In vivo performances, ileal digestibility, and physicochemical characterization of raw and boiled eggs as affected by *Tenebrio molitor* larvae meal at low inclusion rate in laying quail (*Coturnix japonica*) diet

Giulia Secci,[*] Nicola F. Addeo,[†] Lina F. Pulido Rodriguez,[*] Fulvia Bovera,[†,2] Giuseppe Moniello,[‡,1] and Giuliana Parisi[*,1]

[*]*Department of Agriculture, Food, Environment and Forestry, University of Florence, Firenze 50144, Italy;* [†]*Department of Veterinary Medicine and Animal Production, University of Napoli Federico II, Napoli 80137, Italy; and* [‡]*Department of Veterinary Medicine, University of Sassari, Sassari 07100, Italy*

ABSTRACT A total of 120, twelve wk old female Japanese quails were divided into 4 groups (6 replicates of 5 birds each). The control group (**CON**) fed a corn-soybean diet; in the other 3 groups, *Tenebrio molitor* larvae meal (**TML**) replaced 5, 10, and 20% of the soybean protein (**T5, T10**, and **T20**). The laying performance and egg quality were studied for 54 d. The data were processed by a one-way ANOVA; the orthogonal contrast analysis was performed to test the linear, quadratic and cubic effects among the means. The laying rate and egg mass linearly decreased ($P < 0.01$) as the TML inclusion level in the diet increased. The egg weight and feed conversion ratio linearly increased from the control to T20 diet ($P < 0.01$) while the digestibility of dry matter, organic matter, and crude protein linearly decreased ($P < 0.05$). The albumen and yolk weight showed a linear increase ($P < 0.01$) due to dietary TML inclusion, while the eggshell weight showed the opposite ($P < 0.05$). The estimated activity of Δ9-desaturase (C16:0), Δ5+Δ6-desaturase on both polyunsaturated fatty acid n-6 and n-3 linearly increased ($P < 0.05$) as affected by dietary TML. The boiled yolk lightness (***L****) showed higher values in T5 and T10 groups (quadratic contrast, $P < 0.01$). The yolk redness index (***a****) showed lower values in T5 and T20 than control and T10 groups (cubic contrast, $P < 0.01$). The albumen L^*, a^*, and b^* indexes showed a significant effect of the quadratic contrast ($P < 0.05$). In addition, the albumen b^* index showed a significant effect of the cubic contrast ($P < 0.01$). The total lipids showed the highest values (cubic contrast, $P < 0.05$) in the T10 and T20 groups. The total monounsaturated fatty acids linearly increased ($P < 0.05$) according to the increase of dietary TML. The best inclusion level of defatted TML meal for laying quails seems to be 1.4% of diet, corresponding to the T5 diet.

Key words: boiling, fatty acid, laying performance, poultry, yellow mealworm

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INTRODUCTION

The poultry sector grew from 9 to 122 Mt in the last 5 decades (1961−2017) and a further increment is expected (FAO, 2021) despite the overall stagnation of livestock productions. The success of poultry meat is mainly due to its high nutritional value, moderate costs, and because of its consumption not to be limited by religion. Among the "alternative" species, quail (*Coturnix coturnix*) represents approximately 12% of birds involved in meat and egg productions, the second largest one after chickens (Lukanov, 2019). Quail eggs are mainly produced in Asia, being China the largest producer with around 4.64 million tonnes laid in 2019, corresponding to more than 70% of the global production, followed by Thailand, Indonesia and Brazil. Europe, whose quail farming is mainly based on meat production, stands among the top 10 producers only with Russia that provides the 0.39% of the global production (FAOSTAT, 2021). Quail egg production increased its volume during the decade 2009−2019, passing from 5.2 to 6.04 million tonnes with a global export value of 702.96 USD/metric ton (FAOSTAT, 2021). As part of the poultry sector, even laying quails activities need to be fast orientated towards more sustainable horizons, in order to reduce its environmental impact: the livestock sector has been estimated to exceed 5.4 billion tons of

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[1]The authors contributed equally to the paper.

