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Effects of thymol and isoeugenol feed supplementation on quail adult performance, egg characteristics and hatching success

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Abstract 1. A study was conducted to evaluate whether feed supplementation with thymol or isoeugenol can alter Japanese quail growth rate and final body weight, the female onset of puberty, hen-day egg production and the physical and chemical characteristics of the egg, as well as its potential to alter hatchability.

2. From 4 to 16 weeks of age, birds from each cage (1 male: 3 females) were assigned to 1 of 3 treatments that differed in the supplement added to the feed: control, thymol or isoeugenol (400 mg/kg). The average ages (d) at first egg lay (FIRST), at 25% egg production (A25% EP), at 50% egg production (A50% EP) and weekly and cumulative hen-day egg production (HDEP) were calculated. In addition, physical and chemical characteristics of the eggs, their fertility and hatchability were also evaluated for each group.

3. Feed supplementation did not significantly affect growth rate, final body weight, egg production parameters, fertility and physical characteristics of egg or most of the fatty acid components of the yolk.

4. The group treated with isoeugenol showed an increase in the percentage of palmitoleic fatty acid compared to the control, with thymol group showing intermediates values.

5. Both thymol and isoeugenol supplemented groups showed increased hatchabilities, by 18·8% and 11·8%, respectively, compared to their control counterparts.

6. The improvement in the hatching success of the eggs from the thymol and isoeugenol supplemented groups without a negative impact on their performance may have important economic implications for future breeding programmes, particularly if these effects generalise from quail to other more commercially important poultry species, such as chickens or turkeys.

INTRODUCTION

Diet supplementation can be used to administer new compounds with beneficial effects. As pointed out by Acamovic and Brooker (2005), recent public concern about the use of synthetic compounds in animal diets to enhance performance and health and welfare issues, coupled with changes in some countries in regulations on the use of synthetic medicaments, has stimulated interest and research in the use and effects of phytochemicals in the diets of farmed animals. Herbs and spices can help to sustain good health and welfare of the animals and improve their performance and thus they could also help to increase the resistance of the animals exposed to different stress situations (Windisch *et al*., 2008; Brenes and Roura, 2010; Hashemi and Davoodi, 2010; Borazjanizadeh *et al*., 2011). Essential oils (EO) are complex mixtures of plant secondary metabolites consisting of low-boiling-point phenylpropenes and terpenes (Bakkalia *et al*., 2008; Brenes and Roura, 2010; Cross *et al*., 2011) and are used in the flavour and fragrance markets

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(Van De Braak and Leijten, 1999). Many effects and uses have also been reported for EOs during the last years (Lee *et al.*, 2004) and are summarised by Brenes and Roura (2010) and Hashemi and Davoodi (2010). Among their properties, they can be antimicrobial (Dorman and Deans, 2000; Lambert *et al.*, 2001; Rota *et al.*, 2004), hypolipidaemic (Srinivasan, 2004), anticholesterolaemic (Crowell, 1999), antioxidant (Botsoglou *et al.*, 1997, 2002; Kempaiah and Srinivasan, 2002; Gulcin *et al.*, 2004; Lee *et al.*, 2004; Govaris *et al.*, 2005; Luna *et al.*, 2010), digestive stimulant (Platel and Srinivasan, 2004; Cross *et al.*, 2007, 2011), antiviral (Bishop, 1995), antimycotic (Mari *et al.*, 2003; López *et al.*, 2004; Dambolena *et al.*, 2010), antitoxigenic (Ultee and Smid, 2001; Juglal *et al.*, 2002), antiparasitic (Santoro *et al.*, 2007), insecticidal or pest-repellent (Gillij *et al.*, 2008; Kembro *et al.*, 2009; Gleiser *et al.*, 2011), inhibitor of odour and ammonia control (Varel, 2002), as well as modulator of the GABA (stress control related neurotransmitter) receptor (Perillo *et al.*, 1999; Garcia *et al.*, 2006; Garcia *et al.*, 2008; Reiner *et al.*, 2009). Hence, further research on the potential beneficial use of EO as feed supplements may be of practical relevance. However, it has been shown that the biological activity and the EO chemical composition may vary with the geographic location, growth conditions and the part of the plant used to extract the EOs, as well as the extraction and isolation methods used to obtain them (Cosentino *et al.*, 1999; Faleiro *et al.*, 2005; Koroch *et al.*, 2007). Indeed, these EO chemical variations may explain the lack of consistency found between some studies evaluating bioactive effects of EO feed supplementation on avian performance (i.e. Botsoglou *et al.*, 2002; Lee *et al.*, 2004; Giannenas *et al.*, 2005; Barreto *et al.*, 2008). Therefore, evaluating the effects of single EO components would help to identify which molecules are responsible for the observed bioactive effects. The present study will focus on the effects of thymol and isoeugenol. These natural phenols are the main components of, for example, ''oregano'' and ''clove'', aromatic plants widely used in cooking (Sivropoulou *et al.*, 1996; Badger *et al.*, 2002; Gulcin *et al.*, 2004; Jirovetz *et al.*, 2006; Alma *et al.*, 2007).

