Accepted Article

Thymol feed supplementation in quail alters the percentages of nutritionally relevant egg yolk fatty acids: effects throughout incubation[†]

Running title: Thymol supplementation in quail alters egg yolk fatty acids throughout incubation

Maria E Fernandez[a,b], Raul H Marin[a,b], Agustin Luna[a,b], Maria P Zunino[b,c], Maria C Labaque[a,b,*]

[a]Instituto de Investigaciones Biológicas y Tecnológicas (CONICET-Universidad Nacional de Córdoba), Av. Vélez Sársfield 1611 (X5016GCA), Córdoba, Argentina.

[b]Instituto de Ciencia y Tecnología de los Alimentos (FCEFyN-Universidad Nacional de Córdoba)

[c]Instituto Multidisciplinario de Biología Vegetal (CONICET-Universidad Nacional de Córdoba), Córdoba, Argentina

[*]**Corresponding author:** maria.carla.labaque@unc.edu.ar

[†] The paper was given partially at the Poultry Science Association Annual Meeting 2015

ABSTRACT

BACKGROUND: PUFA are crucial components of the yolk and particularly prone to oxidative damage generating losses of nutrients for embryonic development and influencing the quality of eggs for human consumption. We evaluated whether dietary thymol (a natural antioxidant) is related to changes in quail egg yolk total, triglycerides and phospholipids fatty acid composition (T, TG, PL, respectively) at different stages of embryo development. Thus, female Japanese quail (100d) were assigned to 1 of 2 dietary

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jsfa.8407

This article is protected by copyright. All rights reserved.

Accepted Article

treatments (12 individuals each): CON (basal diet) or THY (0.0016mol of thymol day^{-1} $animal^{-1}$). After 2 weeks of supplementation, eggs were incubated and samples were obtained at 0, 4 and 16d of embryonic development.

RESULTS: In 0d-THY-eggs, alpha-linolenic acid and n-3 PUFA in T and TG, docosahexaenoic acid and PUFA in T, and arachidonic acid in TG were increased, while SFA in T was reduced. From 4d on, PUFA, n-3 PUFA and SFA from T and TG in THY-eggs, were found similar to CON-eggs. The changes in PL throughout incubation were similar in both dietary treatments.

CONCLUSION: Thymol would provide the embryo with PUFA for synthesis/deposition in membranes and/or derive to supply energy. Additionally, thymol supplementation would be advisable for the production of healthier table-eggs.

Keywords: poultry; maternal diet; embryonic development; lipid fractions; natural antioxidants

INTRODUCTION

The triglycerides (TG) and phospholipids (PL), both of which are highly unsaturated, constitute almost all the yolk lipids.[1] The yolk lipids are not only the major energy source for embryo development but also provide fatty acids (FA), particularly long-chain (LC) polyunsaturated fatty acids (PUFA), with defined roles in the functional development of certain tissues.[2] In this manner, FA and the energy derived from its beta oxidation are partitioned between different purposes (e.g. energy source and functional development of the embryo) depending on the stage of development, resulting in relevant changes of the FA composition of yolk lipids as incubation proceeds.[1-4] Avian embryos undergo extremely

This article is protected by copyright. All rights reserved.

Accepted Article

rapid development over a relatively short period of time and thus PUFA are likely to suffer great levels of oxidative damage unless this is mitigated by sufficient maternal allocation of appropriate antioxidants.[5]

Maternal traits and its interaction with external factors influence circulating levels and its body stores of lipids and lipid-soluble antioxidants which eventually shall be allocated to the egg.[6,7] At the same time, diet composition stands out among external factors that influence the quality and quantity of FA and antioxidants that female birds allot for egg formation.[8] Like other animals, birds are unable to synthesize PUFA *de novo*, therefore the presence of adequate amounts of its precursors in maternal diet (and in the yolk) is essential to satisfy the demands of the developing embryo.[8]

Diet supplementation can be used as a strategy in animal nutrition to administrate compounds with beneficial effects on health. Indeed, by this mean, a lipophilic antioxidant can be uniformly incorporated into lipidic molecules where it can effectively inhibit the oxidative reactions at their localized sites.[9,10] Essential oils (EO) supplemented into the diet have received increased attention in the last decades due to their antioxidant properties and beneficial influence on lipid metabolism, performance, health and welfare issues.[10-14] Particularly, the antioxidant potential of some natural phenolic components of EO would be similar to that of conventional synthetic compounds and its use in animal feed would not raise safety concerns for consumers of animal products.[15-17] The present study focuses on the effects of thymol (THY, 2-Isopropyl-5-methylphenol), the main component of ‘‘*oregano*’’ and ‘‘*thyme*’’ essential oils.[11] This component is considered a compound of interest in food industry and has the "generally recognized as safe" (GRAS) status of United States government-approved food additives, with safety levels calculated in a wide

This article is protected by copyright. All rights reserved.

Accepted Article

range of animal species by the European Food Safety Authority.[17] Thymol is an effective reactive oxygen species scavenger with minimum pro-oxidant effects.[11,15] The antioxidant activity of THY has been explained to be due the presence of OH phenolic group which donates hydrogen to the peroxy radicals produced during the first step in lipid oxidation, thus retarding the hydroxyl peroxide formation.[10,11]

THY can cause effects at various levels and its magnitude may be related to the amount of the component that is actually incorporated, redistributed into the body (including allocation to the egg yolk) and excreted.[16,18-20] Metabolism of this monoterpenoid has been studied in several species. For example, in humans THY is quickly absorbed after oral, pulmonary or dermal administration and is metabolized and eliminated by the kidneys.[10] In piglets, it has been demonstrated that THY is mainly and almost completely absorbed in the stomach and the proximal small intestine.[11] It has been demonstrated also that THY and/or its metabolites (mainly sulfate and glucuronide conjugates, and to a lesser extent thymoquinol) are rapidly excreted through urine and/or faeces in quail, rats, rabbits, dogs and humans.[11,21] Of great interest to our study are the recent findings by Haselmeyer et al.,[18] who demonstrated that dietary THY is efficiently absorbed and eliminated since only traces were found in tissues as a result of thyme supplementation in broiler diets. Also, studies in hens fed with thyme-extract[22] and in quails fed with THY,[21] have shown that dietary THY is allocated into the egg yolk. In hens, approximately, 0.006% of the ingested THY was transferred to egg yolk after 12 days of feeding.[22] Regarding effect on FA, it has been demonstrated that dietary supplementation with THY led to an increment in unsaturated FA in female quail liver,[19] where egg yolk lipids are formed. In addition, thyme and THY supplementation showed to improve the oxidative stability of chicken eggs,[23,24] and broiler

This article is protected by copyright. All rights reserved.

Accepted Article

meat during storage.[16] In these studies, malondialdehyde concentrations in THY or thyme treated samples were lower than in untreated ones. Moreover, in female quail, the dietary supplementation with THY also showed an improved hatching success.[25] Further, in tissues such as plasma, brain and muscle of mammalian and avian species, THY has increased PUFA precursors, i.e. linoleic acid and alpha-linolec acid.[11,20]

Taking into account that THY can be transferred to the egg yolk, along with its lipophilic and antioxidant properties in vivo, we propose that THY supplementation will enhance the PUFA of the egg yolk that should be revealed in the total fatty acids (T) as well as in both main lipid fractions, TG and PL. Thus, providing a source of high quality FA not only for embryo development but also for human consumption. The aim of the present study is to determine whether THY feed supplementation on female Japanese quail diet is related to changes in the egg yolk FA composition at different stages of embryo development.

EXPERIMENTAL

Ethics

Experiments were carried out in accordance with international standards of care and use of laboratory animals and approved by the Institutional Committee on Care and Use of Animals of Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba (ACTA 4/2015 Resolución 571-HCD-2014).

