Use of anise seed and/or α-tocopheryl acetate in laying Japanese quail diets

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Abstract

An experiment was conducted to investigate the possible use of anise (*Pimpinella anisum*) and/or α-tocopheryl acetate as dietary supplements on the performance and some egg quality characteristics of laying Japanese quail. One hundred and eight *Coturnix japonica* quail (72 females and 36 males), 149 days old, were randomly allocated to four equal groups with three subgroups of nine birds each (six females and three males). A commercial laying diet was fed to the control group (Group A). The remaining three groups received the same diet supplemented with anise seed at 10 g/kg (Group B) or 20 g/kg (Group C), or additional 600 mg α-tocopheryl acetate/kg (Group D). The birds were given feed and water *ad libitum* for a period of 29 days, while being kept under commercial conditions. During the experiment, egg production, feed consumption and mortality were recorded daily. Also, at the end of experiment, egg weight, egg yolk, albumen and shell weight percentages, egg yolk colour (using the L*a*b* colour space), blood serum total cholesterol and triglycerides concentrations were determined. Neither the supplementation of anise nor that of α-tocopheryl had any effect on the performance of the birds or the quality of their eggs, except for a significant change of the colour of the egg yolk. Cholesterol concentration in the serum tended to decrease with the addition of anise to the diet.

Keywords: *Pimpinella anisum*, vitamin E, *Coturnix japonica*, performance, egg characteristics, serum total cholesterol, serum triglycerides
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Introduction

For centuries aromatic plants have been used worldwide as food and for medicinal purposes. Various biological activities, such as antioxidative (Botsoglou *et al.*, 2002; Giannenas *et al.*, 2005; Florou-Paneri *et al.*, 2006), anticoccidial (Christaki *et al.*, 2004; Florou-Paneri *et al.*, 2006) or antimicrobial (Govaris *et al.*, 2007; Botsoglou *et al.*, 2010) properties have been identified in these plants. Consequently, an increasing interest in the use of these products in poultry nutrition has been experienced, especially since the complete ban by the European Union countries in 2006 (EU, 2005) on the use of antibiotics as growth promoters in animals.

Anise (*Pimpinella anisum* L.), a member of the Apiaceae family, is an annual aromatic plant, native to the eastern Mediterranean and southwestern regions of Asia. The part of the plant used, is the fruit, in particular the seed and its essential oil. Anise seed is listed by the Council of Europe as a natural source of feed flavouring and in the USA it is considered as GRAS, i.e. Generally Recognized As Safe, (Franz *et al.*, 2005; Al-Beitawi *et al.*, 2009).

Anise has been examined for its antiparasitic and digestion stimulating properties (Cabuk *et al.*, 2003), as well as its antibacterial (Tabanca *et al.*, 2003), antifungal (Soliman & Badea, 2002), antipyretic (Afifi *et al.*, 1994), antioxidant (Gulcin *et al.*, 2003), antimicrobial (Al-Kassie *et al.*, 2008), anthelmintic (Bhatti *et al.*, 1996) and hypocholesterolemic (Craig, 1999) activities. Additionally, anise is reported to possess anticonvulsant (Pourgholam *et al.*, 1999), antiepileptic (Janahmadi *et al.*, 2008) and muscle relaxant (Albuquerque *et al.*, 1995) properties. Some studies have been conducted to evaluate the use of anise seed or

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 222-4062 (online)
Publisher: South African Society for Animal Science

oil in poultry nutrition, especially to replace antibiotics as growth promoters (Ciftci *et al.*, 2005; Soltan *et al.*, 2008; Al-Beitawi *et al.*, 2009).

It is well documented that dietary vitamin E, in the form of α-tocopheryl acetate, is a strong antioxidant in the body; though limited research has been conducted on its potential to improve performance and egg quality traits of laying Japanese quail (Sahin *et al.*, 2006). Antioxidants in poultry diets perform an important role in the good health and performance of poultry and the oxidative stability of their products, and poultry diets are routinely supplemented with antioxidants. Nevertheless, it remains unclear whether the antioxidant properties of aromatic plants are comparable to that of α-tocopheryl acetate, which is usually included in commercial poultry diets (Windisch *et al.*, 2008).

The aim of the present study was to investigate the dietary use of anise seeds and α-tocopheryl acetate on performance and some egg quality characteristics of laying Japanese quail.

Materials and Methods

Anise seeds were dried, milled and incorporated into the experimental diet. The chemical analysis of the anise seeds, performed according to the guidelines of AOAC (2005), is presented in Table 1.