Thymol, as an animal feed supplement, has been shown in mammals (rats) to help maintain higher levels of polyunsaturated fatty acids in the liver, brain, kidney and heart (Youdim and Deans, 1999*a*,*b*, 2000), suggesting that this compound acts as an effective free radicals scavenger and may influence the *in vivo* antioxidant defence systems. In birds, dietary supplementation with thymol has also shown to improve the oxidative stability of chicken eggs (Botsoglou *et al.*, 1997) and meat during storage (Luna *et al.*, 2010). Indeed, there is also evidence that thymol can be transferred to egg yolk (Krause and Ternes, 1999). On the other hand, little is known about the use of isoeugenol as a feed additive for animals. A few reports have also given information about the potential antioxidant activity of isoeugenol administration (Rajakumar and Rao, 1993; Tuckey *et al.*, 2009). In a previous Japanese quail study in our laboratory, isoeugenol feeding supplementation showed a reduced lipid oxidation in on fresh laid eggs (Labaque *et al.*, 2008). Nevertheless, given that thymol and isoeugenol are molecules that may have intrinsic bioactivities on animal physiology and metabolism (George *et al.*, 2001; Badger *et al.*, 2002; Reiner *et al.*, 2009) and even with similar reported molar equivalent antioxidant capacities (Dorman and Deans, 2000), it is conceivable that these two compounds could have a positive effect on several different aspects of birds' performance.

This study evaluated whether feed supplementation with thymol or isoeugenol can alter Japanese quail growth rate and final body weight, the female onset of puberty, hen-day egg production and physical characteristics of the eggs including fatty acid composition, as well as its potential to alter hatchability. Growth rate, final body weight and the egg production parameters were studied as a first approach to assess the potential consequences of these new enriched feeds on bird's development and productivity. The lipid composition of yolk is evaluated because of their importance regarding the nutritional requirement of the avian embryo (Noble and Speake, 1997; Speake *et al.*, 1998) and also the nutritional quality of eggs for human consumption (Logan, 2003). For example, lipids are required for the biogenesis of the cell membranes, and also to provide the avian embryo with almost all energy needed to sustain development within the egg (Noble and Cocchi, 1989; Noble and Speake, 1997; Speake *et al.*, 1998). A particular requirement during avian embryo development is the supply of certain long-chain highly-unsaturated fatty acids to the developing tissues in order to maintain appropriate functional development (Noble and Cocchi, 1989; Noble and Speake, 1997; Speake *et al.*, 1998). However, because animals are unable to synthesise polyunsaturated from saturated or monounsaturated fatty acids, the presence of adequate amounts in diet of both fatty acids types in the lipids of the yolk is essential to satisfy the demands of the developing tissues (Noble and Cocchi, 1989; Surai and Sparks, 2001). We used Japanese quail not only because it is considered an important agricultural species for meat and egg production in many countries (Caron *et al.*, 1990; Baumgartner, 1994; Jones, 1996) but also

because it is considered a useful animal model for the extrapolation of data to chickens and other commercially important poultry species (Mills and Faure, 1992; Jones, 1996; Minvielle, 2009).

