



Comparative Evaluation of Dietary Oregano, Anise and Olive Leaves in Laying Japanese Quails

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ABSTRACT

Aim of the present study was the comparative evaluation of the effect of ground oregano, anise and olive leaves as feed additives on performance and some egg quality characteristics of laying Japanese quails. A total of 189 *Coturnix japonica* quails (126 females and 63 males), 149 days old, were randomly allocated into seven equal groups with three subgroups of 9 birds each (6 females and 3 males). A commercial laying diet was fed to the control group. The remaining six groups were fed the same diet supplemented with oregano at 10 g/kg or 20 g/kg, anise at 10 g/kg or 20 g/kg and olive leaves at 10 g/kg or at 20 g/kg. The birds were offered feed and water *ad libitum* for a period of 29 days, while being kept under commercial conditions. During the experiment, egg production, feed intake and mortality were recorded daily. At the end of the feeding period egg weight, egg yolk, albumen and eggshell weight percentages, egg yolk color (using the L*a*b* color space) and blood serum triglycerides were determined. The diets supplemented with olive leaves (10 g/kg or 20 g/kg) resulted in a tendency ($p = 0.054$) for higher egg production percentage. Also, the color parameter a* was significantly ($p = 0.001$) higher in the eggs of quails that consumed oregano (10g/kg or 20 g/kg) or olive leaves (10g/kg or 20 g/kg).

INTRODUCTION

Herbs and spices have a traditional history of use in the nutrition of humans and animals. Today, in the search for natural feed additives in animal diets, many studies focus on the antioxidant (Botsoglou *et al.*, 2002; Giannenas *et al.*, 2005; Florou-Paneri *et al.*, 2006), anticoccidial (Christaki *et al.*, 2004; Florou-Paneri *et al.*, 2004) or antimicrobial (Govaris *et al.*, 2007; Botsoglou *et al.*, 2010) properties of the plants. Consequently, such plants have attracted increasing interest as an alternative feeding strategy to replace antibiotic growth promoters.

Oregano (*Origanum vulgare* subsp. *Hirtum*) is a spice belonging to the Labiatae family, well known in Mediterranean countries. It contains a variety of bioactive components as carvacrol, thymol, γ -terpinene and p-cymene and exhibits considerable antimicrobial, antifungal and antioxidant activity (Giannenas *et al.*, 2004, 2005; Florou-Paneri *et al.*, 2005; Bampidis *et al.*, 2006). In previous studies ground oregano has proven to be a promising dietary supplement mainly in poultry nutrition (Bampidis *et al.*, 2005; Botsoglou *et al.*, 2005).

Anise (*Pimpinella anisum* L.) is an annual aromatic plant belonging to the Apiaceae family. It is cultivated mainly in Southern Europe and Southeast Asia. Anise fruits or the so-called seeds are the used parts of the plant (Al-Beitawi *et al.*, 2009). According to Franz *et al.* (2005) they contain 2-6% essential oils, phenolic acids, eugenol, estragole



and trans-anethole – a powerful phytoestrogen which is the main component of the oil (80-95%). Anise has been used over the years for its antioxidant (Gulcin *et al.*, 2003), antimicrobial (Al-Kassie, 2008), antibacterial (Tabanca *et al.*, 2003) and antifungal (Soliman & Badea, 2002) properties.

Olive leaves are agricultural residues from the beating of olive trees (*Olea europea* L.) for fruit harvest (Delgado Pertinez *et al.*, 1998). They contain many substances, such as oleuropein, which is a bitter monoterpene glycoside and their most active compound, as well as verbascoside, lingstroside, tyrosol or hydroxytyrosol, oleanic and maslinic acids, luteolin, arigenine, olivine, olivine-diglucoside (Silva *et al.*, 2006; Dekanski & Janicijevic-Hudomal, 2007). Most of the phenolic compounds have been shown to possess hypoglycemic and hypocholesteremic 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).

This study was designed to evaluate and compare the effects of the dietary use of oregano, anise and olive leaves in natural form on the performance and some egg quality characteristics of laying quails.

MATERIALS AND METHODS

Plant material collection

In this study, oregano consisted of flower tops and leaves of the plant. Moreover, from the anise plant, the seeds were used, whereas the olive leaves were collected from trees that were not treated with any chemicals for the last six months. All above plants of Greek origin were dried and ground before inclusion in the diets. Proximate analysis of oregano, anise and olive leaves, performed according to the guidelines of AOAC (2005), is presented in Table 1.

Table 1 - Chemical analysis of oregano, anise and olive leaves.