Table 1 Chemical analysis of anise seed

	g/kg
Dry matter	935
Crude protein	129
Crude fat	138
Crude fibre	202
Ash	82

One hundred and eight *Coturnix japonica* quail (72 females and 36 males), 149 days old, were randomly allocated to four groups with three replications of nine birds each (six females and three males). All the quail were weighed individually at the time of placement in the cages, and average body weights did not differ (P >0.100) between the four treatments. For an acclimatization period of 10 days before the onset of the investigation the birds were fed a commercial layer diet (Table 2) *ad libitum.* This diet contained 30 mg α-tocopheryl acetate/kg. After this preliminary period the birds in the control group (Group A) remained on the same basal diet. The birds of the remaining three groups were fed the basal diet supplemented with either ground anise seed at 10 g/kg (Group B), or 20 g/kg (Group C) or an additional 600 mg α-tocopheryl acetate/kg feed (Group D). The α-tocopheryl acetate used was obtained from Roche Products Ltd. (Hertfordshire, UK). For a period of 29 days the birds received feed and water *ad libitum*, and were kept under commercial conditions for laying hens. The quail were handled according to the principles of the Greek Directorate General of Veterinary Services for the care of animals in experimentation.

During the experimental period, egg production, feed intake and mortality were recorded daily. At the last day of the experiment, egg weight and egg yolk, albumen and shell (with shell membrane) weight percentages were determined in 10 eggs per replication. Furthermore, egg yolk colour was measured in a mixture of 10 egg yolks from each replication, using the L*a*b* colour space (L = lightness, a = redness, b = yellowness) according to Herber-McNeill & Van Elswyk (1998), with the aid of a Konica Minolta Chroma Meter CR-410 (Japan).

At the end of the feeding period, total cholesterol concentration in blood serum was measured according to the method of Roeschlau *et al.* (1974), and the concentration of blood serum triglycerides was measured according to the method of Fossati & Prencipe (1982). For both of the above measurements a biochemical analyser, Flexor E, Vital Scientific N.V. (Holland), was used.

The statistical analysis was performed using the SPSS (2007) 16.0.1 statistical package (SPSS Inc., Chigaco, IL, USA). The one-way analysis of variance (ANOVA) for the four groups of the experimentation was performed using the general linear model function of SPSS. Furthermore, regression analysis of the

effect of the anise was performed using the curve estimation function of SPSS. For the mortality the Pearson's chi square test was used. A value of $P \leq 0.050$ was considered significant and a value of $0.050 < P \leq 0.100$ was considered a tendency. Levene's test was applied to test the homogeneity of the variances. The Tukey's test was applied to determine statistical differences between the means.

Table 2 Composition of the basal diet

Ingredients	g/kg	Chemical composition (analysed)	g/kg
Maize	456.7	Dry matter	900
Soybean meal	305.4	Crude protein	198
Wheat	100.0	Crude fat	45
Calcium carbonate	62.1	Crude fibre	34
Soybean oil	30.0	Ash	94
Maize gluten meal	27.7		
Dicalcium phosphate	10.4	Calculated analysis	
Vitamin and trace mineral premix[1]	3.5	Calcium	26
Salt	2.1	Total phosphorus	6
Sodium bicarbonate	1.9	Lysine	10.2
Methionine	0.2	Methionine & cystine	7.2
		Metabolisable energy, MJ/kg	12.13

[1]Supplying per kg feed: 14000 IU vitamin A; 5000 IU vitamin D_3; 30 mg vitamin E; 13 mg vitamin K; 3 mg vitamin B_1; 8 mg vitamin B_2; 3 mg vitamin B_6; 20 µg vitamin B_{12}; 85 mg vitamin niacin; 20 mg pantothenic acid; 2 mg folic acid; 200 µg biotin; 10 mg vitamin C; 960 mg choline chloride; 100 mg Zn; 116 mg Fe; 120 mg Mg; 20 mg Cu; 0.2 mg Co; 1 mg I; 0.3 mg Se.

Results

At the completion of the trial, feed intake and mortality did not differ ($P > 0.100$) between the groups (Table 3). Similarly, the regression analysis of the effect of anise on the performance parameters (Table 6) showed no ($P > 0.100$) differences.

Table 3 Performance of laying quail (mean ± SD)

Group	Egg production %	Daily feed intake g	Mortality %
A	70.9 ± 11.51	32.3 ± 4.3	0
B	73.4 ± 13.32	28.6 ± 4.0	3.7 ± 6.4
C	76.3 ± 3.17	29.3 ± 0.5	0
D	59.4 ± 10.54	31.5 ± 0.5	0
P value	0.282	0.419	0.387

Groups: A = control; B = 10 g anise/kg; C = 20 g anise/kg; D = 600 mg vitamin E/kg.
Column means did not differ significantly at $P \leq 0.050$.