Animals and husbandry

Female Japanese quail (*Coturnix coturnix* -Linnaeus 1758-) were used in the present study. The birds studied were taken from a population of a single 210-bird hatch. Egg incubation, chick brooding, and lighting procedures were similar to those described by Labaque et al.,

This article is protected by copyright. All rights reserved.

Accepted Article

[26] At 28d of age, 24 females were housed in pairs in 12 cages measuring 20cm × 45cm × 25cm (length × width × height), meanwhile 6 males were housed individually in separated cages (without visual contact with females) (see details below). From hatch to 28d of age, all birds were fed a starter ration (24% of Crude Protein and 2900kcal of Metabolizable Energy kg diet^{-1}). From this age on and until feed supplementation was initiated (100d of age), birds were fed with a layer ration (20% of Crude Protein and 2900kcal of Metabolizable Energy kg diet^{-1}). At all ages feed and water were provided *ad libitum*. Birds were provided a 14h light (0600–2000h; approximately 180 cd), 10h dark cycle. Daily maintenance and feeding chores were done at the same time each day (0900h).

To ensure that all females were habituated to the presence of males and laid fertile eggs before starting the supplementation protocol, from 80d of age and until the end of the study they were visited by males during 10min three times per week (non-consecutive days).[27] During the study, all males were incorporated randomly in all cages to minimize potential male effects.

Diets

At 100d of age, females within each cage were randomly assigned to 1 of 2 feed treatments (12 individuals each one): Control (CON; basal diet) or THY (0.042mol of THY kg of basal diet^{-1}). THY was commercially obtained from Sigma Aldrich, SAFC®, ≥99%, FCC, USA) per kg of supplemented feed which is equivalent to a dose of 0.0016mol of THY day^{-1} animal^{-1}. Female body weight did not differ ($P=0.90$) between treatment groups prior to feed supplementation assignments (335±48g and 337±37g for CON and THY, respectively). The dose of THY was selected because is within the concentration range to

This article is protected by copyright. All rights reserved.

Accepted Article

which its transference to the egg has been proved both in hen[22] and quail. [21] Birds were fed daily *ad libitum*.

Both THY supplemented and CON layers diets had corn, soybean disabled, wheat bran, soybean pellets, sunflower pellets, calcium, salt, vitamins, minerals and phosphate in identical compositions. Nutrient composition of the diets is shown in table 1.

THY diet was prepared according to Labaque et al.,[26] Feed was prepared weekly and stored (maximum of 7d) at room temperature (23±2°C) and relative humidity (40±5%) in airtight and photoresistant containers. FA composition of control and THY diet are shown in table 2.

Egg handling and artificial incubation

Following two weeks of feed supplementation, to guarantee that transference of THY to the egg reached the concentration plateau pointed out by Krause and Ternes,[22] egg from both treatments were collected during 10 consecutive days and stored at 16ºC for a maximum of 9d until incubation (temperature: 37.8ºC; humidity: 55.2%; eggs turning every hour)[25] to obtain samples at three stages of embryonic development: 0, 4 and 16d of incubation, where initial-, early- and final- embryo development were respectively expected. We selected these periods of incubation to include both the changes that take place during early development, which not always are considered in developmental studies, and the later stages, characterized by a sheer intensity of lipid transfer from the yolk to the embryo.[1] Eggs from each treatment combination (diet and incubation day) were then stored at -20°C for later analysis of T, TG and PL (see below).

Fatty acids analysis

This article is protected by copyright. All rights reserved.

Accepted Article

Egg lipids were extracted from the yolk following homogenization in a suitable excess of chloroform/methanol (2:1).[28] Solvents were removed under reduced pressure in a rotary evaporator. Methyl esters of T, PL and TG of the yolk were obtained. All chemicals used in this study were reagent-grade commercial products.

Lipids were subjected to alkaline saponification (1N potassium hydroxide in methanol) and the unsaponifiable matter extracted with n-hexane. The fatty acid methyl esters (FAME) were prepared by transmethylation through treatment with N sulphuric acid in methanol and analyzed by gas chromatography (GC-MS) according to Labaque et al.[29]

Thin-layer chromatography (TLC) on silica gel G, using a Hexane/Diethyl ether/Acetic acid (80:20:1 v v^{-1}) solvent system, was used to separate the total lipid extract into two of its major fractions (TG and PL) according to the technique described by Royle et al.[30] The separated bands were visualized with iodine vapor and were identified by comparison with the migration of lipid standards in this system. The bands were scrapped from the plates and the lipids were eluted from the silica by vortex mixing with 3 x 5 ml of either chloroform/methanol/water (5:5:1 v v^{-1}; PL) or diethyl ether (TG). The isolated TG and PL fractions were subjected to transmethylation as described above.

FAMEs were analyzed by gas chromatography on a 60m fused capillary column with an internal diameter of 0.25mm (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 ionization detector. Helium was used as carrier (constant flow of 49.6 psi). The injection port temperature was 250ºC and the detector temperature was 250ºC. Oven program temperature started on 180ºC for 5min and increased to 200ºC at 4ºC min^{-1} keeping at that temperature for 5 min. After that, the temperature was increased

This article is protected by copyright. All rights reserved.

Accepted Article

until 230ºC at 3ºC min^{-1} and kept there for 25min. Quantification was carried out by normalization and relative area percentages of each fatty acid methyl ester detected. The FA content was expressed as a percentage of the T of the chromatographic run.

Statistical Analysis

Data were analyzed as 2 x 3 factorial arrangements with diet (THY and CON) and stage of incubation (0, 4 and 16d of incubation) as main factors, using the two-way ANOVA procedure on InfoStat.[31] Means were compared for significant differences ($P<0.05$) by using the LSD Fischer Test. To better fit ANOVA assumptions, variables were transformed to ranks prior to analysis.[32]

RESULTS

Total Fatty Acids

Table 3 summarizes statistical information regarding total fatty acid composition of quail eggs at 0, 4 and 16d of incubation laid by females fed on diets supplemented with THY or CON. Thymol dietary supplementation led to 0d egg yolks with increased percentages of alpha-linolenic acid, docosahexaenoic acid, arachidonic/linoleic acids and docosahexaenoic acid/alpha-linolenic acids ratios, PUFA and n-3 PUFA, and reduced percentages of n-6 PUFA/n-3 PUFA ratio, SFA, SFA/UFA ratio, compared to the CON. As incubation proceeded in THY-eggs the initial percentages of alpha-linolenic acid, docosahexaenoic acid, PUFA and n-3 PUFA decreased and the SFA and SFA/UFA ratio increased by the 4d measurement and remained unchanged until the end of the incubation. In THY-treatment, the ratio arachidonic/linoleic acids transiently decreased by 4d while the ratio docosahexaenoic/alpha-linolenic remained unchanged. Otherwise, the ratio n-6 PUFA/n-3

This article is protected by copyright. All rights reserved.

Accepted Article

PUFA was increased at later developmental stages in THY-group (reaching similar values to the CON). The percentage of palmitic acid was similar among all treatments (diet/incubation) except for the 4d measurement in THY-eggs, when the percentage transiently increased. Equally for both dietary treatments, palmitoleic acid showed a transient decrease by 4d, while stearic acid and thus stearic/oleic acids ratio increased by the 4d measurement.

Fatty acids of triglycerides fraction

Table 4 summarizes statistical information regarding TG FA composition of quail eggs at 0, 4 and 16d of incubation laid by females fed on diets supplemented with THY or CON. When THY was supplemented into the maternal diet, 0d egg yolks had increased percentages of alpha-linolenic acid, arachidonic acid, PUFA and n-3 PUFA, compared to the CON. Initial percentages of alpha-linolenic acid and arachidonic acid decreased in the THY-group by the 4d (reaching similar values to the CON) and remained unchanged until the end of the incubation. In both dietary treatments, docosahexaenoic acid, while the SFA/PUFA, n-6 PUFA/n-3 PUFA stearic and stearic/oleic acids ratio increased through development.

