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Effect of Dietary Supplementation of Olive Leaves and/or α-Tocopheryl Acetate on Performance and Egg Quality of Laying Japanese Quail (*Coturnix japonica*)

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ABSTRACT

Nowadays, there is an increasing interest in the identification of natural substances that can increase animal production. In this experiment performance parameters and egg quality of laying Japanese quail dietary supplemented with olive leaves and/or α-tocopheryl acetate were studied. For this reason 108 *Coturnix japonica* quail (72 females and 36 males), 149 days old, were divided into four groups with three replicates of 9 birds each (6 females and 3 males). One group was given a basal commercial diet and served as control. The diets given to the other three groups were also based on the commercial diet with the addition of dried olive leaves powder at 10 g kg^{-1} or at 20 g kg^{-1} or α-tocopheryl acetate at 300 mg kg^{-1}. The quail were kept for 29 days under commercial conditions. Egg production, feed consumption and mortality were calculated. Also, egg weight, egg yolk, albumen and shell percentages, egg yolk colour (L*a*b* colour space), blood serum total cholesterol and blood serum triglycerides were determined at the end of the experiment. The dietary addition of olive leaves increased the egg production ($p \leq 0.001$), affected the egg yolk colour ($p \leq 0.05$) and modified the blood serum total cholesterol ($p \leq 0.01$). Moreover, regression analysis showed that the dietary addition of olive leaves had a linear effect on egg production, egg yolk colour and blood serum triglycerides. The dietary addition of α-tocopheryl acetate affected ($p \leq 0.05$) the egg yolk colour. In conclusion, dietary olive leaves could be used in Japanese quail nutrition without any adverse effect on their performance and egg quality.

Key words: Olive leaves, oleuropein, vitamin E, egg laying percentage, egg quality

INTRODUCTION

The Japanese quail (*Coturnix japonica*) has been widely used for biological and genetic studies because of its small body weight, easy management and ability to keep large numbers in limited area (Ayasan *et al*., 2005). Also, it has early sexual maturity and the turnover of generations is rapid (Yannakopoulos *et al*., 1995). Its egg production is high and as a result many off spring can be produces from a limited number of parents (Ayasan *et al*., 2005).

The olive tree (*Olea europaea* L.) is believed to have originated around the shores of the Mediterranean well over 5,000 years ago and is widely cultivated for the production of edible fruits and oil. This tree shows strong resistance against various microorganisms (Kubo *et al*., 1995) whereas, the olive leaves have traditionally been used to treat a variety of diseases (Benavente-Garcia *et al*., 2000). Olive leaves are agricultural residues from the beating of olive

trees for fruit harvest and represent around 10% of the total weight of the collected material, while their production is estimated to be 25 kg per olive tree (Delgado-Pertinez *et al.*, 1998). According to Silva *et al.* (2006) fresh olive leaves have total phenolic content (g kg^{-1}) between 11.6-17.4, while dried leaves between 11.7-40.1. The most active compound that has been identified in the olive leaves is oleuropein, which is a bitter monoterpene glycoside belonging to the secoiridoids class (Silva *et al.*, 2006). Furthermore, the olive leaves contain verbascoside, lingstroside, tyrosol or hydroxytyrosol, oleanic and maslinic acids, luteolin, arigenine, olivine, olivine-diglucoside (Silva *et al.*, 2006; Dekanski and Janicijevic-Hudomal, 2007) as well as a significant amount of tocopherol (Paiva-Martins *et al.*, 2009). According to Malik and Bradford (2008), the best method for processing and storage of olive leaves is simply drying them at room temperatures (25°C) in order to take advantage of health benefits from their polyphenols. Most of the phenolic compounds have been shown to possess hypoglycaemic and hypocholesterolemic activities (Romani *et al.*, 1999) to be potent antioxidants with anti-inflammatory properties (Benavente-Garcia *et al.*, 2000) to have antimicrobial properties (Bisignano *et al.*, 2001) and antiviral activity against DNA or RNA viruses (Fredrickson, 2000).

There are some reports on the use of olive leaves on animal nutrition. Molina-Alcaide and Yanez-Ruiz (2008) have used the leaves in the diets of goats and sheep and found that they could increase the efficiency of microbial protein synthesis in the rumen, while for lactating animals olive leaves resulted in an improvement in milk fat quality. Moreover, other researchers examined olive leaves or olive leaves extract in growing pigs (Paiva-Martins *et al.*, 2009) and in rabbits (Wang *et al.*, 2008). Concerning the poultry, the dietary olive leaves supplementation inhibited microbial growth and delayed lipid oxidation of turkey breast fillets during refrigerated storage (Botsoglou *et al.*, 2010).