MATERIALS AND METHODS

Animals and husbandry

Japanese quail (*Coturnix coturnix japonica*) were used in the present study. The birds evaluated (see below) were taken from a population of a single 402-bird hatch. Incubation, chick brooding, and lighting procedures were similar to those described by Kembro *et al*. (2008), with the exception that chicks were brooded from d 1 in mixed-sex groups of 50 within each of 8 brooder boxes, each measuring $90 \times 90 \times 60$ cm (length × width × height). Brooding temperature was set at 37·8°C during the first week of life, with a weekly decline of 2·8°C until final room temperature of 25 ± 2°C was achieved. Water and a starter feed ration (280 g/kg crude protein; 11·6 MJ ME/kg) were provided *ad libitum*. At 28 d of age, quail were sexed by plumage coloration, individually weighed, wing-banded for later identification, and 36 males and 72 females were randomly housed in groups of 1 male and 3 females into cages within two 6-tier cage battery units, each battery comprising 24 cages. Each cage measured $20 \times 45 \times 25$ cm (length × width × height). At this time, birds were also switched to a breeder ration (215 g/kg CP; 11·4 MJ ME/kg) with feed and water continuing *ad libitum*. Birds were subjected to a daily photostimulatory cycle of 14L : 10D with a light intensity of approximately 350 lux during the lighted portion of the day and lights-on occurring at 0600 h daily. Daily maintenance and feeding chores were carried out at the same time each day (0900 h).

Treatments and traits measured

From 4 to 16 weeks of age, birds from each cage were assigned to 1 of 3 treatments that differed in the supplement added to the feed: control (CON), 400 mg/kg of thymol (THY), and 400 mg/kg of isoeugenol (ISO). A 0·5% ethanol in water solution of those supplements was pulverised weekly then added to fresh commercial feed. The compounds used as supplements were obtained commercially: thymol (SAFC®, ≥ 99%, FCC, USA) and isoeugenol (SAFC®, ≥ 99%, mixture of cis and trans, FCC, USA).

All birds were also weighed at the end of the study (16 weeks of age) to assess final body weight and calculate the growth rate during feed supplementation. Growth rate was calculated by dividing the body weight reached at the end of the period of supplementation (16 weeks) on the initial (4 weeks) weight of birds (at the time of incorporating the feed supplemented). Daily egg production was recorded for 10 weeks beginning with the day on which the first bird laid an egg; this occurred at 41 d of age and was considered d 1 of lay. To assess the onset of puberty, the average ages (d) at first egg lay (FIRST) and at 25% and 50% egg production (A25%EP and A50%EP, respectively) were calculated for each group. A cumulative hen-day egg production (HDEP) was also calculated.

Following 6 weeks of feed supplementation, 480 eggs from the three supplement treatments (160 eggs each) were randomly collected during 10 consecutive days and individually marked with pencil, their fresh weight was obtained with a scale (to the nearest 0·1 g), and their length (major axis) and breadth (minor axis) were measured with callipers (to the nearest 0·1 mm). A total of 30 eggs from each treatment were stored at 4°C to perform *a posteriori* analyses of their physical and chemical characteristics (see below). The other 130 eggs per treatment were used to establish fertility and hatchability parameters (see below for details).

Half of the eggs collected to analyse their physical and chemical characteristics (15 eggs per treatment) were broken out and the yolk, albumen and shell were weighed in order to calculate the percentages of each component. The other half was used to assess fatty acid composition. All chemicals used in this study were reagent-grade commercial products. The fatty acid methyl esters were analysed according to the technique described by Young (1995). Briefly, samples of yolk (0·2 g) were suspended in 2·0 ml of methanol, placed into a 17 ml culture tube, treated with 0·5 ml of 2 M aqueous sodium hydroxide, and tightly sealed with a Teflon-lined screw cap. The culture tubes were then placed within screw-capped 250 ml plastic bottles and tightly sealed. This combination was then placed at the centre of a domestic microwave oven (ATMA, Model MD809, NEWSAN S.A., Argentina), operating at 2450 MHz and 800 W maximum output and heated at 80% power for 14 s. After heating and left to reach ambient temperature, each mixture was extracted with hexane (3× *ca*. 2 ml), all within the culture tube, and the apolar phase (hexane) was collected and discarded (unsaponifiable fraction), then, 2·5 ml of 2 M methanolic sulphuric acid solution were added. This solution was prepared with 196·2 g (108·7 ml) of sulphuric acid that were carefully added to 500 ml methanol. The solution was mixed and cooled in an ice bath, and finally completed to 1000 ml. The heating procedure was repeated and then