Fatty acids of phospholipids fraction

Table 5 summarizes statistical information regarding PL FA composition of quail eggs at 0, 4 and 16d of incubation laid by females fed on diets supplemented with THY or CON. In both dietary treatments, the alpha-linolenic acid, docosahexaenoic acid and n-3 PUFA declined while the stearic acid, SFA, SFA/PUFA, SFA/UFA and n-6 PUFA/n-3 PUFA increased throughout development. Palmitoleic acid decrease by 16d of incubation in CON, while it remained with no changes in THY-eggs. Oleic acid and MUFA exhibited lower

This article is protected by copyright. All rights reserved.

Accepted Article

percentages in egg yolks from THY-diet than in CON-diet at all stages of incubation. Instead, the ratio SFA/MUFA showed an opposite pattern. Nevertheless, the stearic/oleic acids ratio increased as incubation proceeded in both dietary treatments.

DISCUSSION

The T profile of CON 0d egg yolk was found consistent with those previously reported in the literature for *C. coturnix* (Linnaeus, 1758).[25,33,34] With reference to the T profile of 0d egg yolk from THY treatment, differences between THY doses utilized in the current and previous studies[25] suggest that the higher is the THY supplied, the greater is the increase in the percentage of UFA with a consistent detriment in SFA. The TG and PL profiles were found approximately similar to those reported for hen eggs.[1,35] Overall changes regarding the T, TG and PL from CON and THY eggs along incubation are consistent with the already reported findings during developing of embryos from parental birds fed with conventional diets.[1,3,6,36,37]

Our results suggest that THY could be involved in oxidative processes (as antioxidant) before being deposited in the egg, considering that the increase of PUFA was seen at 0d and that this discrepancy from the CON was not given by differences in the composition of the diets actually provided (see table 2). This proposal is consistent with previous evidence indicating that the chain reaction included in the oxidation of the consumed lipids could be inhibited by the transfer of natural antioxidants such as THY into the female by feeding, and consequently decreasing the oxidation of constituents transferred into the egg yolk, helping maintain PUFA.[15] Furthermore, several authors have studied the *in vivo* effect of thymol on antioxidant parameters in tissues such as plasma, brain and muscle of mammalian and avian species. Findings have been consistent with each other, indicating

This article is protected by copyright. All rights reserved.

Accepted Article

that THY supplementation leads to a great increase in antioxidant enzymes activity involving glutathione peroxidase and superoxide dismutase and a decrease in malondialdehyde levels, associated with greater concentration of PUFA precursors such as linoleic acid and alpha linolenic acid as well as its long chain derivates such as arachidonic acid and docosahexaenoic acid,[11,20] as observed herein. Additionally, in vitro and in vivo studies have demonstrated that THY as well as EO containing THY supplementation alters several lipid metabolic pathways including bile acid, cholesterol synthesis and fatty acid metabolism, indicating that thymol plays multiple modulatory roles in lipid metabolism[10,38-40]. One of the mechanisms by which supplementation with THY or EO containing THY would reflect an increase in the antioxidant capacity/status of the supplemented females is based on its inhibitory effect on lipogenesis and its hypolipidemic actions[39,41]. Inhibiting lipogenesis and/or hyperlipidemia has been demonstrated to decrease the plasma levels of oxygen free radicals and thus the production of oxidized compounds such as malondialdehyde as well[38], helping maintain PUFA levels. Although further study is needed to clarify the mechanism of hypolipidemic or antilipogenic actions of EO containing THY and its effect on PUFA, our results are consistent with the background to date.

Newly laid eggs (0d of incubation) from THY group were found to contain higher relative amounts of docosahexaenoic and arachidonicacids compared to CON eggs, even though feed did not contain appreciable quantities of them in any diet. Given these findings, whether THY supplementation might help in a more efficient conversion of PUFA precursors, i.e. linoleic and alpha-linolenic acids, into their primary long chain derivates should be explored in the future. Indeed, from the ratio arachidonic:linoleic acids and

This article is protected by copyright. All rights reserved.

Accepted Article

docosahexaenoic:alpha-linolenic acids in the 0d egg yolk, it is possible to roughly estimate the activity of the desaturases and elongases.[42] As demonstrated by other authors,[20] supplementation with THY could allow greater bioavailability of the two precursors (linoleic and alpha-linolenic acids) through increasing digestive enzyme activities and feed efficiency, leading to enhancements in docosahexaenoic and arachidonic acids as the observed herein, without discarding the other hypothesis already mentioned.

Several authors have demonstrated that bioconversion of alpha-linolenic acid into long chain metabolites in the body is limited, thus dietary alpha-linolenic acid is less effective than preformed docosahexaenoic acid (i.e. present as docosahexaenoic acid in the yolk) at supporting deposition of docosahexaenoic acid in nervous tissue of developing embryo.[2,43] Consequently, it could be positive to the developing embryo that THY supplementation herein has increased not only the percentage of alpha-linolenic acid, but also docosahexaenoic acid as well as arachidonic acid, considering the benefits of the incorporation of these essential FA for the functional and structural development of the avian embryo.[1,44]

Independent of the diet provided to parental birds, several authors have provided evidence of preferential absorption of both arachidonic and docosahexaenoic acids from yolk lipids, specifically from TG and PL, during chick embryo formation.[1,3,4,6,45] This process would occur at a higher rate in the early development where FA deposition is in structural fats.[1,46] Therefore, the observed decline from 0d to 4d of incubation in alpha-linolenic and docosahexaenoic acids in T and arachidonic acid in TG of THY treatment, as well as docosahexaenoic acid in PL from CON and THY, may represent an indirect evidence of preferential absorption by the yolk sac membrane into the embryo, conducive to a proper

This article is protected by copyright. All rights reserved.

Accepted Article

development and synthesis/deposition of structural lipids in membranes.[1,4,36,45,46] On the other hand, as has been raised by many authors, the greater increase in stearic acid in the 16d yolk detected both in the CON and the THY group in the T, TG and PL fractions, may reflect that virtually all PUFA have been incorporated into the embryo, which would lead to the increase of the SFA in complementary manner.[1,46,47]

The question still arises, why as embryo development proceeds, changes in PUFA from T and TG are not evident in the CON treatment. In other words, what could these changes imply for the metabolism of embryos in the THY treatment. Some authors have observed a diet-induced difference in the metabolism of FA under a variety of dietary conditions, such as enrichment with n-9, n-6 and n-3 FA in chicken.[2] Among *de novo* synthesized FA, UFA can be converted to SFA, but the SFA cannot be changed to unsaturated forms in embryonic tissues, such as the liver.[1] Thus, it seems that THY embryo can afford the utilization/derive of PUFA into oxidative pathways to supply energy for embryo formation if necessary while CON embryos cannot.[2] Naturally, this hypothesis should be investigated, but it is consistent with the observed differences between dietary treatments. It is unlikely that the decline observed in the PUFA from THY eggs was due to peroxidation since shell eggs are inherently resistant to oxidative deterioration upon this conditions and because it has been proved that dietary thyme and oregano, where THY is the main component, also increases the oxidative stability of shell eggs.[23,24] Furthermore, the addition of THY to the yolk has been shown to inhibit lipid oxidation in the yolk even under extreme pro-oxidant conditions.[23] Considering the whole embryonic development, the major proportion of the transferred FA is utilized for energy purposes via beta-oxidation in the embryonic tissues.[43] The lipid-based metabolism occur since days 4-5 of the quail embryonic development,[48]

This article is protected by copyright. All rights reserved.