Dietary vitamin E, used as α-tocopheryl acetate is a well known antioxidant, but its efficacy on performance and egg quality traits of laying quail has little been investigated (Sahin *et al.*, 2006). Supplementation of antioxidants in animal nutrition has an important role either for the oxidative stability of their products or the good health and improvement of their performance (Giannenas *et al.*, 2005).

Therefore, the present study was conducted to investigate the potential use of olive leaves and/or α-tocopheryl acetate as dietary supplements on performance and egg quality of laying Japanese quail.

MATERIALS AND METHODS

Olive leaves procurement: Olive leaves were obtained at a local oil press in Northern Greece during the oil production. These leaves were from olive trees not treated with any chemicals in the last 6 months. They were dried, milled and incorporated into the experimental diets. The chemical analysis of the olive leaves, performed according to the guidelines of AOAC (2005) is presented in Table 1.

Animals and diets: In this study, which was performed in 2009, a total of 108 *Coturnix japonica* quail (72 females and 36 males)149 days old, were divided into four groups with three replicates of 9 birds each (6 females and 3 males). All birds were individually weighted before placing them in the cages and the average body weight did not differ ($p>0.100$) between the four groups. The birds were fed a commercial layer diet (Table 2) *ad libitum* for an acclimatization period of 10 day. The commercial diet contained 30 mg α-tocopheryl acetate kg^{-1}. After this period the birds in the control group (CONTROL) remained on the same diet. The other three groups were fed the same diet with the addition of olive leaves powder at 10 g kg^{-1} (OLIVE10 group) or 20 g kg^{-1}

Table 1: Chemical analysis of olive leaves

Parameters	Values (g kg^{-1})
Dry matter	937
Crude protein	79
Crude fat	21
Crude fiber	191
Ash	49

Table 2: Composition of basal commercial diet

Item	%	Item	%
Ingredients		**Chemical analysis**	
Maize	45.67	Dry matter	90.00
Soybean meal	30.54	Crude protein	19.80
Wheat	10.00	Crude fat	4.50
Calcium carbonate	6.21	Crude fiber	3.40
Soybean oil	3.00	Ash	9.40
Coru gluten meal	2.77		
Dicalcium phosphate	1.04	**Calculated analysis**	
Vitamin and trace mineral premix*	0.35	Calcium	2.60
Salt	0.21	Total phosphorus	0.60
Sodium bicarbonate	0.19	Lysine	1.02
Methionine	0.02	Methionine and Cystine	0.72
		Metabolisable energy (kj kg^{-1})	12139

*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 g vitamin B_{12} 85 mg vitamin niacin, 20 mg pantothenic acid, 2 mg folic acid, 200 g 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

(OLIVE 20 group) or α-tocopheryl acetate at 300 mg kg^{-1} (TOCO group). The α-tocopheryl acetate product used was from Roche Products Ltd, Hertfordshire, UK. The birds were given feed and water *ad libitum* for a period of 29 days, while being kept under commercial conditions. The quail were handled according to the principles of the Greek Directorate General of Veterinary Services for the care of animals in experimentation.

The egg production, feed consumption and mortality were measured on a daily basis. At the end of the experiment, feed to egg conversion ratio (kg feed/kg of eggs) was calculated. Moreover, egg weight and egg yolk, albumen and shell (with shell membrane) percentages were measured in ten eggs per replicate. Also, the egg yolk colour was determined in a mixture of ten egg yolk from each replicate, using the L*a*b* colour space (L = Lightness, a = Redness, b = Yellowness) according to Herber-McNeill and Van Elswyk (1998) with the aid of a Konica Minolta Chroma Meter CR-410 (Japan).

At the last day of the experiment, blood serum total cholesterol of quail was measured according to Roeschlau *et al.* (1974) and blood serum triglycerides were measured according to Fossati and Prencipe (1982). For these measurements a biochemical analyzer Flexor E, Vital Scientific N.V. (Holland) was used.

Statistical analysis: The experimental data were subjected to statistical analysis with the aid of SPSS 16.0.1 statistical package (SPSS Inc., Chigaco, IL, USA). The general linear model function was used for the analysis of variance (ANOVA). Furthermore, the curve estimation function was used for regression analysis of the effect of the olive leaves. Pearson's chi square test was applied for the mortality analysis. A value of $p \leq 0.05$ was considered significant and a value of $0.05 < p \leq 0.10$

was considered a tendency. The homogeneity of the variances was examined with Levene's test (Olkin, 1960) and Tukey's test (John, 1998) was used to determine statistical differences between the means.