extracted with hexane (3× *ca*. 2 ml). The combined hexane extracts (fatty acids methyl esters) were evaporated to dryness and dissolved in hexane with the appropriate volume (100–500 µl) prior to Gas-Liquid chromatography analysis. Fatty acid profiles were analysed according to Maestri and Guzmán (1993).

Fatty acid identification

Fatty acid methyl esters were analysed by gas chromatography on a 60 m fused capillary column with an internal diameter of 0·25 mm (Polyethylene Glycol, Perkin Elmer Elite-WAX). The analysis was performed on a Perkin-Elmer Clarus® 600 Gas Chromatograph/Mass Spectrometer (GC/MS) equipped with a flame ionisation detector. Helium was used as carrier (constant flow of 49·6 psi). The injection port temperature was 250°C and the detector temperature was 250°C. Oven program temperature started on 180°C for 5 min and increased to 200°C at 4°C/min keeping at that temperature for 5 min. After that, the temperature was increased until 230°C at 3°C/min and kept there for 25 min. Quantification was carried out by normalisation and relative area percentages of each fatty acid methyl ester detected. All chemical determinations were conducted in duplicate for each egg.

Fertility and hatching success

Eggs were stored at 16°C for a maximum of 9 d until incubation. Eggs were placed into a preheated, forced-draft incubator designed for 900 quail eggs. The incubator temperature was set at 37·8°C, the humidity was held at 55 ± 2%, and eggs were turned every hour. At 14 d of incubation, eggs were transferred to hatching baskets and temperature and humidity maintained at 38°C and 65 ± 2%, respectively. At the end of d 17 of incubation, new hatchlings were removed from the incubator and non hatched eggs were opened to determine if they were fertile or not (i.e. presence or absence of an embryo). Eggs with no embryonic development were regarded as infertile. Hatchability was determined as the number of hatched eggs relative to the number of fertile eggs.

Statistical analysis

A one-way ANOVA was used to assess differences in final body weight, growth rate, puberty parameters (FIRST, A25%EP, and A50%EP), HDEP, and physical and chemical characteristics of eggs from different supplement treatments. Where appropriate, Fisher LSD tests were used for post-hoc comparisons. Chi-squared contingency table tests (Siegel, 1984) were used to compare the fertility and hatchability of eggs from each supplement treatment. A P value of <0·05 was considered to represent significant differences.

RESULTS

Results of final quail body weight and growth rate are presented in Table 1. ANOVA showed that at the end of the experimental period, neither body weight nor growth rate were affected by feed supplementation ($F_{2,101} = 0{\cdot}28$; $P = 0{\cdot}75$ and $F_{2,100} = 0{\cdot}27$; $P = 0{\cdot}76$, respectively). As expected for this species, females were heavier ($F_{1,101} = 68{\cdot}21$; $P < 0{\cdot}0001$) and had a higher growth rate ($F_{1,100} = 34{\cdot}88$; $P < 0{\cdot}0001$) than males (259·19 ± 3·58 and 2·91 ± 0·06 *vs* 217·49 ± 3·99 and 2·46 ± 0·07, respectively). There was no mortality during experiment and no bird showed any abnormal development.