Accepted Article

when the vascular system is developed and access to O_2 supports a complete FA oxidation.[47] This phenomena occurs in a great extent during the last third up until one or two days before hatching, when oxygen availability is again limited.[47] Herein, PUFA percentages were found higher than SFA only in THY eggs at 0d of incubation in the T. Consequently, the SFA, that are the most favorable energy source for completion of the embryo development due to their greater caloric value[47] were not suppressed in the T neither in the TG fraction during the period of unlimited oxygen availability.[47] On the other hand, the initial PUFA enhancements induced by THY supplementation were not again observed as development proceeded suggesting that the embryo might be able to capitalize these FA, as we already pointed out.

The percentages of stearic and oleic acids found as well as its ratio in the T of both dietary treatments is within a reported range which would not compromise hatchability,[33] which is consistent with the increased hatching success we already have shown with THY supplementation.[25]

Different strategies (mainly dietary enrichment with FA) to produce animal food products with higher concentration of n-3 PUFA have been attempted in the past decade due to its relation with a number of physiological and health beneficial effects for human.[49,50] In this context, consumption of egg yolks (0d) from THY-group could help to better meet adequate intakes of preformed long-chain n-3 PUFA along with reduced SFA percentages.[51] Nevertheless, a series of studies should be developed to assess the feasibility of this proposal (e.g. toxicological and sensorial testing, etc.).

In conclusion, this study provides the first description of the effects of THY on yolk T, TG and PL FA composition during avian embryo development. THY promotes an improved

This article is protected by copyright. All rights reserved.

Accepted Article

nutritional quality of newly laid eggs as well as changes throughout incubation which could favor successful embryonic development. Moreover, from a human nutrition perspective, the use of THY would be advisable for the production of healthier eggs. All these findings contribute to the set of positive results that we already reported, which configure THY-dietary supplementation of poultry as a simple strategy to improve diverse aspects related to the birds´ management, productivity and quality of their products.[16,25,26,52]

ACKNOWLEDGEMENTS

This research was supported by grants from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), FONCyT (Fondo para la Investigación Científica y Tecnológica, Préstamo BID-PICT 2014-2764) and SECyT, UNC (Secretaría de Ciencia y Tecnología, Universidad Nacional de Córdoba) Argentina. We thank MA Palacio for technical assistance with gas chromatography analysis and MJ Ortiz for technical assistance with animal husbandry.

REFERENCES

1. Noble RC and Cocchi M, Lipid metabolism and the neonatal chicken. *Prog Lipid Res* **29**:107-140 (1990).

2. Speake BK, Murray AM and Noble RC, The utilization of yolk lipids by the chick embryo. *Worlds Poult Sci J* **54**:319-334 (1998a).

3. Yalçin S, Bagdatlioglu N, Bruggeman V, Babacanoglu E, Uysal I, Buyse J, Decuypere E and Siegel PB, Acclimation to heat during incubation. 2. Embryo composition and residual egg yolk sac fatty acid profiles in chicks. *Poult Sci* **87**:1229-1236 (2008).

4. Koppenol A, Buyse J, Everaert N, Willems E, Wang Y, Franssens L and Delezie E, Transition of maternal dietary n-3 fatty acids from the yolk to the liver of broiler breeder progeny via the residual yolk sac. *Poult Sci* **94**:43-52 (2015).

5. Deeming DC and Pike TW, Embryonic growth and antioxidant provision in avian eggs. *Biol Lett* ***9***, 20130757,. DOI:10.1098/rsbl.2013.0757 (2013).

6. Speake BK, Murray AMB and Noble RC, Transport and transformations of yolk lipids during development of the avian embryo. *Prog Lipid Res* **37**:1-32 (1998b).

7. Blount JD, Houston DC and Møller AP, Why egg yolk is yellow. *Trends Ecol Evol* **15**:47–49 (2000).

This article is protected by copyright. All rights reserved.

Accepted Article

8. Surai PF and Sparks NHC, Comparative evaluation of the effect of two maternal diets on fatty acids, vitamin E and carotenoids in the chick embryo. *Brit Poult Sci* **42**:252-259 (2001).

9. Reiner GN, Labuckas DO and García DA, Lipophilicity of some GABAergic phenols and related compounds determined by HPLC and partition coefficients in different systems. *J Pharm Biomed Anal* **49**:686-691 (2009).

10. Brenes A and Roura E, Essential oils in poultry nutrition: Main effects and modes of action. *Anim Feed Sci Technol* **158**:1–14 (2010).

11. Abd El-Hack ME, Alagawany M, Farag MR Tiwari R, Karthik K, Dhama K, Zorriehzahra J and Milad A, Beneficial impacts of thymol essential oil on health and production of animals, fish and poultry: a review. *J Essent Oil Res* **28**:365-382 (2016).

12. Han M, Song P, Huang C, Rezaei A, Farrar S, Brown MA and Ma X, Dietary grape seed proanthocyanidins (GSPs) improve weaned intestinal microbiota and mucosal barrier using a piglet model. *Oncotarget* **7**(49):80313-80326 (2016). doi:10.18632/oncotarget.13450.

13. Song P, Zhang R, Wang X, He P, Tan L and Ma X, Dietary grape-seed procyanidins decreased postweaning diarrhea by modulating intestinal permeability and suppressing oxidative stress in rats. *J Agric Food Chem* **59**(11):6227-6232 (2011). doi: 10.1021/jf200120y.

14. Fan P, Song P, Li L, Huang C, Chen J, Yang W, Qiao S, Wu G, Zhang G and Ma X, Modulation of Biogenic Amines on Intestinal Signaling Proteins and Its Detection. *Curr Protein Pept Sci* **17**:1-9 (2016)

15. Yanishlieva NV, Marinova EM, Gordon MH and Raneva VG, Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chem* **64**:59–66 (1999).

16. Luna A, Labaque MC, Zygadlo JA and Marin RH, Effects of thymol and carvacrol feed supplementation on lipid oxidation in broiler meat. *Poult Sci* **89**:366-370 (2010).

17. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) Scientific Opinion on safety and efficacy of CRINA® Poultry Plus (preparation of benzoic acid and essential oil compounds) as feed additive for chickens for fattening. *EFSA J* **10**:2620-2642 (2012).

18. Haselmeyer A, Zentek J and Chizzola R, Effects of thyme as a feed additive in broiler chickens on thymol in gut contents, blood plasma, liver and muscle. *J Sci Food Agric* **95**:504-508 (2015).

19. Luna A, Labaque MC, Zygadlo JA and Marin RH, Use of natural phenols as feed supplements with antioxidant effects on poultry products. *World Vet Poult Assoc* 516 (2013).

20. Hashemipour H, Kermanshahi H, Golian A and Veldkamp T, Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. *Poult Sci* **92**:2059–2069 (2013).

21. Fernandez ME, Palacio MA and Labaque MC, Thymol detection and quantitation by solid-phase microextraction in faeces and egg yolk of Japanese quail. *J Chromatogr B Analyt Technol Biomed Life Sci* **1044-1045**:39-46 (2017). doi: 10.1016/j.jchromb.2016.12.042.

22. Krause EL and Ternes W, Bioavailability of the antioxidative thyme compounds thymol and p-cymene-2,3-diol in eggs. *Eur Food Res Technol* **209**:140–144 (1999).

This article is protected by copyright. All rights reserved.

Accepted Article

23. Botsoglou NA, Yannakopoulos AL, Fletouris DJ, Tserveni-Goussi AS and Fortomaris PD, Effect of dietary thyme on oxidative stability of egg yolk. *J Agric Food Chem* **45**:3711-3716 (1997).

