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## Data Article

# Dynamics of thymol dietary supplementation in quail (*Coturnix japonica*): Dataset on thymol bioavailability, egg yolk fatty acids profile and performance traits



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## ABSTRACT

Dietary supplementation with thymol (2-isopropyl-5-methylphenol) has been proposed as a strategy to improve modern intensive poultry production. Moreover, its antioxidant properties and potential beneficial influence on lipid metabolism have fostered current research focusing on enhancing nutritional quality of meat and egg products. In general, studies have focused on the overall effects of dietary supplementation once the supplementation protocol has finished and using only one potential dose,

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PUFA  
 MUFA  
 SFA  
 Liver histopathology  
 Healthy  
 Eggs  
 Poultry nutrition

without actually measuring bioactive compounds' concentration in the diet supplied or target tissues. Herein, we provide a unique dataset of the dynamics of thymol bioavailability and biological action, optimal dosage and duration of supplementation needed to achieve meaningful effects, as well as persistence of induced changes after chronic supplement withdrawal. Specifically, during a month-long supplementation period, 5 sampling points were evaluated separated by at least 1 week. Then, a last sampling point was studied after a 3-week withdrawal period. Three increasing doses of dietary thymol were used, and approximately 80 variables assessed. The measured variables were associated with free thymol concentration in feed, egg yolk and droppings, feed and egg yolk fatty acids profile (saturated, unsaturated, and poly-unsaturated fatty acids), performance traits (body weight, feed intake, egg laying rate, egg physical characteristics), general welfare quality assessment (plumage state) and liver histopathology. The data can provide insights on the link between the dynamics of free thymol concentration and the changes in fatty acids profile in quail egg yolk, both during chronic thymol dietary supplementation and after supplement withdrawal. The comprehensive approach used herein for studying thymol supplementation outcome could help understanding the scope of its effects on a whole organism level.

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#### Specifications table

Subject area	Animal Science and Zoology; Agricultural and Biological Sciences (General)
More specific subject area	Poultry metabolism and nutrition; Feed additives; Natural Products; Healthy table eggs; Fatty acids profile
Type of data	Tables
How data was acquired	Thymol concentration was assessed using head-space solid phase microextraction followed by gas chromatography-mass spectrometry (HS-SPME/GC-MS in a Perkin Elmer Clarus® 600). Fatty acid methyl esters were analyzed by gas chromatography mass-spectrometry (GC-MS in a Perkin Elmer Clarus® 600). Body weight and feed intake were assessed using a balance (OHAUS Scout-Pro® SP601), egg physical characteristics with an analytical balance (OHAUS Adventurer® AR2140 and a digital caliper) and general welfare quality with a procedure adapted Welfare Quality consortium protocol. Histopathological alterations were recorded with a light microscope (Olympus X-785) coupled to a digital camera (Moticam Camera 2300, 3 Megapixels).
Data format	Raw and processed data
Experimental factors	Husbandry under standard laboratory protocol for quail
Experimental features	A bi-factorial design combining the effects of the diet supplied (five levels) and time of sampling (five levels) was established for the variables studied: >Five experimental diets (two controls: the basal diet and the basal diet with vehicle solution; and three thymol doses: 2, 4 and 6.25 g/kg of feed, respectively). >Five times of sampling (one initial sample point; three sample points along the supplementation period and one at the end of the post-supplementation period).
Data source location	Instituto de Investigaciones Biológicas y Tecnológicas (IBYT), Córdoba, Argentina.
Data accessibility	Data are presented in excel format file and stored in the public repository figshare: DOI: <a href="https://doi.org/10.6084/m9.figshare.7589066.v1">https://doi.org/10.6084/m9.figshare.7589066.v1</a>
Related research article	M.E. Fernandez, R.H. Marin, A. Luna, M.P. Zunino, M.C. Labaque, Thymol feed supplementation in quail alters the percentages of nutritionally relevant egg yolk fatty acids: effects throughout incubation, J. Sci. Food Agric. 97 (2017) 5233–40. <a href="https://doi.org/10.1002/jsfa.8407">https://doi.org/10.1002/jsfa.8407</a>

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**Value of the data**

- The data can provide insights on the link between the dynamics of free thymol concentration and the changes in fatty acids (FA) profile in quail egg yolk, both during chronic thymol dietary supplementation and after supplement withdrawal.
  - The data can be used for the establishment of optimal dosage and duration of thymol supplementation needed to achieve meaningful effects, as well as persistence of induced changes after supplement withdrawal.
  - The dataset could be used by the poultry industry for initiating exploratory studies for the use of thymol in dietary supplements.
  - The dataset could be used by other researchers for comparative studies for understanding in vivo thymol effects on a whole organism level.
  - The data can be used in the context of toxicological evaluation of chronic thymol dietary supplementation.
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## 1. Data

Data on quantitative changes promoted by three increasing doses of dietary thymol (2-isopropyl-5-methylphenol, at 2, 4 and 6.25 g/Kg of feed) during a month-long supplementation period and after a 3-week withdrawal period were assed (i.e. 5 times of sampling separated by at least 1 week). For schematic representation of experimental design see Fig. 1.