	Oregano g/kg	Anise g/kg	Olive leaves g/kg
Dry matter	883	935	937
Crude protein	108	129	79
Crude fat	64	138	21
Crude fiber	129	202	191
Ash	91	82	49

Birds and diets

A total of 189 *Coturnix japonica* quails (126 females and 63 males), 149 days old, were randomly allocated

into seven equal groups with three subgroups of 9 birds each (6 females and 3 males). All quails were individually weighed when placed in the cages, and their average weight did not differ ($p > 0.100$) between the seven groups. Quails were allowed to acclimatize for a period of 10 days, consuming a commercial laying diet in mash form *ad libitum* (Table 2). The same diet was fed to the control group (CONTR) during the experimental period. The remaining six groups were fed the above diet supplemented with 10 g oregano/kg (OREGA10), 20 g oregano/kg (OREGA20), 10 g anise/kg (ANISE10), 20 g anise/kg (ANISE20), 10 g olive leaves/kg (OLIVE10), or 20 g olive leaves/kg (OLIVE20). Birds were offered feed and water *ad libitum* for a period of 29 days, while being kept under commercial conditions. Quails were handled according to the principles of the Greek Directorate General of Veterinary Services for the care of animals in experimentation.

Table 2 - Composition of basal diet.

Ingredients	g/kg	Chemical analysis	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 fiber	34
Soybean oil	30.0	Ash	94
Corn gluten meal	27.7		
Dicalcium phosphate	10.4	Calculated analysis	
Vitamin and trace mineral premix ¹	3.5	Calcium	26.0
Salt	2.1	Total phosphorus	6.0
Sodium bicarbonate	1.9	Lysine	10.2
Methionine	0.2	Methionine & Cystine	7.2
		Metabolizable energy, kcal/kg	2900

1 - Supply per kg feed: 14000 IU vitamin D³, 30 mg vitamin E, 13 mg vitamin K, 3 mg vitamin B₁, 8 mg vitamin B₂, 3 mg vitamin B₆, 20 µg vitamin B₁₂, 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.

Throughout the experimental period, egg production, feed intake and mortality were recorded daily. At the end of the study, egg weight and egg yolk, albumen and eggshell (with shell membrane) weight percentages were determined in ten eggs per subgroup. Moreover, the egg yolk color was measured in a mixture of ten egg yolks from each subgroup using the L*a*b* color 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, blood serum triglycerides were measured in six quail per group (two quail from each subgroup), according to Fossati & Prencipe (1982), using a biochemical analyser Flexor E, Vital Scientific N.V. (Holland).

Statistical analysis

The statistical analysis was performed using the SPSS 16.0.1 statistical package (SPSS Inc., Chicago, IL, USA). The one-way analysis of variance (ANOVA) for the seven groups of the experimentation was performed using the general linear model function of SPSS. Pearson's chi square test was used to analyze mortality. 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. Duncan's test was applied to determine statistical differences between the means.

RESULTS AND DISCUSSION

The effect of oregano, anise and olive leaves on egg production, daily feed intake and mortality of quails at the last day of the experimentation are presented in Table 3. A beneficial effect ($p = 0.054$) was noticed on egg production of the groups OLIVE10 and OLIVE20 as compared to the remaining groups, except for the group OREGA10. Moreover, there was no egg production difference ($p > 0.100$) among OREGA10, OREGA20, ANISE10, ANISE20 and CONTR groups. There are no published data concerning the comparative use of oregano, anise and olive leaves in laying quail diets. As far as the use of olive leaves in quail nutrition is concerned, direct comparison with other studies cannot be made due to lack of pertinent reports. However, similar results were reported in laying hens fed olive pulp (Christaki *et al.*, 1994). The benefits of dietary olive leaves are possibly due to the presence of polyphenols and particularly oleuropein, the main active component in this material (Malik & Brandford, 2008). In previous studies relative to the supplementation of quail feeds with oregano only, no effect on egg production was found (Cetingul *et al.*, 2007, 2009), whereas El-Deeb *et al.* (2007) reported that dietary anise decreased quail egg production.

As indicated in Table 3, there were no differences ($p > 0.100$) in daily feed intake between the dietary treatments over the experimental period. According to other researchers (Cetingul *et al.*, 2007, 2009; Handl *et al.*, 2008), oregano did not affect the daily feed intake in quails. On the other hand, Bayram *et*

al. (2007) found that when anise was added to quail diets, feed intake increased. Moreover, there were no significant ($p > 0.100$) changes in mortality between the experimental groups, which is in agreement with Cetingul *et al.* (2009), who examined the dietary use of oregano in laying quails.

Table 3 - Performance of laying quail (mean \pm s.d).

Group ¹	Egg Production	Daily Feed Intake	Mortality
	% ²	g	%
CONTR	70.88 ^a \pm 11.51	32.6 \pm 4.3	0.0 \pm 0.0
OREGA10	79.89 ^{ab} \pm 14.36	31.2 \pm 2.6	3.7 \pm 6.4
OREGA20	77.78 ^a \pm 12.93	30.0 \pm 1.5	0.0 \pm 0.0
ANISE10	73.38 ^a \pm 13.32	28.6 \pm 4.0	3.7 \pm 6.4
ANISE20	76.25 ^a \pm 3.17	29.3 \pm 0.5	0.0 \pm 0.0
OLIVE10	95.01 ^b \pm 3.27	31.3 \pm 1.6	0.0 \pm 0.0
OLIVE20	94.14 ^b \pm 2.79	33.6 \pm 1.4	3.7 \pm 6.4
P value	0.054	0.319	0.668

1 - Groups: CONTR = control; OREGA10 = 10 g oregano/kg; OREGA20 = 20 g oregano/kg; ANISE10 = 10 g anise/kg; ANISE20 = 20 g anise/kg; OLIVE10 = 10 g olive leaves/kg; OLIVE20 = 20 g olive leaves/kg. 2Values in the same column with a superscript in common do not differ significantly at $P \leq 0.100$.