[2]Corresponding author: bovera@unina.it

CO_2-eq, equal to 14.5% of the total anthropogenic greenhouse gas emissions (Grossi et al., 2019) and the poultry industry is responsible for the production of 0.1 giga ton of CO_2-eq (in comparison, dairy cattle provide 1 G ton). As recently underlined, feed production and processing are hot spots for the livestock sector, whose contribute in terms of greenhouse gas emissions has been esteemed at 45% of the overall impact (Grossi et al., 2019). In addition, the most common ingredients for poultry diets, as maize and soybean meal, are harshly criticized due to the water and broad spaces exploitations, as well as their competition with human consumption that is a product suggested to be integrated while evaluating the environmental impact (Costantini et al., 2021).

Despite the Food and Agriculture Organisation has endorsed edible insects in human diet, people from Western cultures still do not promote entomophagy but they can accept insect derived products as animal feeds. The increasing interest in insect meal as alternative protein source in poultry sector has been demonstrated both by the escalation of published works in the last decade and the expected approval of its use by the European Commission. The EU Regulation 2017/893 admitted the use of *Tenebrio molitor* (**TM**), however, to date, only the aquaculture sector can benefit of insects as ingredient for feed. However, very few studies specifically considered the use of insect meals in laying quails and they are divided into black soldier fly (Dalle Zotte et al., 2019), *T. molitor* (Shariat Zadeh et al., 2020) or other local insect species (Das and Mandal, 2014). The preliminary evaluation of Shariat Zadeh et al. (2020) found a positive correlation between the inclusion of TM larvae meal, the egg productive performance and the egg-related indices. Graded substitution level of fishmeal with TM (included at 15 and 22.5% as fed) significantly lowered the feed conversion ratio (**FCR**) values, but egg quality traits such as yolk height and shell weight were significantly reduced. In general, Shariat Zadeh et al. (2020) highlighted the safety of using TM meal for quails' health, however additional data are necessary to evaluate the possibility to include TM in the diet of laying quails. Indeed, other studies aimed to substitute the conventional protein sources including the insect meal (TM larvae) in feeds for laying hens at 1, 2, and 3% (Ko et al., 2020) or 2.5 and 5% (Sedgh-Gooya et al., 2021) finding that the TM larva inclusions were effective in improving the FCR, but the authors suggest to limit the inclusion of TM at the 3% to avoid modification in blood parameters of laying hens and in egg quality characteristics.

Our hypothesis that *T. molitor* larvae meal could represent a potential ingredient in laying quail diet and its inclusion can have similar effects than in laying hens. Thus, the present trial aimed to study the effect of low inclusion levels of a *T. molitor* defatted larvae meal on laying performance and egg physical and chemical characteristics of quails. Moreover, considering that in some countries, such as Japan, the market volume of boiled quail egg, generally preserved in cans, amounted to more than 1,000 tons in 2019 (www.statista.com), a cooking trial was assessed to evaluate the boiled-egg quality.

MATERIALS AND METHODS

Growing Trial, Egg Collection, Apparent Ileal Digestibility

All the animals were humanely treated according to the principles of the animal welfare stated by the Directive 63/2010/EEC regarding the protection of the animals used for experimental and other scientific purposes. The experimental procedures were approved by the Ethical Animal Care and Use Committee of the Department of Veterinary Medicine and Animal Production of the University of Napoli Federico II, Italy (prot. N. 2017/0017676). The trial was carried out in a commercial quail farm located in the province of Sassari (Sardinia, Italy).

A total of 120, 12-wk-old female Japanese quails (*Coturnix japonica*, average weight 299.85 ± 15.87 g) were equally divided into 4 groups (30 birds per groups, each containing 6 replicates of 5 birds). Each replicate was housed in galvanized metal cages (100 × 50 × 25 cm high) equipped with 3 feeding points and 3 nipples, providing feed and drinking water ad libitum, respectively. The cages were located in an environmental controlled room at 23.8 ± 0.7°C temperature and 58.5 ± 5.7% relative humidity, under 16:8 h dark:light lighting regimen. The groups were submitted to different dietary treatments. The control group (**CON**) fed a corn-soybean diet formulated to meet or exceed the nutritional requirements of the birds, according to NRC (1994) and Arif et al. (2010). For the other 3 groups, indicated as T5, T10, and T20, an aliquot of soybean, respectively equal to 2.4, 4.1, and 9.6% (corresponding to 1.05, 1.80, and 4.20% of crude protein and around to 5, 10, and 20% of the protein of the control diet) was replaced with the protein from defatted *Tenebrio molitor* larvae meal (**TML**), respectively. The inclusion level of the TML was 1.4, 2.8, and 5.6% for T5, T10, and T20 groups, respectively (corresponding to 0.96, 1.93, and 3.96% of crude protein). The defatted insect meal was purchased at the ENTOMO Farm Company (Libourne, France). To measure nutrient digestibility, an indigestible marker (Celite, Sigma-Aldrich, St. Louis, MO) was added to the dosage of 5 g/kg during the last 10 d of the trial to each diet at the expense of corn.