The results concerning the effect of the dietary addition of anise and α-tocopheryl acetate on some egg quality parameters are presented in Table 4. Egg weight (g), yolk, albumen and shell weight (%) did not

differ (P >0.100) between the treatment groups. The egg yolk colour was affected (P ≤0.001) since the eggs of group D had a lower L* parameter value compared to those of groups B and C. Also, the eggs of group D had a higher a* parameter value compared to those of groups A, B and C, whereas no difference (P >0.100) between treatments was found in the b* parameter. Furthermore, regression analysis of the effect of anise (Table 6) showed a tendency (P ≤0.100) for a linear increase of the L* parameter with $R^2 = 0.349$ and also a linear increase (P ≤0.050) of the b* parameter with $R^2 = 0.484$.

Table 4 Egg quality parameters (mean ± SD)

Group	Egg weight	Egg yolk	Egg albumen	Egg shell	Yolk colour		
	g	%	%	%	L*	a*	b*
A	11.95 ± 0.02	31.39 ± 0.48	54.10 ± 1.41	14.52 ± 0.99	68.80^{ab} ± 0.41	2.16^{a} ± 0.74	64.13 ± 0.35
B	11.63 ± 0.26	32.59 ± 0.85	53.04 ± 0.82	14.38 ± 0.19	70.19^{a} ± 0.31	1.85^{a} ± 0.65	65.85 ± 1.01
C	11.82 ± 0.04	32.01 ± 0.95	54.07 ± 1.62	13.92 ± 0.79	69.97^{a} ± 0.90	2.37^{a} ± 0.13	66.25 ± 1.42
D	11.84 ± 0.2	32.27 ± 1.14	53.73 ± 2.68	14.00 ± 1.80	67.65^{b} ± 0.58	3.72^{b} ± 0.10	65.11 ± 0.72
P value	0.263	0.444	0.869	0.888	0.003	0.008	0.106

Groups: A = control; B = 10 g anise/kg; C = 20 g anise/kg; D = 600 mg vitamin E/kg.
Column means with different superscripts differ significantly at P ≤0.010.

Table 5 presents the effect of the dietary addition of anise and α-tocopheryl acetate on blood serum total cholesterol and triglyceride concentrations. Total cholesterol values had a tendency (P ≤0.100) to be lower for the quail in group B compared to those in groups A and D, while triglycerides values did not differ (P >0.100) between treatments. In addition, regression analysis of the effect of the anise on the above parameters (Table 6) showed a tendency (P ≤0.100) for a linear decrease of total cholesterol concentration with $R^2 = 0.406$.

Table 5 Blood serum total cholesterol and triglycerides (mean ± SD) concentrations

Group	Total cholesterol mg/dL	Triglycerides mg/dL
A	204.7^{a} ± 35.5	257.0 ± 74.8
B	156.7^{b} ± 8.5	131.0 ± 17.3
C	160.0^{ab} ± 14.1	213.3 ± 94.5
D	204.3^{a} ± 32.0	259.3 ± 116.2
P value	0.077	0.282

Groups: A = control; B = 10 g anise / kg; C = 20 g anise / kg; D = 600 mg vitamin E/kg.
Column means with different superscripts differ significantly at P ≤0.100.

Discussion

The objective of this study was to investigate the use of anise as a feed additive in laying Japanese quail nutrition. According to Franz *et al.* (2005), anise seeds contain 2 - 6% essential oil and also phenolic acids, flavonol and flavone glycosides. The predominant constituent of the essential oil is *trans*-anethole (80 - 95% of total oil), a powerful flavouring which belongs to the phytoestrogens (Albert-Puleo, 1980).

Table 6 Regression analysis of the effect of dietary anise on laying percentage and egg quality parameters

Parameter	*P*	R^2	Linear regression equations
Egg production, %	0.514	0.063	= 70.822 + 0.268 x Anise (g/kg)
Daily feed intake, g	0.317	0.142	= 31.510 – 0.148 x Anise (g/kg)
Egg weight, g	0.457	0.081	= 11.866 – 0.006 x Anise (g/kg)
Egg yolk, %	0.408	0.100	= 31.684 + 0.031 x Anise (g/kg)
Egg albumen, %	0.984	0.000	= 553.747 – 0.001 x Anise (g/kg)
Egg shell, %	0.321	0.140	= 14.570 – 0.030 x Anise (g/kg)
Yolk colour, L*	0.094	0.349	= 69.135 + 0.055 x Anise (g/kg)
Yolk colour, a*	0.664	0.029	= 2.020 + 0.011 x Anise (g/kg)
Yolk colour, b*	0.037	0.484	= 64.354 + 0.106 x Anise (g/kg)
Total cholesterol, mg/dL	0.065	0.406	= 196.111 – 2.233 x Anise (g/kg)
Triglycerides, mg/dL	0.552	0.053	= 222.278 – 2.183 x Anise (g/kg)