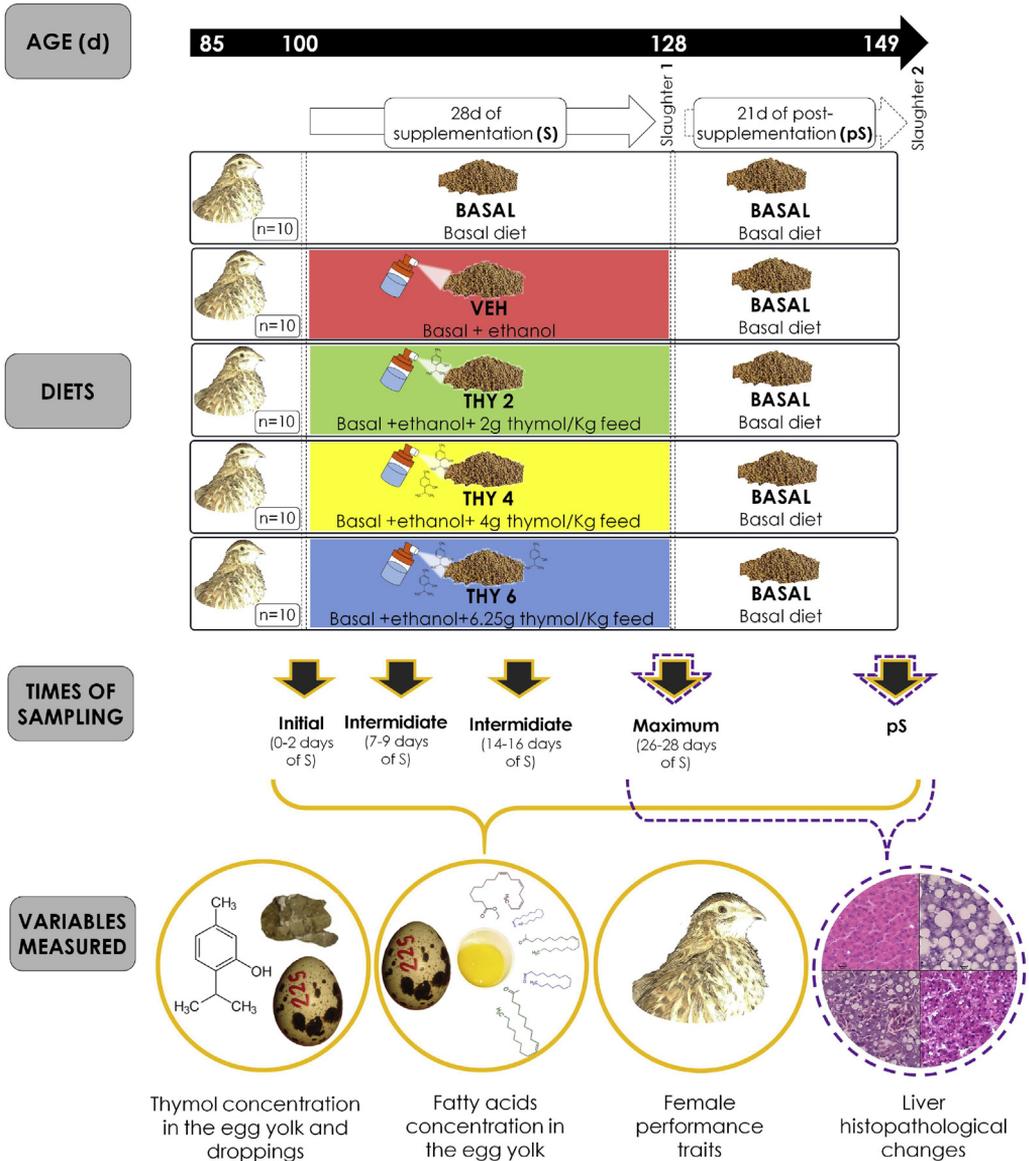
Data corresponding to the approximately 80 variables assessed are presented as raw data in 11 excel files and stored in the public repository figshare, at <https://doi.org/10.6084/m9.figshare.7589066.v1>. In each file, the headers of the columns representing the variable analyzed for each animal (rows). In this article, information describing the registered variables is summarized in four tables (see data records section Tables 1–4). In Table 1, information on free thymol concentration (in the feed, egg yolk and droppings), fatty acids concentrations (i.e. saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in the feed and egg yolk) and performance traits (weekly body weight, feed intake, egg laying and egg physical characteristics) are shown. Table 2, summarizes information regarding variables related to the general welfare assessment (female skin lesions and plumage state). Finally, Tables 3 and 4 show information regarding liver histopathological alterations and histological indices, respectively.

## 2. Experimental design, materials, and methods

### 2.1. Animals and husbandry

All experimental procedures were in compliance with the *Guide for the Care and Use of Laboratory Animals* issued by the National Institute of Health [1]. The experimental protocol was approved by the Institutional Committee for the Care and Use of Laboratory Animals (Comité Institucional para el Cuidado y Uso de Animales de Laboratorio (CICUAL)) of the Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba (ACTA 4/2015 Resolución 571-HCD-2014).