The effect of dietary treatments on some egg quality traits is shown in Table 4. There were no significant ($p > 0.100$) differences in egg weight and yolk, albumen and shell weight percentage between the seven groups. Cetingul *et al.* (2007, 2009) also reported no significant effect on egg weight when oregano was incorporated in quail diets. Bayram *et al.* (2007) observed that egg weight was reduced when anise was used as dietary supplement in laying quails. Eggs from quails fed either oregano or olive leaves at levels of 10 g/kg and 20 g/kg presented significantly ($p = 0.001$) different yolk color as compared to the other groups, since a* color value was higher, shifting towards red. This increase in yolk a* values might be due to the passage of pigments contained in the plants included in the diet into the egg yolk. No significant ($p > 0.100$) differences on L* and b* values were observed between all the dietary treatments. There is little information published on the effect of dietary oregano, on yolk color index of quail eggs. Cetingul *et al.* (2009) mentioned that the addition of oregano in quail feeds had no influence on yolk color. Moreover, there are no literature data relative to the effect dietary anise and olive leaves on yolk color of laying quails.

Serum total triglycerides values (mg/dl) were 257.0 \pm 74.8, 219.7 \pm 33.7, 144.7 \pm 109.0, 131.0 \pm 17.3, 213.3 \pm 94.5, 262.3 \pm 30.3 and 163.3 \pm 35.1 (mean \pm sd) for the quails of groups CONTR, OREGA10, OREGA20, ANISE10, ANISE20, OLIVE10 and OLIVE20, respectively,



Table 4 - Quail egg quality parameters (mean \pm s.d).

Group ¹	Egg weight	Egg yolk g	Egg albumen%	Egg shell%	Yolk Colour%		
					L*	a*2	b*
CONTR	11.95 \pm 0.02	31.39 \pm 0.48	54.10 \pm 1.41	14.52 \pm 0.99	68.80 \pm 0.41	2.16a \pm 0.74	64.13 \pm 0.35
OREGA10	12.27 \pm 1.03	32.42 \pm 1.06	53.35 \pm 0.97	14.24 \pm 1.36	69.24 \pm 2.46	4.30b \pm 0.29	66.65 \pm 3.01
OREGA20	11.64 \pm 0.48	31.79 \pm 0.83	53.67 \pm 1.08	14.53 \pm 0.71	67.50 \pm 0.76	3.76b \pm 0.97	64.68 \pm 0.65
ANISE10	11.63 \pm 0.26	32.59 \pm 0.85	53.04 \pm 0.82	14.38 \pm 0.19	70.19 \pm 0.31	1.85a \pm 0.65	65.85 \pm 1.01
ANISE20	11.82 \pm 0.04	32.01 \pm 0.95	54.07 \pm 1.62	13.92 \pm 0.79	69.97 \pm 0.90	2.37a \pm 0.13	66.25 \pm 1.42
OLIVE10	11.99 \pm 0.25	31.64 \pm 0.48	53.54 \pm 0.54	14.82 \pm 0.82	69.60 \pm 1.70	3.60b \pm 0.75	66.00 \pm 1.32
OLIVE20	12.04 \pm 0.47	31.53 \pm 0.94	53.49 \pm 0.78	14.98 \pm 0.44	69.56 \pm 1.26	4.25b \pm 0.53	67.38 \pm 1.69
P value	0.695	0.515	0.888	0.764	0.288	0.001	0.243

1 - Groups: CONTR = control; OREGA10 = 10 g oregano/kg; OREGA20 = 20 g oregano/kg; ANISE10 = 10 g anise/kg; ANISE20 = 20 g anise/kg; OLIVE10 = 10 g olive leaves/kg; OLIVE20 = 20 g olive leaves/kg. 2 - Values in the same column with a superscript in common do not differ significantly at $P \leq 0.001$.

and did not differ significantly ($p > 0.100$) between the groups.

CONCLUSIONS

Results of this study showed that there were no adverse effects from the dietary inclusion of oregano, anise and olive leaves in laying quail feeds. However, dietary olive leaves (10 g/kg and 20 g/kg) improved egg production and increased the a* value of the yolk colour. Also, dietary oregano (10 g/kg and 20 g/kg) increased the a* value of the yolk colour. Therefore, the use of herbal natural feed additives to benefit laying quail performance and egg quality seems to be promising.

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XXIV

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