24. Radwan Nadia L, Hassan RA, Qota EM and Fayek HM, Effect of natural antioxidant on oxidative stability of eggs and productive and reproductive performance of laying hens. *Int J Poult Sci* **7**(2): 134-150 (2008).

25. Luna A, Dambolena JS, Zygadlo JA, Marin RH and Labaque MC, Effects of thymol and isoeugenol feed supplementation on quail adult performance, egg characteristics and hatching success. *Brit Poult Sci* 53:631-639 (2012).

26. Labaque MC, Kembro JM, Luna A and Marin RH, Effects of thymol feed supplementation on female Japanese quail (*Coturnix coturnix*) behavioral fear reponses. *Anim Feed Sci Technol* **183**:67–72 (2013a).

27. Brillard JP, Practical aspects of fertility in poultry. *Worlds Poult Sci* J **59**:441-446 (2003).

28. Folch J, Lees M and Sloane Stanley GH, A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**:497-509 (1957).

29. Labaque MC, Martella MB, Maestri DM and Navarro JL, The influence of diet composition on egg and chick traits in captive Greater Rhea. *Brit Poult Sci* **54**:374-380 (2013b).

30. Royle NJ, Surai PF, McCartney RJ and Speake BK, Parental investment and egg yolk lipid composition in Gulls. *Funct Ecol* **138**:298-306 (1999).

31. Infostat. Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. Grupo InfoStat, FCA, Univ. Nac. de Córdoba, Argentina. URL: http://www.infostat.com.ar (2016).

32. Quinn GP and Keough MJ. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, New York, pp. 64 (2002).

33. Aydin R and Cook ME, The effect of dietary conjugated linoleic acid on egg yolk fatty acids and hatchability in Japanese quail. *Poult Sci* **83**:2016-2022 (2004).

34. Arantes da Silva W, Naiverti Elias AH, Aricetti JA, Sakamoto MI, Murakami AE, Marques Gomes ST, Visentainer JV, de Souza NE and Matsushita M, Quail egg yolk (*Coturnix coturnix japonica*) enriched with omega-3 fatty acids. *Food Sci Technol* **42**:660–663 (2009).

35. Jiang ZR, Ahn DU and Sim JS, Effects of feeding flax and two types of sunflower seeds on fatty acid compositions of yolk lipid classes. *Poult Sci* **70**:2467-2475 (1991).

36. Vilchez C, Touchburn SP, Chavez ER and Chan CW, Effect of feeding palmitic, oleic, and linoleic acids to Japanese quail hens (*Coturnix coturnix japonica*). 2. Maternal diets and stage of incubation on the lipid metabolism of quail embryos. *Poult Sci* **71**:1032-1042 (1992).

37. Sahan U, Ipek A and Sozcu A, Yolk sac fatty acid composition, yolk absorption, embryo development, and chick quality during incubation in eggs from young and old broiler breeders. *Poult Sci* **93**:2069-2077 (2014).

38. Souri H, Khatibjoo A, Taherpour K, Hassanabadi A, Fatahnia F, Askari M, Effect of Thymus vulgaris and Satureja khuzestanica ethanolic extracts on broiler chickens' performance and immune response. *Iranian J App Anim Sci* **5**(2): 437-446 (2015).

39. Choi JH, Kim SW, Yu R and Yun JW, Monoterpene phenolic compound thymol promotes browning of 3T3-L1 adipocytes. *Eur J Nutr* PMID 27431894 DOI: 10.1007/s00394-016-1273-2 (2016).

This article is protected by copyright. All rights reserved.

Accepted Article

40. Haque MR, Ansari SH, Najmi AK and Ahmad MA, Monoterpene phenolic compound thymol prevents high fat diet induced obesity in murine model. *Toxicol Mech Methods* **24**(2):116-123 (2014) doi:10.3109/15376516.2013.861888

41. Aragonès G, Ardid-Ruiz A, Ibars M, Suárez M and Bladé C, Modulation of leptin resistance by food compounds. *Mol Nutr Food Res* **60**(8):1789-1803 (2016) DOI 10.1002/mnfr.201500964

42. Nain S, Renema RA, Korver DR and Zuidhof MJ, Characterization of the n-3 polyunsaturated fatty acid enrichment in laying hens fed an extruded flax enrichment source. *Poult Sci* **91**:1720-1732 (2012).

43. Maldjian A, Farkas K, Noble RC, Cocchi M and Speake BK, The transfer of docosahexaenoic acid from the yolk to the tissues of the chick embryo. *Biochim Biophys Acta* **1258**:81-89 (1995).

44. Cherian G, Nutrition and metabolism in poultry: role of lipids in early diet. *J Anim Sci Biotechnol* **6**:28 (2015).

45. Cherian G and Sim JS, Net transfer and incorporation of yolk n-3 fatty acids into developing chick embryos. *Poult Sci* **72**:98-105 (1993).

46. Speake BK, Deans EA and Powell KA, Differential incorporation of docosahexaenoic and arachidonic acids by the yolk sac membrane of the avian embryo. *Comp Biochem Physiol B Biochem Mol Biol* **136**:357-367 (2003).

47. Moran ET Jr, Nutrition of the developing embryo and hatchling. *Poult Sci* **86**:1043-1049 (2007).

48. Ainsworth SJ, Stanley RL and Evans DJ, Developmental stages of the Japanese quail. *J Anat* **216**(1):3-15. (2010).

49. Hargis PS and Van Elswyk ME, Manipulating the fatty acid composition of poultry meat and eggs for the health conscious consumer. *Worlds Poult Sci J* **49**:251-264 (1993).

50. Palmquist DL, Omega-3 Fatty Acids in Metabolism, Health, and Nutrition and for Modified Animal Product Foods. *The Prof Anim Scientist* **25**:207-249(2009).

51. Burdge GC and Calder PC, Conversion of alpha-linolenic acid to longer-chain polyunsaturatedfatty acids in human adults. *Reprod Nutr Dev* **45**:581-597 (2005).

52. Lynch Ianello I, Battan M, Labaque C, Luna A, Marin RH and Gleiser RM, Fly emergence from manure of japanese quail fed thymol- or isoeugenol-supplemented diets. *Poult Sci* **93**:2449-2456 (2014).

This article is protected by copyright. All rights reserved.

Accepted Article

Table 1. Nutrient composition of administrated diets as g $kg^{-1}$of diet

Nutrient	**g kg^{-1}**
Min. crude protein	200
Min. fat matter	55
Max. crudefiber	51,5
Max. total minerals	75
Max. calcium	29
Min. calcium	25
Max. phosphorous	8.5
Min. phosphorous	7.5

This article is protected by copyright. All rights reserved.