Adult female Japanese quail (*Coturnix japonica*) taken from a population of a single 230-bird hatch were used in this study. Chicks were brooded from day 1 in mixed-sex groups of ~40 within each of 6 brooder boxes, each measuring 90 cm × 90 cm × 60 cm (length × width × height). Each box had two feeders covering the front part of the box and 16 automatic nipple drinkers. A wire mesh floor (1 cm grid) was raised 5 cm to allow the passage of droppings and a lid prevented the birds from escaping. Brooding temperature was 37.5 °C during the first week of life, with a weekly decline of 3 °C until room temperature (24–27 °C) was achieved. At 28 days of age, females were sexed by plumage coloration, individually weighted and wing banded to identify each bird. At this age, female birds were individually housed in cages measuring 20 cm × 45 cm × 25 cm (length × width × height), allowing them to establish visual and auditory contact with each other, while permitting individual measurements of feed consumption and egg production. An individual feeder and an automatic nipple drinker were positioned in each cage. From hatch to 28 d of age, all birds were fed a starter ration (24% of Crude



**Fig. 1. Timeline scheme.** The days of age (d) of the birds is indicated by the time line in the upper box. All birds received basal diet between 85 and 100 days of age. From 100 to 128 days of age (Supplementation (S) period), females were provided a diet according to their dietary treatment assignment. Five diets were administrated (10 individuals randomly assigned to each one): two controls (BASAL and VEHICLE) and three increasing doses of thymol (THY2, THY4 and THY6, corresponding to 2, 4 and 6.25 g of THY/kg feed, respectively). Administered diets are indicated with boxes of rounded edges. Vertical dotted lines indicate changes in the diet of each experimental group. Once finished the supplementation period, half of the quail were slaughtered for histological analysis (Slaughter 1). The other half of the quail was subjected to a post-supplementation period of 21 days (pS) during which the basal diet was reestablished. Finished de pS period, the remaining birds were slaughtered for the same purpose as the first group (Slaughter 2). To assess changes in the variables studied, five times of sampling were defined along S and pS periods: one initial sample point; three sample points along the S period and one at the end of the pS period. Thymol concentration in the egg yolk and droppings, fatty acids concentration in the egg yolk and female performance traits were evaluated along the five sample points (horizontal bracket and circles of solid line). Histopathological changes of liver were assessed at the sample point of maximum Sand in the point of pS (horizontal bracket and a circle of dotted line).

**Table 1**

Description of variable column headers for excel files corresponding to data on thymol (2-isopropyl-5-methylphenol) concentration in feed, egg yolk and droppings, concentration of feed and egg yolk total fatty acids, weekly body weight and feed intake, egg laying and egg physical characteristics publicly available [18].

File name	Variable	Description
Fernandez 2019 Thymol in feed.xls	Concentration (ng/g)	Thymol concentration (ng/g) in quail feed
Fernandez 2019 Thymol in egg yolk.xls	Concentration (ng/g)	Thymol concentration (ng/g) in quail egg yolk
Fernandez 2019 Thymol in droppings.xls	Concentration (ng/g)	Thymol concentration (ng/g) in quail droppings
Fernandez 2019 Concentration of egg yolk total fatty acids.xls	Concentration (g/100 g FAME)	Concentration of fatty acids (g/100 g of FAME) in quail egg yolk. Column headers indicate the name of fatty acid analyzed
Fernandez 2019 Concentration of feed total fatty acids.xls	Concentration (g/100 g FAME)	Concentration of fatty acids (g/100 g of FAME) in quail feed. Column headers indicate the name of fatty acid analyzed
Fernandez 2019 Weekly body weight and feed intake.xls	Weekly body weight (g) Weekly feed intake (g)	Weekly body weight of quail Weekly feed intake (WFI) of quail. For example, WFI at the time of sampling "7 days of S" correspond to the summation of individual daily feed intake between 1 and 7 days of S; of WFI at the time of sampling "21 days of pS" correspond to the summation of individual daily feed intake between 15 and 21 days of pS.
Fernandez 2019 Cumulative number of eggs.xls	Cumulative number of eggs	Cumulative number of eggs laid from the beginning of S until the end pS.
Fernandez 2019 Egg physical characteristics.xls	Egg weight (g) Egg shell (%)  Egg albumen (%)  Egg yolk (%)  Egg shape index	Quail egg weight (g) Percentage of the total weight of the egg that corresponds to the shell  Percentage of the total weight of the egg that corresponds to the albumen.  Percentage of the total weight of the egg that corresponds to yolk  Ratio between the egg width and the egg length, expressed as a percentage

S = supplementation period, pS = post-supplementation period.

Protein and 2900 kcal of Metabolizable Energy kg diet<sup>-1</sup>). From this age on and until feed supplementation was initiated (100 d of age), birds were fed with the BASAL diet, which was a layer ration (20% of Crude Protein and 2900 kcal of Metabolizable Energy kg diet<sup>-1</sup>). At all stages feed and water were provided *ad libitum*. The photoperiod was 14 h light and 10 h dark (0600–2000 h; approximately 300–320 lx). The environmental temperature was maintained at 24 ± 2 °C.

## 2.2. General procedure

A total of 50 eighty-five days-old female quail of homogeneous body weights (251 ± 1 g; Mean ± SE) and in their egg-laying peak were selected from an initial group of 96 female individuals. All birds continued receiving a basal diet between 85 and 100 days of age and all variables (see below for details) were monitored along this period (Fig. 1). From 100 to 128 days of age (Supplementation period, S; Fig. 1), females were randomly assigned to either a chronic dietary supplementation with one of 3 THY doses (THY2, THY4 and THY6; 10 females each) or to one of 2 control groups (basal diet, BASAL, and basal diet with vehicle solution, VEHICLE; 10 females each). Once finished the S period, half of the quail

**Table 2**

Description of variable column headers for excel file "Fernandez 2019 General welfare.xls" [18] regarding welfare assessment variables in adult female quail supplemented with dietary thymol (2-isopropyl-5-methylphenol).