Onset of puberty and cumulative HDEP are shown in Table 2. ANOVA showed that feed supplementation did not significantly affect any of the three parameters used to assess female

Table 1. *Mean (± SE) final body weight and growth rate in Japanese quail fed on diets supplemented with either thymol (THY, 400 mg/k basal diet), isoeugenol (ISO, 400 mg/k basal diet) or control (CON)*

	Feed supplementation treatment		
	CON	THY	ISO
Body weight	248·44 ± 6·04	243·71 ± 4·76	242·35 ± 5·66
Growth rate	2·76 ± 0·06	2·67 ± 0·05	2·78 ± 0·09

Table 2. *Mean (±SE) age at first egg lay (FIRST), 25% (A25% EP) and 50% (A50% EP) egg production and cumulative (10 weeks of lay) hen day egg production in Japanese quail fed on diets supplemented with either thymol (THY, 400 mg/k basal diet), isoeugenol (ISO, 400 mg/k basal diet) or control (CON)*

Feed supplementation	FIRST	A25% EP	A50% EP	Cumulative (10 w) HDEP
CON	42·83 ± 2·04	44·17 ± 1·56	51·17 ± 1·30	0·86 ± 0·02
THY	43·50 ± 2·26	45·33 ± 1·67	49·33 ± 2·60	0·85 ± 0·02
ISO	42·67 ± 1·86	43·67 ± 1·20	48·17 ± 2·74	0·87 ± 0·02

onset of puberty ($F_{2,15}=0{\cdot}27$; $P=0{\cdot}76$; $F_{2,15}=0{\cdot}33$; $P=0{\cdot}72$ and $F_{2,15}=0{\cdot}43$; $P=0{\cdot}66$ for FIRST, A25%EP and A50%EP, respectively). Similarly, cumulative HDEP (full 10 weeks of lay) was not affected by the quail diet supplementation ($F_{2,15}=0{\cdot}39$; $P=0{\cdot}68$).

Neither THY nor ISO affected the measured physical characteristics of the quail egg (Table 3) or most of the fatty acid components of the yolk (Table 4). However, ANOVA showed a significant main effect ($F_{2,25}=4{\cdot}32$, $P=0{\cdot}02$) of the feed supplementation for the palmitoleic (16:1) fatty acid content. Post-hoc analysis showed that the group treated with ISO showed an increase ($P<0{\cdot}01$) in the content of palmitoleic acid compared to the CON group, while the THY group showed intermediate values (Table 4).

Fertility of eggs was not significantly influenced by feed supplementation ($\chi^2=2{\cdot}44$, df. $=2$; $P=0{\cdot}30$) (Table 5). Nevertheless, a significant effect of feed supplementation was observed for hatchability ($\chi^2=14{\cdot}35$, df. $=2$; $P=0{\cdot}001$) (Table 5). Specifically, the analysis showed that both THY and ISO supplemented groups showed an increased number of hatchlings compared to the CON group (18·8% and 11·8% increase, respectively) ($\chi^2=13{\cdot}76$, df. $=1$; $P<0{\cdot}001$; $\chi^2=4{\cdot}84$, df. $=1$, $P=0{\cdot}03$, respectively). No difference in the hatching success was found between the THY and ISO supplemented groups ($\chi^2=2{\cdot}46$, df. $=1$, $P=0{\cdot}12$).

Table 3. *Mean (±SE) of physical characteristics of quail eggs laid by females fed on diets supplemented with either thymol (THY, 400 mg/k basal diet), isoeugenol (ISO, 400 mg/k basal diet) or control (CON)*

Physical characteristics of eggs	Feed supplementation		
	CON	THY	ISO
Weight (g)	12·84 ± 0·20	12·81 ± 0·25	13·02 ± 0·21
Length (cm)	3·47 ± 0·02	3·40 ± 0·04	3·41 ± 0·03
Breadth (cm)	2·60 ± 0·01	2·61 ± 0·01	2·64 ± 0·01
Eggshell (%)	13·23 ± 0·46	13·87 ± 0·34	12·95 ± 0·36
Yolk (%)	29·36 ± 0·57	27·48 ± 0·83	29·48 ± 1·61
Albumen (%)	53·62 ± 0·99	52·37 ± 1·02	53·3 ± 0·33