Samples of the 2 main protein sources (soybean meal and TML) and of the diets were analysed for chemical-nutritional characteristics. The chemical composition (dry matter, ash, crude protein, ether extract, and fiber fractions) was determined according to the AOAC (2005) methods. For the only insect meal, the nitrogen-to-crude protein conversion factor was 4.97, according to Janssen et al. (2017). The acid detergent fiber (**ADF**) and the residual nitrogen in ADF (**N-**

Table 1. Analyzed nutrient composition (% as fed) of the soybean and *Tenebrio molitor* larvae meals used in the trial.

	Soybean meal	*Tenebrio molitor* larvae meal
Dry matter	90.21	94.4
Ash	5.97	3.21
Crude protein	43.9	68.9
Ether extract	1.15	7.50
ADF[1]	5.47	6.97
N-ADF[2]	11.5	2.46
Chitin	-	4.51
Ca	0.30	1.97
P	0.63	1.25
Methionine, % CP[3]	0.59	1.35
Lysine, % CP	2.87	4.69

[1]ADF, acid detergent fiber.
[2]N-ADF, nitrogen linked to ADF.
[3]CP, crude protein.

ADF; AOAC, 2005) were determined and used to estimate the amount of chitin according to Marono et al. (2015). The levels of calcium, phosphorous and amino acids as methionine, lysine, and cysteine were determined according to Addeo et al. (2021). The metabolizable energy content of the diets was calculated from their chemical composition according to NRC (1994) equations; the apparent metabolizable energy for insect meals used in the present trial was calculated using the apparent digestibility coefficients of the total tract (**CTTAD**), as measured by De Marco et al. (2015). The chemical-nutritional characteristics of the protein sources and the ingredients and chemical-nutritional characteristics of the diets are reported in the Tables 1 and 2, respectively. The fatty acid profile of the diets (Table 3) was determined according to the method described below for the eggs.

Table 2. Ingredients and chemical-nutritional characteristics of the diets used in the trial.

Diet[1]	CON	T5	T10	T20
Ingredients, %				
Corn meal	52.1	53.7	55.0	57.0
Soybean meal 44%	34	31.6	29.2	24.4
Insect meal	-	1.4	2.8	5.6
Vegetable oil	6	5.5	5.3	5.3
Calcium carbonate	5.5	5.4	5.3	5.3
Dicalcium phosphate	1.5	1.5	1.5	1.5
Mineral Vitamin premix[2]	0.5	0.5	0.5	0.5
Methionine	0.1	0.1	0.1	0.1
Salt	0.3	0.3	0.3	0.3
Chemical composition				
Dry matter, %	90.48	90.77	90.55	90.42
Ash, % DM[3]	12.01	11.45	12.38	11.88
Crude protein, % DM	21.74	22.10	22.48	22.02
Ether extract, % DM	8.46	7.75	8.14	8.36
Ca, % DM	2.53	2.52	2.52	2.53
AvP[4], % DM	0.36	0.35	0.38	0.39
Crude fiber, % DM	9.94	8.16	9.12	7.23
NDF[5], % DM	9.68	11.16	10.45	9.73
ADF[6], % DM	6.26	6.58	6.07	6.91
ADL[7], % DM	2.51	2.77	2.88	3.01
Methionine+Cysteine, % DM	0.83	0.86	0.86	0.88
Lysine, % DM	1.16	1.22	1.24	1.28
ME, Kcal/kg[8]	2,890	2,890	2,890	2,891

[1]T5, T10, T20: diets in which 5, 10, and 20% of the protein of the control diet (CON) have been replaced with the protein from defatted *Tenebrio molitor* larvae meal.
[2]Provides per kg of product: Fe, 50,000 mg; Co, 200 mg; Cu, 8,500 mg; Mn, 75,000 mg; Zn, 70,000 mg; Se, 250 mg; I, 1,500 mg; folic acid, 500 mg; pantothenic acid, 13.5 g; niacin, 30 g; vit. A, 10,000,000 IU; cholecalciferol 50,000 μg; vit. K3, 4,000 mg; vit. B2, 5,000 mg; vit. B6, 2,000 mg; vit., B12 10,000 μg; vit. E (dl-α-tocopheryl acetate) 21,978 IU. Celite was added at the dosage of 5 g/kg during the last 10 days of the trial to each diet at the expense of corn meal.
[3]DM, dry matter.
[4]AvP, available phosphorous.
[5]NDF, neutral detergent fiber.
[6]ADF, acid detergent fiber.
[7]ADL, acid detergent lignin.
[8]ME, metabolizable energy, calculated value.