Variable name	Description
Lesions legs	Legs skin lesions score (range 0–2)
Lesions rear end	Rear end skin lesions score (range 0–2)
Lesions chest	Chest skin lesions score (range 0–2)
Lesions cloacae	Cloacae skin lesions score (range 0–2)
Lesions wings	Wing skin lesions score (range 0–2)
Plumage neck	Neck plumage damage score (range 0–2)
Plumage head	Head plumage damage score (range 0–2)
Plumage wings	Wings plumage damage score (range 0–2)
Plumage rear end	Rear end plumage damage score (range 0–2)
Plumage chest	Chest plumage damage score (range 0–2)
Foot Pad dermatitis	Score assigned to lesion observed in the foot (range 0–2)
Eye pathologies	Presence or absence of eye pathologies (1 or 0, respectively)
Dirt legs	Presence or absence of dirt in the legs (1 or 0, respectively)
Dirt cloacae	Presence or absence of dirt in the cloacae (1 or 0, respectively)
Dirt belly	Presence or absence of dirt in the belly (1 or 0, respectively)

**Table 3**

Description of histopathological variable recorded in the liver of in adult female quail supplemented with dietary thymol (2-isopropyl-5-methylphenol) presented in the columns of the excel file "Fernandez 2019 Frequency of liver histological alterations.xls" [18].

Variable	Description
Hemorrhage	Extent and severity of hemorrhage (a*w). RP1
Dilation of sinusoids	Extent and severity of dilation of sinusoids (a*w). RP1
Vascular congestion	Extent and severity of vascular (a*w). RP1
Hydropic degenerat.	Extent and severity of hydropic degeneration (a*w). RP2
Steatosis, fatty change	Extent and severity of steatosis or fatty change (a*w). RP2
Pycnotic hepatocytes	Extent and severity of pycnotic hepatocytes (a*w). RP2
Fibrosis	Extent and severity of fibrosis (a*w). RP2
Necrosis	Extent and severity of necrosis (liquefactive or focal) (a*w). RP2
Oval cells	Extent and severity of the area occupied by oval cells (a*w). RP3
Hypertrophy	Extent and severity of the hypertrophy (acute cellular swelling) (a*w). RP3
Hyaline degeneration	Extent and severity of hyaline degeneration (Intracellular eosinophilic bodies). RP3
Leucocyte infiltration	Extent and severity of the leucocyte infiltration (a*w). RP4
Average hemorrhage	Average of "a*w" values for hemorrhage within a treatment. RP1
Av. Dilation sinusoids	Average of "a*w" values for dilation of sinusoids within a treatment. RP1
Av. Vascular congest.	Average of "a*w" values for vascular congestion within a treatment. RP1
Av. hydropic degen.	Average of "a*w" values for hydropic degeneration within a treatment. RP2
Av. Steatosis, fatty ch.	Average of "a*w" values for steatosis or fatty change within a treatment. RP2
Av. Pycnotichepatoc.	Average of "a*w" values for pycnotic hepatocytes within a treatment. RP2
Av. Fibrosis	Average of "a*w" values for fibrosis within a treatment. RP2
Av. Necrosis	Average of "a*w" values for necrosis within a treatment. RP2
Av. Oval cells	Average of "a*w" values for area occupied by oval cells within a treatment. RP3
Av. Hypertrophy	Average of "a*w" values for hypertrophy within a treatment. RP3
Av. Hyaline nfiltrate.	Average of "a*w" values for hyaline degeneration within a treatment. RP3
Av. Leucocyte infiltrate.	Average of "a*w" values for leucocyte infiltration within a treatment. RP4
Rel. hemorrhage	Relativized "a*w" values for hemorrhage within a treatment. RP1
Rel. dilation of sinus.	Relativized "a*w" values for dilation of sinusoids within a treatment. RP1
Rel. vascular congest.	Relativized "a*w" values for vascular congestion within a treatment. RP1
Rel. hydropic degener.	Relativized "a*w" values for hydropic degeneration within a treatment. RP2
Rel. steatosis, fatty	Relativized "a*w" values for steatosis or fatty change within a treatment. RP2
Rel. pycnotichepatoc.	Relativized "a*w" values for pycnotic hepatocytes within a treatment. RP2
Rel. fibrosis	Relativized "a*w" values for fibrosis within a treatment. RP2
Rel. necrosis	Relativized "a*w" values for necrosis within a treatment. RP2
Rel. oval cells	Relativized "a*w" values for area occupied by oval cells within a treatment. RP3
Rel. hypertrophy	Relativized "a*w" values for hypertrophy within a treatment. RP3
Rel. hyaline degener.	Relativized "a*w" values for hyaline degeneration within a treatment. RP3
Rel. leucocyte infiltr.	Relativized "a*w" values for leucocyte infiltration within a treatment. RP4

Av = Average, Rel. = Relativized. RP = Contributes to specified reaction pattern.

**Table 4**

Description of variable column headers for excel file “Fernandez 2019 Liver histopathological indices.xls” regarding liver histopathology indices in adult female quail supplemented with dietary thymol (2-isopropyl-5-methylphenol) [18].