Accepted Article

Table 2. Total fatty acid composition (Mean percentage ± Standard Error) from diets control (CON) and supplemented with thymol (THY, 0.0016molday^{-1}animal^{-1})

Fattyacid	CON	THY	P value
14:0 (Miristic)	0.07 ± 0.00	0.06 ± 0.01	0.78
15:0 (Pentadecilic)	0.00 ± 0.00	0.00 ± 0.00	>0.99
16:0 (Palmitic)	11.69 ± 0.08[a]	11.78 ± 0.09[a]	0.46
16:1 n-7 (Palmitoleic)	0.09 ± 0.01	0.09 ± 0.00	0.7
18:0 (Stearic)	5.07 ± 0.52	5.02 ± 0.46	0.94
18:1 n-9 (Oleic)	30.76 ± 0.21	30.95 ± 0.24	0.56
18:2 n-6 (Linoleic)	42.95 ± 1.01	43.99 ± 0.98	0.47
18:3 n-3 (Alpha-Linolenic)	5.43 ± 0.05	5.51 ± 0.08	0.40
20:4 n-6 (Arachidonic)	0.04 ± 0.00	0.05 ± 0.01	0.58
SFA	16.83 ± 0.57	16.87 ± 0.46	0.96
MUFA	30.84 ± 0.21	31.04 ± 0.24	0.55
PUFA	48.42 ± 0.97	49.56 ± 0.91	0.41
UFA	79.26 ± 0.87	80.59 ± 0.72	0.26
n-3 PUFA	5.43 ± 0.05	5.51 ± 0.08	0.40
n-6 PUFA	42.99 ± 1.01	44.05 ± 0.98	0.47
SFA/MUFA	0.55 ± 0.02	0.54 ± 0.01	0.93
SFA/PUFA	0.35 ± 0.02	0.34 ± 0.01	0.75
SFA/UFA	0.21 ± 0.01	0.21 ± 0.01	0.79
n-6/n-3 PUFA	7.93 ± 0.25	8.00 ± 0.30	0.83
18:0/18:1	0.16 ± 0.02	0.16 ± 0.01	0.90

[ab]Values without a common letter in the same row differ significantly ($P < 0.05$). N=6 in each case. D= Diet. S= Storage time. DxS= Interaction.

SFA = saturated fatty acids; were calculated as 14:0 + 15:0 + 16:0 + 18:0.
MUFA = monounsaturated fatty acids; were calculated as 16:1 n-7 + 18:1 n-7.
PUFA = polyunsaturated fatty acids; were calculated as 18:2 n-6 + 18:3 n-3 + 20:4 n-6.
UFA = unsaturated fatty acids; were calculated as MUFA + PUFA.
n-3 PUFA was equal to 18:3 n-3, in this case.
n-6 PUFA was calculated as 18:2 n-6 + 20:4 n-6.

This article is protected by copyright. All rights reserved.

Accepted Article

Table 3. Total fatty acid composition (Mean percentage± Standard Error) of quail eggs at 0, 4 and 16 days (d) of incubation laid by females fed on diets control (CON) or supplemented with thymol (THY, 0.0016mol $day^{-1}animal^{-1}$)

	0d of incubation		4d of incubation		16d of incubation		P value		
Fattyacid	CON	THY	CON	THY	CON	THY	D	I	DxI
14:0 (Miristic)	0.24 ± 0.10	0.22 ± 0.09	0.19 ± 0.12	0.18 ± 0.11	0.11 ± 0.07	0.12 ± 0.08	0.92	0.47	0.95
15:0 (Pentadecilic)	0.23 ± 0.10	0.19 ± 0.08	0.15 ± 0.11	0.17 ± 0.11	0.10 ± 0.06	0.11 ± 0.07	0.95	0.46	0.92
16:0 (Palmitic)	27.14 ± 2.56^{ab}	24.79 ± 3.10^{a}	24.50 ± 1.01^{a}	31.92 ± 2.50^{b}	24.43 ± 0.99^{a}	25.92 ± 1.99^{a}	0.52	0.28	0.04
16:1 n-7 (Palmitoleic)	3.49 ± 0.30^{c}	3.29 ± 0.39^{c}	2.10 ± 0.31^{b}	0.91 ± 0.28^{b}	2.58 ± 0.14^{a}	2.96 ± 0.69^{a}	0.16	<0.001	0.40
18:0 (Stearic)	7.68 ± 1.62^{a}	2.82 ± 1.41^{a}	10.08 ± 3.19^{b}	8.40 ± 1.79^{b}	8.83 ± 1.97^{b}	11.33 ± 2.43^{b}	0.47	0.05	0.29
18:1 n-9 (Oleic)	41.16 ± 1.79	40.84 ± 3.40	39.85 ± 2.88	36.05 ± 7.80	39.33 ± 2.88	39.36 ± 2.75	0.72	0.78	0.99
18:2 n-6 (Linoleic)	15.48 ± 1.99	13.84 ± 1.02	15.83 ± 1.02	18.63 ± 7.14	10.84 ± 3.37	16.92 ± 1.94	0.64	0.91	0.17
18:3 n-3 (alpha-Linolenic)	3.55 ± 1.45^{ab}	9.00 ± 2.70^{c}	3.91 ± 1.17^{abc}	3.01 ± 1.81^{a}	7.74 ± 2.38^{bc}	0.41 ± 0.10^{a}	0.30	0.48	0.01
20:4 n-6 (Arachidonic)	1.22 ± 0.34	1.73 ± 0.65	2.05 ± 0.43	0.98 ± 0.46	1.92 ± 0.96	2.60 ± 0.83	0.70	0.74	0.14
22:6 n-3 (Docosahexaenoic)	0.17 ± 0.08^{a}	3.16 ± 0.84^{c}	1.47 ± 0.25^{bc}	0.11 ± 0.04^{a}	0.81 ± 0.45^{ab}	0.49 ± 0.20^{a}	0.82	0.36	<0.001
SFA	35.29 ± 3.83^{b}	28.03 ± 2.77^{a}	34.92 ± 4.10^{b}	40.66 ± 2.20^{b}	33.47 ± 2.22^{ab}	37.48 ± 1.26^{b}	0.98	0.10	0.05
MUFA	44.65 ± 1.75	44.13 ± 3.72	42.16 ± 2.79	37.26 ± 8.03	43.27 ± 3.19	42.33 ± 2.39	0.84	0.84	0.96
PUFA	20.68 ± 2.79^{a}	28.44 ± 1.87^{b}	23.35 ± 2.25^{ab}	22.82 ± 8.55^{a}	23.82 ± 3.65^{a}	20.50 ± 1.43^{a}	0.95	0.49	0.05
UFA	65.33 ± 3.92	72.57 ± 3.34	65.51 ± 3.69	60.09 ± 1.91	69.76 ± 5.61	62.83 ± 1.42	0.42	0.33	0.07
n-3 PUFA	3.98 ± 1.47^{a}	12.86 ± 2.42^{b}	5.48 ± 0.99^{a}	3.22 ± 1.79^{a}	10.70 ± 1.52^{b}	0.98 ± 0.15^{a}	0.29	0.09	<0.001
n-6 PUFA	16.69 ± 2.28	15.57 ± 1.50	17.87 ± 1.43	19.60 ± 7.59	12.76 ± 2.86	19.52 ± 1.51	0.65	0.74	0.15
SFA/MUFA	0.80 ± 0.10	0.68 ± 0.13	0.86 ± 0.14	2.13 ± 1.21	0.80 ± 0.11	0.90 ± 0.08	0.73	0.38	0.40
SFA/PUFA	1.94 ± 0.44	1.00 ± 0.10	1.58 ± 0.28	2.85 ± 1.00	1.64 ± 0.39	1.86 ± 0.14	0.93	0.40	0.09
SFA/UFA	0.56 ± 0.08^{b}	0.39 ± 0.06^{a}	0.55 ± 0.09^{b}	0.69 ± 0.06^{b}	0.50 ± 0.08^{ab}	0.60 ± 0.03^{b}	0.90	0.12	0.05
n-6 PUFA/n-3 PUFA	17.27 ± 9.37^{b}	1.40 ± 0.31^{a}	3.58 ± 0.49^{b}	28.87 ± 11.30^{b}	1.25 ± 0.28^{a}	23.87 ± 6.99^{b}	0.09	0.13	<0.001
18:0/18:1	0.19 ± 0.05^{a}	0.07 ± 0.04^{a}	0.28 ± 0.10^{b}	0.23 ± 0.01^{b}	0.24 ± 0.06^{b}	0.31 ± 0.08^{b}	0.58	0.04	0.39
20:4/18:2	0.07 ± 0.02^{ab}	0.12 ± 0.04^{c}	0.13 ± 0.02^{c}	0.04 ± 0.01^{a}	0.05 ± 0.02^{ab}	0.19 ± 0.09^{bc}	0.98	0.68	<0.01
22:6/18:3	0.03 ± 0.01^{a}	0.87 ± 0.59^{b}	0.89 ± 0.54^{b}	0.35 ± 0.23^{ab}	0.14 ± 0.09^{ab}	2.75 ± 1.79^{ab}	0.21	0.72	0.04

[abc]Values without a common letter in the same row differ significantly (*P<0.05*). N=5 in each case. D= diet. I= Days of incubation. DxI= Interaction

SFA = saturated fatty acids; SFA levels were calculated as 14:0 + 15:0 + 16:0 + 18:0 + 20:0.