Table 3. Fatty acid profile of the administered diets (g/100 g total fatty acid methyl esters).

Diet[1]	CON	T5	T10	T20
C12:0	0.02	0.02	0.03	0.02
C14:0	0.09	0.09	0.13	0.14
C15:0	0.04	0.02	0.03	0.02
C16:0	22.38	15.56	16.01	14.39
C16:1n-9	0.09	0.08	0.11	0.08
C16:1n-7	0.21	0.17	0.32	0.20
C17:0	0.14	0.09	0.10	0.10
C18:0	3.82	2.73	3.05	2.60
C18:1n-9	40.61	34.19	33.28	31.00
C18:1n-7	0.98	0.79	0.80	0.73
C18:2n-6	27.63	43.10	43.27	46.87
C18:3n-3	0.68	1.03	1.02	1.12
C20:0	0.67	0.48	0.45	0.43
C20:1n-11	0.28	0.12	0.09	0.11
C20:1n-9	0.76	0.38	0.32	0.32
C20:4n-6	0.02	0.00	0.11	0.010
C20:5n-3	0.11	0.10	0.09	0.06
C22:0	0.42	0.21	0.21	0.23
C22:1n-11	0.30	0.30	0.17	0.20
C22:1n-7	0.32	0.26	0.16	0.20
C24:0	0.43	0.26	0.27	0.20
ΣSFA[2]	*27.60*	*19.24*	*20.06*	*17.89*
ΣMUFA[3]	*43.55*	*36.29*	*35.24*	*33.83*
ΣPUFA[4]*n-6*	*27.65*	*43.10*	*43.38*	*46.87*
ΣPUFAn-3	*0.79*	*1.16*	*1.11*	*1.18*

[1]T5, T10, T20: diets in which 5, 10, and 20% of the protein of the control diet (CON) have been replaced with the protein from defatted *Tenebrio molitor* larvae meal.
[2]SFA, saturated fatty acids.
[3]MUFA, monounsaturated fatty acids.
[4]PUFA, polyunsaturated fatty acids.

The trial was carried out for 54 d, from May 5 to June 28, 2019. The live weight and the feed intakes of the quails were recorded weekly per replicate. The amount of the administered feed and that of the leftover feed were measured daily to calculate the birds feed intake. The number of eggs produced, and the individual egg weights were recorded per replicate every week. Per each replicate of each group, the egg mass was calculated by multiplying the egg weight by the egg production percentage and the FCR was calculated as the amount of feed intake per day divided by the amount of egg mass per day.

Overall, 288 eggs were collected during the 6 wk of the trial (2 eggs per replicate, 12 eggs per group each week), they were weighed and stored at −80°C until analyses (see section *Physicochemical analyses of raw and boiled eggs*).

At the end of the trial, the quails were slaughtered in a specialized slaughterhouse. After having measured the intestinal length, the ileum was separated from the Meckel's diverticulum to the ileocecal junction avoiding

contamination of other intestinal contents and the digesta were pooled per replicate, immediately frozen and subsequently freeze-dried. The dried ileal digesta were ground to pass a 1-mm sieve and stored at −20°C until chemical analysis (AOAC, 2005). The amount of acid insoluble ash (**AIA**) in the diets and in the ileal contents of the quails was measured according to Vogtmann et al. (1975). The apparent ileal digestibility of nutrients (dry matter, organic matter, crude protein, ether extract and calcium) was calculated as it follows: 100 − 100 × [(% AIA in the diet/% AIA in the ileal content) × (% nutrient in the ileal content/% nutrient in the diet)].

