietary treatments. The quails receiving 2 g/kg thyme had the lowest small intestinal weight and the highest breast yield percentages, although the differences were not statistically significant ($P > 0.05$). It may have caused lower FCR in treatment quails, although this was not happened. Despite of having lower small intestinal weight, quails receiving thyme utilized feed efficiently as much as other groups did. This might be a result of higher nutrient absorption area or enhanced digestive enzymes.

3.3. Serum biochemistry

The impact of treatments on serum constituents are presented in Table 4. An improved HDL-cholesterol concentration was noted in the group supplemented with thyme relative to the control, antibiotic or ajwain. Treatments did not induce any significant effect on the serum concentration of triglyceride, though it tended to increase in quails fed diets containing ajwain. Maximum concentration of serum protein obtained in quails supplemented with ajwain ($P < 0.05$). An increased serum protein was seen in the group supplemented with thyme compared with control and antibiotic. Minimum concentration of cholesterol was seen in the group supplemented with ajwain ($P < 0.05$). Quails supplemented with antibiotic and thyme had lower total cholesterol compared with control group. The highest concentration of serum albumin

Table 2
Effect of experimental diets on performance indices of quails at different ages.[1]

Item	Dietary treatments				SEM
	Control[2]	Antibiotic[3]	Thyme[4]	Ajwain[5]	
Body weight, g					
14 d	52.8[a]	50.5[b]	52.0[a]	49.5[b]	0.3
21 d	115.9[a]	115.8[a]	113.7[ab]	112.4[b]	0.5
28 d	176.5[ab]	181.3[a]	172.6[ab]	167.4[b]	3.2
35 d	222.5	226.4	223.5	216.4	5.3
Daily feed intake, g/d					
7 to 14 d	8.4	8.4	8.4	8.4	0.3
15 to 21 d	19.8	20.5	19.5	19.9	0.7
22 to 28 d	23.0	22.4	21.0	21.2	1.8
29 to 35 d	24.0	22.6	24.9	23.9	1.85
7 to 35 d	18.8	18.4	18.4	18.3	24.1
FCR, g:g					
7 to 14 d	2.19	2.40	2.26	2.5	0.19
15 to 21 d	2.20	2.19	2.21	2.28	0.07
22 to 28 d	2.66	2.40	2.50	2.60	0.18
29 to 35 d	3.65[a]	3.50[ab]	3.40[b]	3.42[b]	0.09
7 to 35 d	2.67	2.62	2.59	2.70	0.20

FCR = feed:gain ratio; SEM = standard error of mean.
[a,b] Values in the same row not sharing a common superscript differ ($P < 0.05$).
[1] Data are means of 4 replicate cages consisting of 8 birds per replicate cage.
[2] Basal diet with no supplementation.
[3] Basal diet + 55 mg zinc bacitracin/kg.
[4] Basal diet + 2 g thyme/kg.
[5] Basal diet + 2 g ajwain/kg.

Table 3
Effect of experimental diets on carcass composition, yield and internal relative organ weight (%) of Japanese quails at 35 d of age.[1]

Item	Dietary treatments				SEM
	Control[2]	Antibiotic[3]	Thyme[4]	Ajwain[5]	
Carcass	78.4	79.0	78.9	78.2	0.70
Breast yield	41.0	40.6	43.7	40.6	1.85
Legs yield	30.0	29.8	27.8	29.4	2.10
Liver	3.02	3.77	3.34	3.36	0.77
Heart	1.28	1.22	1.10	1.37	0.21
Small intestine	6.58	7.17	5.56	6.83	1.8

SEM = standard error of mean.
[1] Data are means of 4 replicate cages consisting of 8 birds per replicate cage.
[2] Basal diet with no supplementation.
[3] Basal diet + 55 mg zinc bacitracin/kg.
[4] Basal diet + 2 g thyme/kg.
[5] Basal diet + 2 g ajwain/kg.

Table 4
Effect of experimental treatments on serum biochemical parameters of broilers at 35 d of age.[1]

Item	Dietary treatments				SEM
	Control[2]	Antibiotic[3]	Thyme[4]	Ajwain[5]	
Protein, g/mL	3.85[c]	3.72[c]	4.24[b]	4.56[a]	0.06
Albumin, g/mL	1.18[c]	2.10[a]	2.10[a]	1.52[b]	0.09
Triglyceride, mg/100 mL	67	65	70	92	10.2
Total cholesterol, mg/100 mL	221.5[a]	205.7[b]	208.6[b]	161.6[c]	3.23
HDL-cholesterol, mg/100 mL	25.56[b]	19.52[d]	28.39[a]	22.79[c]	1.10

HDL = high density lipoprotein; SEM = standard error of mean.
[a–d] Values in the same row not sharing a common superscript differ ($P < 0.05$).
[1] Data are means of 4 replicate cages consisting of 8 birds per replicate cage.
[2] Basal diet with no supplementation.
[3] Basal diet + 55 mg zinc bacitracin/kg.
[4] Basal diet + 2 g thyme/kg.
[5] Basal diet + 2 g ajwain/kg.

obtained in the quails fed diets containing ajwain ($P < 0.05$). Quails receiving antibiotic or thyme had lower serum concentration of albumin compared with control.

Chemical analysis of blood serum is a labile biochemical system which can reflect the situation of the organism and the changes occurring to it under effect of various external and internal factors. In the present trial total cholesterol concentration was significantly reduced by supplementation of thyme and ajwain, and HDL-cholesterol level was significantly enhanced in quails supplemented with thyme. Consistently, Toghyani et al. (2010) indicated that supplementation of dried aerial part of thyme to broiler diets resulted in significant increase in plasma HDL-cholesterol. Davoodi et al. (2016) reported that addition of different levels of ajwain to broiler diets significantly decreased plasma lipids and increased HDL-cholesterol. In contrast with our results, Ali et al. (2007) reported that supplementing thyme to hen diets resulted in significant reduction of plasma HDL, total cholesterol, triglycerides and total lipids. The reduction of plasma lipids noticed with thyme in research trials was attributed to the lowering influence of thymol or carvacrol on 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme, of cholesterol synthesis (Lee et al., 2003).

4. Conclusion

It was concluded that the addition of 2 g/kg thyme can improve serum biochemistry in quails, although its effects on performance criteria was negligible.

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