Variable	Description
HI <sub>Liv.Rp1</sub>	Liver histopathological index related to reaction pattern 1
HI <sub>Liv.Rp2</sub>	Liver histopathological index related to reaction pattern 2
HI <sub>Liv.Rp3</sub>	Liver histopathological index related to reaction pattern 3
HI <sub>Liv.Rp4</sub>	Liver histopathological index related to reaction pattern 4
HI <sub>Liv</sub>	Total liver histopathological index

were slaughtered for histological analysis ( $n = 25$ ; Slaughter 1, Fig. 1) and the other half were subjected to a post-supplementation (pS) period of 21 days, during which all females received the basal diet -BASAL- (pS period, Fig. 1). After this period, the remaining 25 animals were also slaughtered for histological analysis (Slaughter 2, Fig. 1). In this way, we assessed whether THY induced changes in variables indicative of tissue damage and if so, whether the potential damages could reverse after 3 weeks of supplement withdrawal.

To assess changes in the variables studied, five times of sampling were defined along S and pS periods: one initial sample point (at 0 days of S); three sample points during the S period (at 7, 14 and 28 days of S); and one sample point at the end of the pS period (21 days of pS). It should be noted that for determinations of THY concentration, eggs and quail droppings were collected offset from the other variables measured by 2 days, thus were collected on days 2, 9, 16, and 26 days of S, and 17 days of pS, respectively.

For the variables THY concentration, FA concentration, feed intake, body weight, morphometric variables and percentage of egg constituents, the experimental design contemplates that data could be analyzed using a general linear mixed model including the diet supplied (five levels: BASAL, VEHICLE, THY2, THY4 and THY6), time of sampling (five levels: initial, three sample points during the S period and one sample point at the end of the pS period) and their interaction as the fixed effects, and female identity (categorical variable with levels = N) could be included in the model as a random effect. For each one of the above mentioned variables separately, considering a normal distribution, the mixed model can be represented as:

$$Y = X\beta + Z\mu + \epsilon \quad (1)$$

where,

**Y** is the vector of observations of the response variable **Y** with mean  $E(\mathbf{Y}) = \mathbf{X}\beta$ ;  $\beta$  is an unknown vector of the fixed effects Diet supplied, Time of sampling and their interaction.

$\mu$  is an unknown vector of the random effect female identity, with mean  $E(\mu) = 0$  and variance-covariance matrix  $\text{var}(\mu) = G$ ;  $\mu \sim N(0, G)$ .

$\epsilon$  is an unknown vector of random errors of the random variable female identity, with mean  $E(\epsilon) = 0$  and variance  $\text{var}(\epsilon) = R$ , which reflect **Y** variation non explicated by the fixed effects Diet and Time of sampling nor the random effect female identity;  $\epsilon \sim N(0, R)$ .

**Z** is a known design matrix that denotes belonging of each **Y** observation to a level of the random effect female identity ( $\mu$ ).

Thus,  $Z\mu$  are the random effects resulting from female identity that determine **Y** covariance.

**X** is a known design matrix that denotes belonging of each y observation to a combination of levels of the fixed effects Diet and Time of sampling ( $\beta$ ).

Thus, **X** and **Z** are known design matrices relating the observations **Y** to  $\beta$  and  $\mu$ , respectively.

For histological variables, experimental design would imply fixed effects of diet supplied (the five levels above-mentioned), the time of sampling (including two levels: 28 days of S or 21 days of pS), and

their interaction. For each one of the histological variables, considering a normal distribution and in linear notation, the model can be represented as:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk} \quad (2)$$

$Y_{ijk}$  is the output of an individual  $k$  observation of the response variable for  $i$  level of the fixed effect Diet and  $j$  level of the fixed effect Time of sampling.

$\mu$  is the global mean for response variable  $Y$ .

$\alpha_i$  denotes the fixed effect Diet (i.e.  $\mu_i - \mu$ ),  $i = \text{BASAL, VEHICLE, THY2, THY4, THY6}$ .

$\beta_j$  denotes the fixed effect Time of sampling (i.e.  $\mu_j - \mu$ ),  $j = 28\text{S and } 21\text{pS}$ .

$\alpha\beta_{ij}$  denotes the interactive fixed effect of the factors Diet and Time of Sampling (i.e.  $\mu_{ij} - \mu$ ).

$\varepsilon_{ijk}$  residual errors  $\sim N(0, \sigma^2)$ .

Finally, for egg-laying (as cumulative number of eggs) only Diet could be considered as a fixed effect. Considering a normal distribution and in linear notation, the model can be represented as:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \quad (3)$$

$Y_{ij}$  is the output of an individual  $j$  observation of the response variable for  $i$  level of the fixed effect Diet.

$\mu$  is the global mean for response variable  $Y$ .

$\alpha_i$  denotes the fixed effect Diet (i.e.  $\mu_i - \mu$ ),  $i = \text{BASAL, VEHICLE, THY2, THY4, THY6}$ .

$\varepsilon_{ij}$  residual errors  $\sim N(0, \sigma^2)$ .

### 2.3. Dietary supplementation

Thymol was commercially obtained from Sigma-Aldrich (SAFC<sup>®</sup>,  $\geq 99\%$ ; FCC, Saint Louis, MO, USA). Thymol was prepared in a 2.4, 4.8 and 7.5% w/v ethanolic solution and uniformly sprayed on fresh feed in order to obtain 2, 4 or 6.25 g of THY per kg of feed [2]. Although THY volatilization in the feed supplied to the quail has not yet been characterized, the estimated Henry's Law constant for THY ( $3.5 \times 10^{-6}$  atm-cu m/mole, derived from its vapor pressure, 0.016 mm Hg at 25 °C, and water solubility, 900 mg/L) together with experimental evidence, indicate that this compound volatilizes or could be degraded from water and moist soil surfaces at rates which greatly vary (from days to months) depending on specific conditions of the matrix (For details, please see: [3]). As THY volatilization and actual concentration in the feed supplied potentially affects its transference and activity in target tissues, herein feed was prepared and supplied daily (in order to minimize THY volatilization from feed before its consumption by females) and THY concentration in feed (sampled immediately after being prepared) was determined for the three doses by HS-SPME (see following section).