DISCUSSION

Phytogenic feed additives including herbs and essential oils or their main components have been proposed as alternatives to synthetic growth promoters (Lee *et al*., 2004, Bakkalia *et al*., 2008; Brenes and Roura, 2010; Hashemi and Davoodi, 2010). In the present study, dietary supplementation with THY and ISO did not increase quail weight and growth rate. These results are in agreement with Lee *et al*. (2003*a*, *b*) who showed that feed supplementation with thymol (100 mg or 200 mg/kg of balanced food) did not affect the growth performance of broilers during periods of rapid growth (from 0 d to 40 d) and Botsoglou *et al*. (2002), Florou-Paneri *et al*. (2005) and Papageorgiou *et al*. (2003) who reported that dietary supplementation with oregano essential oil (that also contains thymol as a major component) had no effect on growth performance in turkeys. Furthermore, in chicken diets, Borazjanizadeh *et al*. (2011) incorporated dried cloves leaves (containing eugenol as a main component) in the feed, and showed no changes in the birds' body weight. There is not much information regarding the effects of isoeugenol as additive in poultry diets to allow us to further discuss our performance results. Although there are differences in the species under study, the doses and time of supplementation, the way in

Table 4. *Percentage (±SE) of fatty acid composition of the yolk lipid fraction of quail eggs laid by females fed on diets supplemented with either thymol (THY, 400 mg/k basal diet), isoeugenol (ISO, 400 mg/k basal diet) or control (CON)*

Fatty acid Composition (%)	Feed supplementation treatment		
	CON	THY	ISO
16:0 (palmitic)	22·21 ± 0·43	22·90 ± 0·40	22·42 ± 0·42
16:1 (palmitoleic)	2·56 ± 0·11[a]	2·86 ± 0·13[a]	3·26 ± 0·21[b]
18:0 (stearic)	11·53 ± 0·53	10·64 ± 0·44	10·55 ± 0·67
18:1 oleic	42·02 ± 0·95	41·64 ± 0·95	43·27 ± 0·68
18:2 linoleic	20·68 ± 0·92	20·79 ± 0·68	19·66 ± 0·49
18:3 linolenic	0·91 ± 0·08	1·08 ± 0·18	0·75 ± 0·04
20:4 arachidonic	0·09 ± 0·01	0·10 ± 0·01	0·10 ± 0·01
Total saturated	33·1 ± 0·79	33·37 ± 0·62	32·69 ± 0·62
Total unsaturated	65·04 ± 0·85	66·15 ± 0·62	66·46 ± 0·58
Total monounsaturated	43·74 ± 0·97	44·29 ± 1	46·13 ± 0·79
Total polyunsaturated	21·3 ± 0·98	21·86 ± 0·81	20·33 ± 0·51
Saturated/Unsaturated	0·51 ± 0·02	0·51 ± 0·01	0·49 ± 0·01

[a,b]Values without a common letter differ significantly ($P<0{\cdot}01$).

Table 5. *Performance parameters of quail eggs collected from females fed on diets supplemented with either thymol (THY, 400 mg/k basal diet), isoeugenol (ISO, 400 mg/k basal diet) or control (CON)*

Performance parameters	Feed supplementation		
	CON	THY	ISO
Fertility	94·3	97·7	96·1
Hatchability	63·4[a]	85[b]	78[b]

[a,b]Values without a common letter differ significantly ($P<0·01$).

which they are incorporated into the dietary supplement (dried leaves, essential oil, etc.) and some particular aspects of each investigation, our results are consistent with the majority of the studies on essential oils or their main components that show no negative effects on body weight gain or final body weight (Windisch *et al*., 2008).

Feed supplementation with THY and ISO had no detectable effect on onset of puberty nor cumulative hen production of females and had no impact on physical characteristics of eggs. Although these results cannot be directly compared with literature data (since we are not aware of any previous report on the potential effect of thymol or isoeugenol supplementation on quail female performance), Cetingul *et al*. (2009) supplemented oregano leaf (10 to 50 g/Kg of feed) into quail diet and reported no significant changes in egg production or physical variables of eggs.

In the present study significant statistical differences were found only in the palmitoleic acid (16·1) content of ISO supplementation, although total polyunsaturated fatty acids (PUFAS) content did not show statistical differences among the different groups. This is in agreement with Bölükbasi *et al*. (2010) who reported *Citrus bergamia* essential oil supplementation did not affect total PUFAS content.