MUFA = monounsaturated fatty acids; MUFA levels were calculated as 16:1 n-7 + 18:1 n-7.

PUFA = polyunsaturated fatty acids; PUFA levels were calculated as 18:2 n-6 + 18:3 n-3 + 18:3 n-6 +20:4 n-6 + 20:5 n-3 + 22:5 n-3 +22:2 n-6 + 22:6 n-3.

UFA = unsaturated fatty acids; UFA levels were calculated as MUFA + PUFA.

n-3 PUFA was calculated as 18:3 n-3 + 20:5 n-3 + 22:5 n-3 + 22:6 n-3.

n-6 PUFA was calculated as 18:2 n-6 + 20:4 n-6.

This article is protected by copyright. All rights reserved.

Accepted Article

Table 4. Fatty acid composition (Mean percentage ± Standard Error) of triglycerides of quail eggs at 0, 4 and 16 days (d) of incubation laid by females fed on diets control (CON) or supplemented with thymol (THY, 0.0016mol $day^{-1}animal^{-1}$)

	0d of incubation		4d of incubation		16d of incubation		P value		
Fattyacid	CON	THY	CON	THY	CON	THY	D	I	DxI
14:0 (Miristic)	0.24 ± 0.20	0.24 ± 0.24	0.27 ± 0.17	0.21 ± 0.21	0.25 ± 0.16	0.38 ± 0.10	0.92	0.43	0.75
15:0 (Pentadecilic)	0.41 ± 0.36	0.20 ± 0.20	0.22 ± 0.22	0.31 ± 0.31	0.26 ± 0.19	0.39 ± 0.18	0.76	0.32	0.75
16:0 (Palmitic)	23.80 ± 3.40	29.21 ± 2.10	27.92 ± 0.73	25.98 ± 1.50	26.17 ± 0.59	26.68 ± 0.10	0.85	0.74	0.39
16:1 n-7 (Palmitoleic)	32.25 ± 0.76^{ab}	4.39 ± 0.66^{b}	4.18 ± 0.20^{b}	24.50 ± 0.93^{ab}	1.55 ± 0.25^{a}	2.83 ± 0.86^{ab}	0.67	0.13	0.07
18:0 (Stearic)	7.50 ± 1.55^{a}	3.63 ± 0.72^{a}	3.49 ± 0.85^{a}	3.77 ± 1.44^{a}	8.56 ± 0.86^{b}	16.43 ± 9.77^{b}	0.35	0.01	0.16
18:1 n-9 (Oleic)	50.81 ± 6.99^{ab}	37.78 ± 3.55^{a}	39.47 ± 1.61^{a}	46.35 ± 2.35^{ab}	49.08 ± 0.86^{b}	43.13 ± 8.45^{ab}	0.40	0.48	0.05
18:2 n-6 (Linoleic)	9.26 ± 1.80	10.32 ± 2.32	12.08 ± 0.52	13.01 ± 0.86	13.32 ± 0.48	9.80 ± 1.83	0.71	0.22	0.13
18:3 n-3 (alpha-Linolenic)	2.84 ± 1.29^{a}	12.54 ± 3.25^{b}	11.34 ± 1.15^{b}	6.72 ± 3.20^{ab}	1.07 ± 0.04^{a}	1.06 ± 0.06^{a}	0.65	0.03	0.05
20:4 n-6 (Arachidonic)	0.50 ± 0.25^{a}	2.36 ± 0.41^{b}	1.03 ± 0.31^{a}	0.84 ± 0.50^{a}	0.50 ± 0.29^{a}	0.30 ± 0.10^{a}	0.19	0.15	0.02
22:6 n-3 (Docosahexaenoic)	0.78 ± 0.16^{b}	1.00 ± 0.07^{b}	0.78 ± 0.05^{a}	0.60 ± 0.08^{a}	0.80 ± 0.06^{a}	0.77 ± 0.21^{a}	0.97	0.01	0.16
SFA	31.95 ± 4.79	33.28 ± 2.85	31.90 ± 1.23	30.26 ± 2.56	35.24 ± 0.67	43.87 ± 10.41	0.66	0.16	0.87
MUFA	54.04 ± 6.40	42.17 ± 4.18	43.65 ± 1.74	48.79 ± 1.96	50.58 ± 1.07	45.96 ± 7.60	0.61	0.70	0.08
PUFA	13.37 ± 2.49^{a}	26.22 ± 5.71^{b}	25.24 ± 1.09^{b}	21.16 ± 2.82^{ab}	15.69 ± 0.65^{ab}	11.83 ± 1.78^{a}	0.70	0.04	0.02
UFA	67.41 ± 4.95	68.39 ± 3.01	68.88 ± 1.26	69.95 ± 2.70	66.27 ± 0.88	57.78 ± 9.37	0.67	0.30	0.35
n-3 PUFA	3.62 ± 1.37^{a}	13.54 ± 3.23^{b}	12.13 ± 1.11^{b}	7.31 ± 3.12^{ab}	1.87 ± 0.09^{a}	1.83 ± 0.15^{a}	0.74	0.02	0.03
n-6 PUFA	9.75 ± 1.85	12.68 ± 2.55	13.11 ± 0.68	13.85 ± 0.40	13.82 ± 0.74	10.00 ± 1.63	0.99	0.40	0.11
SFA/MUFA	0.66 ± 0.12	0.82 ± 0.10	0.74 ± 0.05	0.62 ± 0.06	0.70 ± 0.01	1.02 ± 0.39	0.81	0.48	0.44
SFA/PUFA	2.62 ± 0.54^{a}	2.45 ± 1.46^{a}	1.27 ± 0.07^{a}	1.55 ± 0.31^{a}	2.26 ± 0.13^{b}	3.93 ± 1.45^{b}	0.80	0.03	0.08
SFA/UFA	0.51 ± 0.09	0.50 ± 0.07	0.47 ± 0.03	0.44 ± 0.05	0.53 ± 0.01	0.81 ± 0.31	0.52	0.16	0.64
n-6 PUFA/n-3 PUFA	3.08 ± 0.93^{a}	1.11 ± 0.19^{a}	1.12 ± 0.13^{a}	4.83 ± 2.67^{a}	7.47 ± 0.78^{b}	5.43 ± 0.44^{b}	0.68	0.01	0.10
18:0/18:1	0.16 ± 0.03^{a}	0.10 ± 0.02^{a}	0.09 ± 0.02^{a}	0.08 ± 0.03^{a}	0.17 ± 0.02^{b}	0.44 ± 0.31^{b}	0.38	0.03	0.39

[abc]Values without a common letter in the same row differ significantly ($P<0.05$). N=5 in each case. D= diet. I= Days of incubation. DxI= Interaction

SFA = saturated fatty acids; SFA levels were calculated as 14:0 + 15:0 + 16:0 + 18:0 + 20:0.

MUFA = monounsaturated fatty acids; MUFA levels were calculated as 16:1 n-7 + 18:1 n-7.