Nutrient and FA compositions of the diets supplied were identical between each other and FA concentrations are reported in excel file "Fernandez 2019 Concentration of feed total fatty acids.xls".

### 2.4. Thymol quantification in egg yolk, quail droppings and feed

Thymol concentration was immediately measured in egg yolks and droppings obtained at each sample point and in feed by head space-solid phase microextraction followed by gas chromatography-mass spectrometry (HS-SPME/GC-MS) according to Fernandez et al. (2017) [4]. SPME was performed with a manual holder with 100  $\mu\text{m}$  polydimethylsiloxane (PDMS) fiber. An aliquot of egg yolk (3 g), droppings (5 g) or feed (5 g) was put in a sealed 20 mL glass vial, spiked at the corresponding level with the standard m-cresol (Sigma-Aldrich;  $\geq 99\%$  (GC); Saint Louis, MO, USA) stock solution, and vortexed for 5 min. The vials were placed in a water bath and the PDMS fiber was exposed to the headspace for 30 min at 60 °C and 5 min at 40 °C for egg yolk and droppings or feed, respectively. The fiber was then inserted directly into the GC injector for desorption at 250 °C for 10 min in splitless mode.

Chromatography analysis was carried out in a Perkin Elmer Clarus 600 equipped with a PSSI injector and a quadrupole MS detector (Perkin Elmer, USA). Turbo Mass 5.4.2 software was used to control and acquire data from GC–MS. All the separations were performed using a Perkin Elmer fused silica DB 5 MS capillary column (60 m, 0.25 mm ID, 0.25  $\mu\text{m}$  film thickness), with High-purity helium (99.998%) as a carrier gas (49.6 psi). The splitless injection mode was selected. Electron-impact Ionization was carried out in the mass spectrometer under vacuum with 70-eV ionization energy. Samples were analyzed under the following chromatographic and MS detection conditions: initial oven temperature was set at 100 °C (held for 2 min), and then raised to 230 at 10 °C/min rate. A column head pressure of 14.99 psi and an injector temperature of 280 °C were set. The GC transfer line was maintained at 250 °C. The fiber was desorbed in the GC injector port for 10 min. Chromatograms were acquired in scan mode, which scans the quadrupole from  $m/z = 50$  to  $m/z = 300$  (scan time: 0.20 s, inter-scan time: 0.10 s). All quantitative analyses were performed in TIC mode. The compounds were identified by comparing their mass spectra with those of the libraries of the NIST MS search 2.0. The main components were further identified by co-injection of commercial standards.

### 2.5. Total fatty acid analysis in egg yolks

Dataset includes the estimated concentration of saturated (i.e. myristic, pentadecanoic, palmitic and stearic acids), monounsaturated (i.e. palmitoleic and oleic acids) and polyunsaturated (i.e. linoleic, linolenic, arachidonic, docosahexaenoic, eicosapentaenoic and docosapentaenoic acids) fatty acids of quail egg yolk. Eggs obtained at each time of sampling were stored at  $-20$  °C until yolk total fatty acids analysis. Lipids were extracted from 1 g of yolk following homogenization in a suitable excess of chloroform/methanol (2:1 v/v) [5]. The solvents were removed under reduced pressure in a rotary evaporator. Lipids were subjected to alkaline saponification (1 mol/L potassium hydroxide in methanol), and the unsaponifiable matter was extracted with *n*-hexane. The fatty acid methyl esters (FAMES) were prepared by transmethylation through treatment with 1 mol/L sulfuric acid in methanol and analyzed by gas chromatography/mass spectrometry (GC/MS) [2,6]. All chemicals used in this study were reagent-grade commercial products. FAMES were analyzed by gas chromatography on a 60 m fused capillary column with an internal diameter of 0.25 mm (PerkinElmer Elite-WAX Polyethylene Glycol). The analysis was performed on a PerkinElmer Clarus<sup>®</sup> 600 GC/MS system equipped with a flame ionization detector (Waltham, MA, USA). Helium was used as carrier gas (constant flow of 49.6 psi). The injection port temperature was 250 °C and the detector temperature was 250 °C. The oven temperature was initially held at 180 °C for 5 min, then increased at 4 °C/min to 200 °C and held for 5 min and finally increased at 3 °C/min to 230 °C and held for 25 min. Peak identification was carried out by comparing the known retention times for the fatty acids reported with the temperature program and the chromatographic system used. A solution of known concentration of nonadecanoic acid methyl ester (Sigma Aldrich,  $\geq 98.0\%$  (GC); Saint Louis, MO, USA) as was used internal standard to estimate the content of each fatty acid in the sample.