RESULTS

At the end of the experimentation feed intake values and mortality did not differ between the four groups whereas egg production was significantly ($p \leq 0.001$) higher for groups OLIVE10 and OLIVE20, compared to groups CONTROL and TOCO (Table 3). Moreover, according to the regression analysis of the effect of olive leaves on the performance parameters (Table 4) egg production showed a significant ($p \leq 0.05$) linear increase with $R^2 = 0.569$, but daily feed intake was not affected.

Table 5 presents the results concerning the effect of the dietary addition of olive leaves and α-tocopheryl acetate on some egg quality parameters. Egg weight, yolk, albumen and shell percentages did not differ significantly between the four groups. The egg yolk colour was significantly ($p \leq 0.05$) affected since the eggs of groups OLIVE20 and TOCO had higher a* parameter value compared to controls. Also, the eggs of group OLIVE20 had significantly higher b* parameter value compared to controls, whereas no difference ($p > 0.10$) was found for the L* parameter. Furthermore, regression analysis of the effect of olive leaves (Table 4) showed a significant ($p \leq 0.01$) linear increase of the a* parameter with $R^2 = 0.678$ and also a significant ($p \leq 0.05$) linear increase of the b* parameter with a $R^2 = 0.623$.

Regarding the effect of the dietary addition of olive leaves and α-tocopheryl acetate on blood serum total cholesterol and triglycerides these results are presented in Table 6. Total cholesterol

Table 3: Performance of laying Japanese quail in response to dietary supplementation with either olive leaves powder at 10 g kg^{-1} (OLIVE10) or 20 g kg^{-1} (OLIVE20) or α-tocopheryl acetate at 300 mg kg^{-1} (TOCO)

Group	Egg production (%)	Daily feed intake (g)	Mortality (%)
CONTROL	70.88±11.51[a]	32.25±4.27	0.0±0.0
OLIVE10	95.01±3.27[b]	31.32±1.64	0.0±0.0
OLIVE20	94.14±2.79[b]	33.55±1.38	3.7±6.4
TOCO	59.39±10.54[a]	31.50±0.48	0.0±0.0
p-value	0.001	0.670	0.387

Values are presented as Mean±SD. Values in the same column with a superscript in common do not differ significantly at $p \leq 0.001$

Table 4: Regression analysis of the effect of dietary olive leaves on some laying parameters, egg quality parameters and blood serum total cholesterol and triglycerides of laying Japanese quail

Parameter	P	R^2	A	B
Egg production (%)	0.019	0.569	75.05	+1.163
Daily feed intake (g)	0.571	0.048	31.718	+0.065
Egg weight (g)	0.721	0.019	11.95	+0.004
Egg yolk (%)	0.787	0.011	31.447	+0.007
Egg albumen (%)	0.448	0.085	54.013	-0.030
Egg shell (%)	0.460	0.080	14.542	+0.023
Yolk colour (L*)	0.502	0.067	69.008	+0.034
Yolk colour (a*)	0.006	0.678	2.289	+0.105
Yolk colour (b*)	0.011	0.623	64.213	+0.163
Serum total cholesterol (mg dL^{-1})	0.407	0.100	227.333	-1.733
Serum triglycerides (mg dL^{-1})	0.074	0.386	274.389	-4.683

Regression equation in the form of: Parameter = A+B × Olive leaves addition in feed (g kg^{-1}), L = Lightness, a = Redness, b = Yellowness, according to the L* a* b* colour space

Table 5: Egg quality parameters of laying Japanese quail in response to dietary supplementation with either olive leaves powder at 10 g kg^{-1} (OLIVE10) or 20 g kg^{-1} (OLIVE20) or α-tocopheryl acetate at 300 mg kg^{-1} (TOCO)

					Yolk colour		
Group	Egg weight (g)	Egg yolk (%)	Egg albumen (%)	Egg shell (%)	L*	a*	b*
Control	11.95±0.02	31.39±0.48	54.10±1.41	14.52±0.99	68.80±0.41	2.16±0.74^{a}	64.13±0.35^{a}
OLIVE10	11.99±0.25	31.64±0.48	53.54±0.54	14.82±0.82	69.60±1.70	3.60±0.75^{ab}	66.00±1.32^{ab}
OLIVE20	12.04±0.47	31.53±0.94	53.49±0.78	14.98±0.44	69.56±1.26	4.25±0.53^{b}	67.38±1.69^{b}
TOCO	11.84±0.24	32.27±1.14	53.73±2.68	14.00±1.80	67.65±0.58	3.72±0.10^{b}	65.11±0.72^{ab}
p value	0.866	0.583	0.964	0.728	0.194	0.013	0.043