Broiler breeder hatchability ranges from 79 to 82%, turkey egg hatchability ranges from 76 to 80% (Schaal and Cherian, 2007) whereas quail egg hatchability range from 60 to 80% (Mani *et al*., 2008; Dere *et al*., 2009) meaning that there is scope for improving this production parameter. In this context, the most significant effect detected in the present study was the increase in quail hatching success both in the group feed supplemented with thymol and with isoeugenol (18·8% and 11·8% increase, respectively). To our knowledge, this is the first report of an improvement in hatchability as a consequence of diet supplementation with a main component of essential oils in quail. In accordance with this background, Radwan *et al*. (2008) and Ali *et al*. (2007) also found that incorporating oregano (10 g/kg of basal diet) or thyme (2·5 mg/kg of basal diet) herbs into chicken food improved hatchability nearly 18% and 4%, respectively. Consequently, if the results observed herein with quail generalise to other poultry of higher economic relevance such us broiler breeders, this results may have a significant impact on the industry. In this regard, it is noteworthy to highlight that quail is a species widely used as a study model for poultry and results often extrapolate to domestic chicken due to their physiological similarities and the proximity of the two genera *Coturnix* and *Gallus* (Mills and Faure, 1992; Jones, 1996; Minvielle, 2009).

Incorporation of essential oils rich in phenolic compounds such as THY or carvacrol in laying hen's diet have been shown to have a protective antioxidant effect in egg yolk lipids (Botsoglou *et al*., 2005) and improvements in hatchability (Bozkurt *et al*., 2008). This is in agreement with our finding of an increased hatchability by the incorporation of pure main components of EO (THY and ISO) into the diet of laying hens, and with previous results from our laboratory by Lábaque *et al*. (2008) who reported a protective effect on yolk lipids oxidation as a consequence of the ISO feed supplementation (200 mg/Kg of basal diet), showing a lower concentration of yolk malondialdehyde in this group. In this context, the hatchability improvement found in this study could be related to an antioxidant effect in egg yolk lipids as a consequence of diet supplementation. THY and ISO are two natural phenolic compounds of known radical scavenger and lipophilic properties (Dambolena *et al*., 2011) that can interact with biological membranes modifying their permeability (Sanchez *et al*., 2004; Reiner *et al*., 2009). This would allow them to reach the yolk matrix, to exert their radical scavenger activity and to interfere with oxidative process during the lipid metabolism. This is in agreement with Krause and Ternes (1999) who reported that the transfer of the antioxidant constituents of natural supplements into the hen through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids and therefore, decrease the oxidation of components transferred into the yolk.

To our knowledge, there are no results showing the relationship between PUFA content and hatching success in quail eggs. However, Radwan *et al*. (2008) suggested that the improvement in hatchability success related to diet supplementation with natural antioxidant is associated to an antioxidant protective effect of PUFAs in eggs yolk. This is not in concordance with our results, where the hatchability improvement is not associated with an increased PUFAs content. Thus, it is possible that improvement in

hatchability may be explained by other mechanisms not linked to PUFA, linking oxidative stress to hatching success when these compounds are incorporated in the maternal diet. The reduced changes in fatty acid composition on the supplemented groups could be related to newly laid eggs (which are the case in the present study) not having the same fatty acids composition than eggs with embryo development. Indeed, Speake *et al*. (1998) and Noble and Speake (1997) detailed that the development of the chick embryo depends on two separate phases of extremely intensive lipid metabolism. Particularly, the second phase occurs during the end of embryonic life and is associated with the accumulation of highly polyunsaturated fatty acids within the lipids of several embryonic tissues (Noble and Cocchi, 1989; Latour *et al*., 2000). Thus, it is conceivable that during embryo development these compounds may increase the assimilation/absorption and stability of the available polyunsaturated fatty acids in the yolk and consequently, enhance the hatching success of the eggs laid by quail treated with these supplements. Nevertheless, further studies should be performed in order to evaluate the relation between changes of fatty acid content and oxidative stress with hatchability success in different periods of quail embryo development, in order to test the proposed hypothesis.

In conclusion, the present observations, showing an improvement in the hatching success of the eggs from thymol and isoeugenol supplemented groups without a negative impact on their performance, may have important economic and health implications for future breeding programmes, particularly if these effects generalise from quail to other more commercially important poultry species.

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