### 2.6. Female performance traits

Female performance was evaluated through the measurement of body weight, feed intake, egg laying rate, egg physical characteristics and a general welfare quality assessment. Body weight was measured once a week throughout the experimental period. Daily feed intake (DFI) was estimated as the difference between the amount of feed supplied to each animal (60 g) and the rest that remained in the feeders the next day (g of feed/day/quail) [7,8]. Egg laying was registered daily and the weekly laying rate was calculated as: (number of eggs/7 days)  $\times$  100. Egg weight, quality characteristics (such as intact, membranous, soft shell and broken shell), morphometric characteristics (egg shape index = (egg width/egg length)  $\times$  100) and percentage of constituents (egg yolk, albumen and shell) were registered at each time of sampling [9,10].

A welfare assessment based on observations of physical characteristics was made. Female skin lesions and plumage status were evaluated following a procedure proposed by our colleagues [11] that is an adapted version of the protocol developed by the Welfare Quality consortium [12]. Briefly, skin lesions, which include wounds that have not healed in the legs, rear end, chest, cloacae and wings were

determined using a score scale from 0 to 2, where “0” represents no lesions or scratches, “1” represents at least one lesion <0.5 cm diameter or less than 3 pecks (punctiform damage ~ 0.1 cm of diameter) or scratches, and “2” reflects one lesion  $\geq 0.5$  cm of diameter or more than 3 pecks or scratches. Plumage damage was also determined using a score scale from 0 to 2 as follows: “0” represents individuals with no plumage damage or slight wear (only single feathers lacking), “1” represent individuals with one or more body parts that have moderate wear (i.e. damaged feathers worn or deformed) or one or more featherless areas <1.5 cm in diameter at the larger extent and “2” corresponded to individuals that have at least one featherless area >1.5 cm in diameter at the largest extent. Foot pad dermatitis, for which both feet were analyzed and the foot with the worst condition was scored according to the following: “0” representing feet intact, no or minimal proliferation of epithelium, “1” corresponded to necrosis or proliferation of epithelium or chronic bumble foot with no or moderate swelling, and “2” indicated swollen dorsally visible. Eye pathologies, which include swelling of the eyelids and the skin around the eyes, closure of the eye/eyes and discharge from the eyes were classified as “0” when no evidence of eye pathologies were observed or “1” if there were eye pathologies. Additionally, dirt from the legs, cloacae and belly was examined for signs of diarrhea potentially caused by dietary supplementation.

### 2.7. Liver histological analysis

At each slaughter point birds were euthanized by decapitation [13] and livers were removed and stored in buffered formalin at 10%, processed routinely and stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) as described elsewhere [14,15]. Each slide was examined blinded, with a light microscope (Olympus X-785) and photographed with a digital camera (Moticam Camera 2300, 3 Megapixels). To evaluate histological alterations of liver, tissue was divided into 8 random equal areas to ensure there was no overlapping of the studied areas. For each area, the extension and number of each alteration were recorded with a microscope at 40 $\times$  magnitude. Histopathological index of liver ( $HI_{liv}$ ) were estimated using a semi-quantitative protocol following [16], modified by our colleagues [17]. This procedure has advantages for analysis and interpretation of the data, since it performs a standardized quantification of the different alterations. Briefly, alterations were classified into four major reaction patterns each one of those including alterations that concern to different functional units of the liver or to the whole organ: RP1, circulatory disturbances (result from a pathological condition of blood and tissue fluid flow, such as dilatation of sinusoids, vascular congestion, hemorrhage); RP2, regressive changes (include processes leading to a reduction or total loss of organ function, such as steatosis or fatty degeneration, hydropic degeneration, nuclear alteration, fibrosis, necrosis); RP3, progressive changes (refer to processes that lead to an increased activity of cells or tissues, such as cell hypertrophy and hyperplasia, and presence of oval cells); and RP4, liver inflammation (include processes generally associated to alterations belonging to other reaction patterns, such as leucocyte infiltration, oedema, etc.). The relevance of each lesion depends on its pathological importance, that is, how much it affects the functionality of the organ or the survival of the bird. This is taken into account through a factor of importance (W) that is assigned to each histological alteration. This factor (W) can take values of:

W = 1: minimal pathological importance (the lesion is easily reversible when the toxic exposure ends);

W = 2: moderate pathological importance (the lesion may or may not be reversible depending on the severity and extent of the injury);

W = 3: marked pathological importance (the lesion is normally irreversible, leading to a partial or total loss of the organ function).

On the other hand, each observed pathological damage is assigned an Occurrence Value (a), whose values oscillate between 0 (zero) and 8 (eight), depending on the degree and extent of the damage expressed in percentage. Thus, each possible value of the occurrence value (a) indicates:

0: absence of alteration;

2: mild occurrence;

4: moderate occurrence;

- 6: severe occurrence;
- 8: extreme occurrence.

Then, for each RP, an index was calculated based on the two factors: the pathological importance of the lesions (factor of importance, W; range 1–3) and the extension of pathological change (occurrence value, a; range 0–8). Finally, a total liver histopathological index (HI<sub>liv</sub>) was calculated by adding the single RP liver indices of each individual quail. A greater value of (HI<sub>liv</sub>) reflects the most severely affected individual.

### 3. Data records

The data corresponding to the variables assessed are presented in excel format file and stored in the public repository figshare [18] with the headers of the columns representing the variable analyzed for each animal (rows). In all excel files the first 3 columns prior to variable records correspond to animal identification and treatment group. In the first column the animal identification number is stated, in the second the diet supplied (BASAL, VEHICLE, THY2, THY4, THY6) and in the third the time of sampling (0–2 days of S, 7–9 days of S, 14–16 days of S, 26–28 days of S and 17–21 days of pS). In excel files “Fernandez 2019 Thymol in feed.xls” and “Fernandez 2019 Concentration of feed total fatty acids” only the columns feed identification and diet supplied are indicated prior to variable records, due to feed was measured only once immediately after it was prepared. Tables 1–4 indicate the information referred to each excel file, column header abbreviation of variable and its corresponding description.