Physicochemical Analyses of Raw and Boiled Eggs

The eggs of each group were randomly allotted to be analysed as raw or boiled. The physical characterization of raw eggs was conducted on 36 eggs per group which were thawed for 2 h at room temperature before being analysed. First, the egg circumferences were evaluated with a measuring tape, then each egg was carefully broken to separate and weigh (PB503-S/fact, Mettler Toledo, Columbus, OH) its components: eggshell, albumen, and yolk. The eggshells thickness, including the testaceous membranes, was measured with a manual calliper (Salmoiraghi, Milan, Italy) in 3 points (equator, round, and apex). The pH of both the albumens and yolks was measured (SevenGo pH meter, Mettler-Toledo, Columbus, OH), while the yolk color values expressed as lightness (L^*), redness index (a^*) and yellowness index (b^*) (CIE, 2004) were registered through a Chroma Meter CR-200 (Konica Minolta, Chiyoda, Japan). Once ended the physical analyses, the yolks were pooled within the collection week (n = 6) and lyophilized before the chemical characterisation.

The eggs allotted to the cooking trial (n = 36 per group) were peeled still frozen and inserted into silicone egg cooker cups (OmzgxGod, purchased on Amazon, Italy) prior to be immersed in boiling water for 9 min to obtain hard-boiled eggs. The color values of both boiled albumens and yolks were evaluated using Chroma Meter CR-200 (Konica Minolta). Then, the entire boiled eggs of each dietary group were pooled within the collection week (n = 6) and lyophilized for further analyses.

The water content of the pooled raw yolks was calculated by weighing the samples before and after lyophilization. The total lipids were extracted (Folch et al., 1957) from the experimental diets, the raw yolks and the whole boiled egg, then an aliquot of each extract, containing 400 mg of total lipids, was methylated according to Christie (1982). The fatty acid methyl esters (**FAME**) were chromatographically analysed for the fatty acid (**FA**) profile by a Varian GC 430 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with a capillary column Supelco Omegawax 320 (30 m × 0.32 mm × 0.25 μm; Supelco, Bellefonte, PA). The injector was set at 220°C, while the oven temperature was programmed to start at 100°C, reaching 170°C with 10°C/min increment (isotherm 6 min), then rising to 200°C with 4°C/min (isotherm 5 min). A Flame Ionisation Detector (**FID**) set at 300°C was used. Helium was the carrier gas and flowed at 1.5 mL/min. The relative abundance of each single FA on the overall FAME content (g FA/100 g total FAME) was obtained by using tricosanoic acid (C23:0) (Supelco) as internal standard and external calibration curves (standard Supelco 37 component FAME mix; Supelco). The estimation of Δ9, Δ5 and Δ6 desaturase activity was obtained calculating the ratio between the product and the corresponding precursors, as reported by Mattioli et al. (2018). The following equations were used:

$$\Delta 9 \text{ desaturase } (16) = \frac{\text{C16}:1}{\text{C16}:1 + \text{C16}} \times 100$$

$$\Delta 9 \textit{ desaturase } (18) = \frac{C18:1}{C18:1 + C18} \times 100$$

$$\Delta 5 + \Delta 6 \textit{ desaturase } (n-6) = \frac{C20:2n6 + C20:4n6}{C18:2n6 + C20:2n6 + C20:4n6} \times 100$$

$$\Delta 5 + \Delta 6 \textit{ desaturase } (n-3) = \frac{C20:5n3 + C22:5n3 + C22:6n3}{C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3} \times 100$$

The egg oxidative status was assessed quantifying spectrophotometrically conjugated dienes (Srinivasan et al., 1996) and thiobarbituric acid reactive substances (**TBARS**; Vyncke, 1970). The results are expressed as mmol hydroperoxides (mmol **Hp**) and malondialdehyde equivalents (**MDA-eq.**) on 100 g of fresh sample, respectively.

Statistical Analysis

The data were processed by a one-way ANOVA, using the PROC GLM of SAS (2000) according to the following model:

$$Yij = m + PSi + eij$$

where Y is the single observation, m the general mean, PS the effect of the protein source (i = Control vs. *T. molitor*), e the error. The experimental unit was the replicate. The orthogonal contrast analysis was performed to test the linear, quadratic, and cubic effects among the means (SAS, 2000).

RESULTS

The Table 4 shows the weights recorded at 12, 14, 16, 18, and 20 wk of age together with weight changes and the in vivo performance of the quails according to the dietary treatments. No differences have been recorded

Table 4. Weight changes and in vivo performance of quails (n = 24), egg weight and egg mass (n = 72) of quails fed control or *Tenebrio molitor* diets from 12 to 20 wk of age.