Values are presented as Mean±SD. Values in the same column with a superscript in common do not differ significantly at p≤0.05
L = Lightness, a = Redness, b = Yellowness, according to the L* a* b* colour space

Table 6: Blood serum total cholesterol and triglycerides of laying Japanese quail in response to dietary supplementation with either olive leaves powder at 10 g kg^{-1} (OLIVE10) or 20 g kg^{-1} (OLIVE20) or a-tocopheryl acetate at 300 mg kg^{-1} (TOCO)

Group	Total cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})
Control	204.70±35.5^{ab}	257.00±74.8
OLIVE10	255.30±38.3^{a}	262.30±30.3
OLIVE20	170.00±27.8^{b}	163.30±35.1
TOCO	204.30±32.0^{ab}	259.30±116.2
p value	0.080	0.337

Values are presented as Mean±SD. Values in the same column with a superscript in common do not differ significantly at p≤0.10

values had a tendency (p≤0.10) to be lower for the quail of group OLIVE20 compared to group OLIVE10, while triglycerides values did not differ (p>0.10) between the four groups. In addition, regression analysis of the effect of the olive leaves on the above parameters (Table 4) showed a tendency (p≤0.10) for linear decrease of triglycerides with a $R^2 = 0.386$.

DISCUSSION

A major target in this study was to determine whether the olive leaves could be used to improve performance and egg quality of quail when incorporated into regular feed. Feed intake and mortality was not influenced by the dietary addition of olive leaves, whereas egg production was significantly increased with either 10 g kg^{-1} or 20 g kg^{-1} olive leaves. Results obtained in this study are difficult to compare with previous reports, since there are not pertinent *in vivo* studies with olive leaves. Christaki *et al.* (1994) reported that dietary olive pulp increased egg production in laying hens. Possibly a majority of the benefits of olive leaves and olive oil in laying bird diets are in fact due to the presence of a variety of phenolic compounds and particularly oleuropein which is the main active ingredient (Malik and Bradford, 2008). In growing pigs diets olive leaves improved meat quality, but they decreased feed intake and body weight gain and consequently increased feed to gain ratio (Paiva-Martins *et al.*, 2009). According to the above researchers, in pig diets the lower feed intake is induced by a reduction in palatability, due to oleuropein which is a bitter glycoside.

Dietary α-tocopheryl acetate at 300 mg kg^{-1} feed had no adverse influence on feed intake, mortality and egg production compared to the control group where the α-tocopheryl acetate was at 30 mg kg^{-1} feed. In a previous study, Sahin *et al.* (2006) observed that dietary addition of α-tocopheryl acetate in quail diets increased egg production, but did not affect feed intake.

Kucuk *et al.* (2003) and Biswas *et al.* (2010) found increased egg production in laying hens, whereas, Heydari *et al.* (2009) did not observe improvement in laying performance.

The egg weight, yolk, albumen and shell percentage were not significantly affected neither by the dietary addition of olive leaves nor by the dietary α-tocopheryl acetate. In an earlier study, Christaki *et al.* (1994) noticed increased egg, yolk and albumen weight for laying hens fed olive pulp. In addition, Biswas *et al.* (2010) did not find any significant difference on egg weight, yolk and albumen percentage in laying hens fed added vitamin E.

However, the egg yolk colour parameters a* (redness) and b* (yellowness) were significantly affected by the dietary addition of either olive leaves or α-tocopheryl acetate compared to the control diets. The effect of olive leaves on yolk colour is probably due to their chlorophyll content. In contrast, Grobas *et al.* (2002) reported no difference in egg yolk colour (using the Roche scale) of hens fed increased quantities of vitamin E.

The blood serum total cholesterol of quail was lowest in the group OLIVE20, while a tendency for reduction of serum triglycerides was noticed in the regression analysis. According to Wang *et al.* (2008), olive leaves extract in rabbit diets reduced serum total cholesterol and tryglicerides, which are related markers of arteriosclerosis and as a result inhibited experimental atherosclerosis.

CONCLUSIONS

Olive leaves may be an interesting nutritional supplement to include in laying quail diets in order to improve performance or egg quality traits. In this study, the dietary addition of olive leaves increased the egg production, affected the egg yolk colour and modified the blood serum total cholesterol, without any adverse effect. Moreover, regression analysis showed that the dietary addition of olive leaves had a linear effect on egg production, egg yolk colour and blood serum triglycerides.

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