Moreover, small amounts of eugenol, estragole, methyl-chavicol, anisaldehyde, β-caryophilline, anise ketone (methyloxyphenylacetone) and the polymers of anethole are present in the oil (Ciftci *et al.*, 2005; Franz *et al.*, 2005; Al-Beitawi *et al.*, 2009).

In the present study, quail egg production was not significantly affected by the dietary incorporation of anise seed, a result in line with those of Bayram *et al.* (2007). However, El-Deeb *et al.* (2007) reported that feeding anise to quail decreased egg laying rate by 10.3%. In laying hens, Ali *et al.* (2007) recorded higher egg production when anise was supplemented to their diet. Moreover, in the present experiment daily feed intake did not differ significantly between the four groups. Bayram *et al.* (2007) noted an increase of feed intake of laying Japanese quail due to the addition of anise seeds to the diet. Mortality was not significantly affected over the total feeding period, a finding which is in agreement with that of Al-Beitawi *et al.* (2009) who fed anise seed to broilers. The lack of an effect on performance may be explained by the excellent hygienic condition in the present experiment, since it cannot be excluded that beneficial effects of feed additives might be more evident under less hygienic housing conditions and/or when using less digestible diets (Bonos *et al.*, 2010).

The supplementation of the quail diet with α-tocopheryl acetate at 600 mg/kg feed had no adverse influence on feed intake, mortality and egg production compared to the control group where the diet contained 30 mg α-tocopheryl acetate/kg feed. In a previous study, Sahin *et al.* (2006) observed that the dietary addition of α-tocopheryl acetate to quail diets increased egg production, but did not affect feed intake. Kucuk *et al.* (2003) and Biswas *et al.* (2010) reported increased egg production in laying hens, whereas Heydari *et al.* (2009) did not observe any improvement in laying performance.

Egg weight, yolk, albumen and shell percentage were not significantly affected by the dietary addition of either anise seed or α-tocopheryl acetate in the present study. In a previous study, Bayram *et al.* (2007) reported that egg weight was reduced when quail were fed anise seeds at inclusion levels of 3% and 5%. Other researchers (Ali *et al.*, 2007) noticed that egg weight and yolk, albumen and shell weight were not influenced when anise was fed to laying hens. Biswas *et al.* (2010) did not find any significant difference in egg weight, yolk and albumen percentage in laying hens fed added vitamin E.

The egg yolk colour, parameter a* (redness), was significantly increased by the dietary addition of α-tocopheryl acetate, but anise seed did not modify the L*, a* and b* colour parameters. Ali *et al.* (2007) did not record significant differences in egg yolk colour of hens fed anise seed. Similarly, Grobas *et al.* (2002) found no difference in egg yolk colour of hens that consumed increasing quantities of vitamin E.

The results of the present experiment showed that serum total cholesterol concentration was significantly reduced when anise seed were incorporated in the quail diet, a finding that agrees with those of Ali *et al.* (2007) in laying hens. In contrast, Soltan *et al.* (2006) found that anise seed in broiler diets did not affect serum total cholesterol concentration. The cholesterol reducing effect may be attributed mainly to the effect of the *trans*-anethole. According to researchers (Knight & Eden, 1996; Yildiz, 2005) phytoestrogen

consumption can regulate serum cholesterol levels in the human and animals. Moreover, in this study serum triglyceride concentrations were not affected by anise seeds or α-tocopheryl acetate. Ali *et al.* (2007) found that dietary anise decreased the levels of serum triglycerides and total lipids. Also, Arslan *et al.* (2001) did not record significant differences in plasma cholesterol and triglycerides levels when vitamin E levels were increased in the diets of broilers. Nevertheless, Francini *et al.* (1988) reported that the supplementation of vitamin E decreased cholesterol and triglyceride levels in broilers, though this effect was influenced by the age of the birds.

Conclusion

In conclusion, the incorporation of anise seeds in the feed of laying Japanese quail modified egg yolk colour and had a cholesterol lowering effect in blood serum, without having any adverse effect on performance and the other egg quality characteristics.

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