[1]					[3]	Contrast *P* values		
Diet	CON	T5	T10	T20	RMSE	Linear	Quadratic	Cubic
Initial weight (12 wk), g	302.0	298.5	290.8	308.1	10.48	0.423	0.082	0.992
Weight at 14 wk, g	301.9	297.5	287.3	307.2	9.87	0.429	0.165	0.875
Weight at 16 wk, g	297.2	293.2	292.4	304.7	8.67	0.365	0.141	0.668
Weight at 18 wk, g	307.6	307.6	306.5	312.7	10.11	0.372	0.831	0.348
Final weight (20 wk), g	302.3	328.7	309.2	314.3	9.88	0.333	0.044	0.152
Δ weight, g/d	+0.01	+0.54	+0.33	+0.10	0.31	0.940	0.348	0.388
Feed intake	35.06	35.86	35.28	35.83	0.56	0.273	0.726	0.111
FCR[2]	2.92	3.06	3.38	3.63	0.05	0.001	0.313	0.119
Laying, %	93.01	92.42	80.17	72.42	2.12	<0.001	0.219	0.318
Egg weight, g	12.89	12.68	12.98	13.63	0.24	0.004	0.014	0.083
Egg mass	11.99	11.73	10.42	9.87	0.36	<0.001	0.540	0.083

[1]T5, T10, T20: diets in which 5, 10 and 20% of the protein of the control diet (CON) have been replaced with the protein from defatted *Tenebrio molitor* larvae meal.
[2]FCR, feed conversion ratio.
[3]RMSE, root mean square error.

for the weights; however, the contrast analysis shows a significant effect ($P < 0.05$) of the quadratic contrast indicating that low and intermediate inclusion levels of TML induced higher changes in the final weight compared to the other groups (+0.54 and +0.33 for T5 and T10, respectively). The feed intake was unaffected by dietary treatments. The laying rate and egg mass linearly decreased ($P < 0.01$) as the TML inclusion level in the diet increased. The egg weight increased from the control to the T20 diet (linear contrast $P < 0.01$). The FCR linearly increased from the control to the T20 group ($P < 0.01$).

The coefficients of the apparent ileal digestibility for dry matter, organic matter, crude protein, ether extract, and calcium are summarized in the Table 5. The digestibility of dry matter, organic matter, and crude protein linearly decreased ($P < 0.05$) as the TML inclusion level in the diet increased. No effects of the dietary treatments were detected for the digestibility of ether extract and calcium.

In relation to the physical and chemical traits of raw eggs (Table 6), the egg circumference tended to increase ($P < 0.01$) as the level of TML increased; however, the eggs from T5 group had lower circumference than CON (quadratic contrast, $P < 0.01$). The albumen weight showed a linear increasing trend when expressed both in absolute ($P < 0.01$) or relative value ($P < 0.05$). The yolk weight had a linear increase ($P < 0.01$) only when expressed in grams. Conversely, the eggshell weight linearly decreased ($P < 0.05$) when expressed as percentage of the whole egg. A significant effect of the cubic contrast ($P < 0.01$) has been detected for the yolk percentage and pH value. The yolk chemical characteristics (moisture and total lipid content) and oxidative status of the raw eggs were scarcely affected by the dietary treatments. Indeed, among the analyzed parameters, only the conjugated dienes showed a significant cubic contrast ($P < 0.05$).

The physical and chemical characteristics of the boiled eggs (Table 7) highlighted that lightness (L^*) of the yolk showed higher values in T5 and T10 groups (quadratic contrast, $P < 0.01$). The green-red index (a^*) of the yolk showed lower values in T5 and T20 groups (cubic contrast, $P < 0.01$). The L^*, a^* and b^* indexes of the albumen revealed a significant effect of the quadratic contrast ($P < 0.05$). In addition, b^* index of the albumen also showed a significant effect ($P < 0.01$) of the cubic contrast. The total lipids tended to increase with the *T. molitor* larvae meal inclusion in the diets (cubic contrast, $P < 0.05$), having the T10 and T20 eggs the highest values.