PUFA = polyunsaturated fatty acids; PUFA levels were calculated as 18:2 n-6 + 18:3 n-3 + 18:3 n-6 +20:4 n-6 + 20:5 n-3 + 22:5 n-3 +22:2 n-6 + 22:6 n-3.

UFA = unsaturated fatty acids; UFA levels were calculated as MUFA + PUFA.

n-3 PUFA was calculated as 18:3 n-3 + 20:5 n-3 + 22:5 n-3 + 22:6 n-3.

n-6 PUFA was calculated as 18:2 n-6 + 20:4 n-6.

This article is protected by copyright. All rights reserved.

Accepted Article

Table 5. Fatty acid composition (Mean percentage ± Standard Error) of phospholipids of quail eggs at 0, 4 and 16 days (d) of incubation laid by females fed on diets control (CON) or supplemented with thymol (THY, 0.0016mol day^{-1}animal^{-1})

	0d of Incubation		4d of Incubation		16d of Incubation		P value		
Fattyacid	CON	THY	CON	THY	CON	THY	D	I	DxI
14:0 (Miristic)	0.29 ± 0.12	0.38 ± 0.24	0.32 ± 0.20	0.37 ± 0.26	0.52 ± 0.26	0.10 ± 0.06	0.77	0.97	0.66
15:0 (Pentadecilic)	0.33 ± 0.33	0.30 ± 0.24	0.26 ± 0.16	0.82 ± 0.27	0.44 ± 0.22	0.36 ± 0.27	0.36	0.56	0.56
16:0 (Palmitic)	29.04 ± 1.17	26.83 ± 6.98	28.50 ± 1.59	35.56 ± 8.97	25.53 ± 1.32	22.17 ± 4.24	0.83	0.16	0.89
16:1 n-7 (Palmitoleic)	1.91 ± 0.52^{ab}	9.26 ± 6.41^{ab}	4.37 ± 1.20^{b}	0.97 ± 0.67^{a}	0.90 ± 0.30^{a}	1.38 ± 1.69^{ab}	0.59	0.26	0.04
18:0 (Stearic)	6.47 ± 3.19^{a}	12.75 ± 5.84^{a}	5.15 ± 1.73^{a}	8.90 ± 5.34^{a}	33.52 ± 1.99^{b}	31.53 ± 2.36^{b}	0.86	<0.001	0.73
18:1 n-9 (Oleic)	26.05 ± 1.49^{b}	18.03 ± 5.23^{a}	29.48 ± 1.93^{b}	16.73 ± 5.29^{a}	22.60 ± 2.38^{b}	16.89 ± 5.26^{a}	0.05	0.56	0.50
18:2 n-6 (Linoleic)	14.48 ± 1.88	16.18 ± 1.08	10.81 ± 1.68	13.69 ± 2.61	15.73 ± 0.52	15.07 ± 2.81	0.50	0.24	0.51
18:3 n-3 (Alpha-Linolenic)	15.91 ± 4.02^{b}	12.90 ± 4.00^{b}	17.82 ± 3.24^{b}	14.93 ± 7.42^{b}	0.29 ± 0.11^{a}	0.64 ± 0.53^{a}	0.549	0.04	0.94
20:4 n-6 (Arachidonic)	4.51 ± 0.48	2.89 ± 0.81	2.41 ± 1.45	4.95 ± 2.15	2.16 ± 0.57	7.05 ± 3.56	0.23	0.63	0.07
22:6 n-3 (Docosahexaenoic)	1.08 ± 0.07^{c}	0.98 ± 0.06^{c}	0.89 ± 0.03^{b}	0.90 ± 0.06^{b}	0.40 ± 0.08^{a}	0.24 ± 0.04^{a}	0.36	<0.001	0.55
SFA	36.13 ± 2.51^{a}	40.27 ± 5.77^{a}	34.22 ± 3.32^{a}	45.65 ± 7.97^{a}	60.01 ± 2.62^{b}	54.16 ± 6.23^{b}	0.64	0.01	0.47
MUFA	27.97 ± 1.92^{b}	27.29 ± 4.76^{a}	33.85 ± 2.50b	17.70 ± 5.02^{a}	23.20 ± 2.11^{b}	18.27 ± 4.57^{a}	0.05	0.06	0.08
PUFA	35.98 ± 2.07^{b}	32.94 ± 3.95^{b}	31.92 ± 5.8^{b}	34.45 ± 8.56^{b}	18.58 ± 0.78^{a}	23.00 ± 2.67^{a}	0.82	0.03	0.64
UFA	63.95 ± 3.71^{b}	60.23 ± 5.57^{b}	65.77 ± 3.74^{b}	52.15 ± 9.02^{b}	41.78 ± 2.89^{a}	41.27 ± 5.80^{a}	0.48	0.01	0.58
n-3 PUFA	16.99 ± 4.04^{b}	13.87 ± 4.03^{b}	18.70 ± 3.23^{b}	15.82 ± 7.42^{b}	0.68 ± 0.06^{a}	0.87 ± 0.86^{a}	0.50	0.001	0.85
n-6 PUFA	18.99 ± 2.26	19.07 ± 0.36	13.21 ± 3.11	18.63 ± 2.77	17.90 ± 0.83	22.12 ± 2.42	0.09	0.53	0.74
SFA/MUFA	1.35 ± 0.22^{a1}	1.93 ± 0.69^{b1}	1.01 ± 0.07^{a1}	3.50 ± 1.22^{b1}	2.64 ± 0.33^{a2}	3.25 ± 0.69^{b2}	0.04	0.04	0.10
SFA/PUFA	1.04 ± 0.15^{a}	1.39 ± 0.40^{a}	1.29 ± 0.32^{a}	1.83 ± 0.73^{a}	3.25 ± 0.27^{b}	2.49 ± 0.58^{b}	0.96	0.01	0.69
SFA/UFA	0.59 ± 0.09^{a}	0.74 ± 0.18^{a}	0.54 ± 0.08^{a}	1.10 ± 0.44^{a}	1.46 ± 0.16^{b}	1.38 ± 0.30^{b}	0.52	0.01	0.52
n-6 PUFA/n-3 PUFA	6.29 ± 5.49^{a}	5.30 ± 3.97^{a}	0.71 ± 0.10^{a}	6.23 ± 5.17^{a}	26.73 ± 3.46^{b}	66.92 ± 43.54^{b}	0.13	<0.001	0.56
18:0/18:1	0.29 ± 0.17^{a}	1.46 ± 0.87^{a}	0.17 ± 0.06^{a}	0.52 ± 0.23^{a}	1.53 ± 0.23^{b}	2.15 ± 0.47^{b}	0.15	<0.01	0.82

^{abc}Values without a common letter in the same row differ significantly ($P<0.05$). When effects of the two factors separately were observed on the same fatty acid: ^{abc}Values correspond to the diet and ^{12}values to the incubation. N=5 in each case. D= diet. I= Days of incubation. DxI= Interaction

SFA = saturated fatty acids; SFA levels were calculated as 14:0 + 15:0 + 16:0 + 18:0 + 20:0.

MUFA = monounsaturated fatty acids; MUFA levels were calculated as 16:1 n-7 + 18:1 n-7.

PUFA = polyunsaturated fatty acids; PUFA levels were calculated as 18:2 n-6 + 18:3 n-3 + 18:3 n-6 +20:4 n-6 + 20:5 n-3 + 22:5 n-3 +22:2 n-6 + 22:6 n-3.

UFA = unsaturated fatty acids; UFA levels were calculated as MUFA + PUFA.

n-3 PUFA was calculated as 18:3 n-3 + 20:5 n-3 + 22:5 n-3 + 22:6 n-3.

n-6 PUFA was calculated as 18:2 n-6 + 20:4 n-6.

This article is protected by copyright. All rights reserved.