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### Transparency document

Transparency document associated with this article can be found in the online version at <https://doi.org/10.1016/j.dib.2019.103884>.

### References

- [1] National Institute of Health, *Guide for the Care and Use of Laboratory Animals*, eighth ed., 2011.
- [2] M.E. Fernandez, R.H. Marin, A. Luna, M.P. Zunino, M.C. Labaque, Thymol feed supplementation in quail alters the percentages of nutritionally relevant egg yolk fatty acids: effects throughout incubation, *J. Sci. Food Agric.* 97 (2017) 5233–5240. <https://doi.org/10.1002/jsfa.8407>.
- [3] National Center for Biotechnology Information. PubChem Compound Database; CID=6989, <http://pubchem.ncbi.nlm.gov/compound/6989>. (accessed 13 February 2019).
- [4] M.E. Fernandez, M.A. Palacio, M.C. Labaque, Thymol detection and quantitation by solid-phase microextraction in faeces and egg yolk of Japanese quail, *J. Chromatogr. B* 1044–1045 (2017) 39–46. <https://doi.org/10.1016/j.jchromb.2016.12.042>.
- [5] J. Folch, M. Less, G. Sloane Stanley, A simple method for the isolation and purification of total lipides from animal tissues, *J. Biol. Chem.* 226 (1957) 497–509.
- [6] M.C. Labaque, M. Martella, D. Maestri, J. Navarro, The influence of diet composition on egg and chick traits in captive Greater Rhea females, *Br. Poult. Sci.* 54 (2013) 374–380.
- [7] F. Vercese, E.A. Garcia, J.R. Sartori, A. de P.I. Silva, A.B.G.I. Faitarone, D.A.I. Berto, A. de B.I. Molino, K.I.V. Pelícia, Performance and egg quality of Japanese quails submitted to cyclic heat stress, *Brazilian J. Poult. Sci.* 14 (2012) 37–41. <https://doi.org/10.1590/S1516-635X2012000100007>.
- [8] N. Vali, S. Mottaghi, The effect of using different levels of cinnamon and thyme powder on egg characteristics and fatty acids profile in Japanese quail, *CIBTech. J. Zool.* 5 (2016) 2319–3883.
- [9] A. Luna, J.S. Dambolena, J.A. Zygadlo, R.H. Marin, M.C. Labaque, Effects of thymol and isoeugenol feed supplementation on quail adult performance, egg characteristics and hatching success, *Br. Poult. Sci.* 53 (2012) 631–639. <https://doi.org/10.1080/00071668.2012.721536>.

- [10] N. Nikolova, D. Kocovski, Forming egg shape index as influenced by ambiente temperatures and age of hens, *Biotechnol. Anim. Husb.* 22 (2006) 119–125.
- [11] S. Pellegrini, L. Condat, R. Marin, D. Guzman, Can Japanese quail male aggressions toward a female cagemate predict aggressiveness toward unknown conspecifics? *Poultry Sci.* 96 (2017) 133.
- [12] Collection of data for laying hens on farm, in: Harry J. Blokhuis, et al. (Eds.), *Welfare Quality® Consortium. Welfare Quality® Assessment Protocol for Poultry (Broilers, Laying Hens) Consortium*, 2009, pp. 76–98. Lelystad, Netherlands.
- [13] *AVMA Guidelines for the Euthanasia of Animals*, ed. S3.4, 2013, p. 62. N. Meacham Road Schaumburg.
- [14] M.L. Jacobsen, V.L.B. Jaspers, T.M. Ciesielski, B.M. Jenssen, M.E. Løseth, N. Briels, I. Eulaers, P.S. Leifsson, F.F. Rigét, P. Gomez-Ramirez, C. Sonne, Japanese quail (*Coturnix japonica*) liver and thyroid gland histopathology as a result of in ovo exposure to the flame retardants tris(1,3-dichloro-2-propyl) phosphate and Dechlorane Plus, *J. Toxicol. Environ. Health A.* 80 (2017) 525–531, <https://doi.org/10.1080/15287394.2017.1336414>.
- [15] J.H. Lefkowitz, Special stains in diagnostic liver pathology, *Semin. Diagn. Pathol.* 23 (2006) 190–198. <https://doi.org/10.1053/j.semmp.2006.11.006>.
- [16] D. Bernet, H. Schmidt, W. Meier, P. Burkhardt-Holm, T. Wahli, Histopathology in fish: proposal for a protocol to assess aquatic pollution, *J. Fish Dis.* 22 (2001) 25–34.
- [17] G.E. Rautenberg, M.V. Amé, M.V. Monferrán, R.I. Bonansea, A.C. Hued, A multi-level approach using *Gambusia affinis* as a bioindicator of environmental pollution in the middle-lower basin of Suquia River, *Ecol. Indicat.* 48 (2015) 706–720.
- [18] M.E. Fernandez, J.M.K. Kembro, M.L. Ballesteros, J.M. Caliva, R.H. Marin, M.C. Labaque, Data of thymol dietary supplementation in quail (*Coturnix japonica*): linking bioavailability, effects on egg yolk total fatty acids and performance traits, DOI: 10.6084/m9.figshare.7589066.v1.