The fatty acid profile of the raw yolks is shown in the Table 8. Several fatty acids showed a linear increase (C14:0, C14:1n-5, C16:1n-7, C18:1n-7, C18:3n-6, C20:0, C20:4n-6, C22:4n-6, C22:5n-6) or a linear decrease (C15:0, C18:2n-6, C18:3n-3) according to the increase of TML percentage in the diets. For C20:2n-6, C20:3n-6, C22:4n-6, and C22:5n-6 a significant effect ($P < 0.05$) of

Table 5. Coefficients of the apparent ileal digestibility of the nutrients (%) of quails (n = 24) fed control or *Tenebrio molitor* diets at 20 wk of age.

[1]					[2]	Contrast *P* values		
Diet	CON	T5	T10	T20	RMSE	Linear	Quadratic	Cubic
Dry matter	77.4	77.6	76.6	75.4	6.54	0.042	0.337	0.426
Organic matter	79.8	80.1	78.7	77.5	7.11	0.031	0.542	0.559
Crude protein	78.9	77.5	74.7	72.3	7.56	0.013	0.236	0.396
Ether extract	90.1	90.5	89.9	90.3	8.33	0.579	0.665	0.632
Ca	79.2	79.5	79.1	79.7	9.12	0.554	0.570	0.688

[1]T5, T10, T20: diets in which 5, 10, and 20% of the protein of the control diet (CON) have been replaced with the protein from defatted *Tenebrio molitor* larvae meal.
[2]RMSE, root mean square error.

Table 6. Raw egg quality physical (n = 36) and chemical (n = 6 pools) traits according to quails fed control or *Tenebrio molitor* diets from 12 to 20 wk of age.

[1] Diet	CON	T5	T10	T20	[4] RMSE	Contrast *P* values Linear	Quadratic	Cubic
Egg circumference, cm	8.27	8.20	8.31	8.49	0.27	0.000	0.007	0.539
Shell thickness, μm	31.77	31.88	28.67	29.52	5.48	0.059	0.752	0.169
Albumen weight, g	5.48	5.34	6.05	6.30	0.97	<0.001	0.240	0.078
Albumen, %	45.47	45.80	47.64	47.89	5.17	0.02	0.961	0.428
Yolk weight, g	4.03	4.01	4.14	4.40	0.44	<0.001	0.071	0.942
Yolk, %	33.51	34.68	32.83	33.72	2.69	0.550	0.756	0.001
Eggshell weight, g	2.51	2.24	2.45	2.39	0.48	0.681	0.198	0.069
Eggshell, %	21.06	19.51	19.52	18.39	4.17	0.015	0.794	0.395
pH albumen	8.75	8.84	8.74	8.72	0.23	0.231	0.178	0.164
pH yolk	6.49	6.61	6.45	6.59	0.26	0.501	0.755	0.006
L^* yolk	50.67	50.71	50.63	49.79	15.17	0.815	0.864	0.955
a^* yolk	5.63	4.99	5.63	5.82	1.54	0.293	0.115	0.139
b^* yolk	23.15	22.62	23.52	23.64	3.78	0.416	0.610	0.431
Moisture, g/100 g yolk	49.33	50.01	50.60	49.74	2.86	0.741	0.492	0.835
Total lipids, g/100 g yolk	26.40	26.10	25.14	26.28	2.01	0.720	0.390	0.457
CD[2], mmol Hp/kg yolk	0.40	0.43	0.36	0.37	0.05	0.087	0.504	0.039
TBARS[3], mg MDA-eq/kg yolk	0.031	0.002	0.003	0.005	0.02	0.281	0.125	0.913

[1]T5, T10, T20: diets in which 5, 10, and 20% of the protein of the control diet (CON) have been replaced with the protein from defatted *Tenebrio molitor* larvae meal.
[2]CD, conjugated dienes.
[3]TBARS, thiobarbituric acid reactive substances.
[4]RMSE, root mean square error.

the quadratic contrast has been detected, while a significant cubic effect was recorded for C16:0, C20:3n-6 and C22:1n-9 fatty acids. However, no effects of the dietary treatments were detected for the total saturated fatty acids (**SFA**), monounsaturated fatty acids (**MUFA**) and polyunsaturated fatty acids (**PUFA**). A linear increase ($P < 0.05$) of the estimated activity of Δ9-desaturase (C16:0), Δ5+ Δ6-desaturase on both PUFAn-6 and PUFAn-3 was calculated as affected by TML inclusion in the quails' diet.

The fatty acid profile of the boiled eggs according to the dietary treatments is reported in the Table 9. C12:0, C14:1n-5, C18:0 and C18:3n-3 linearly decreased as the TML percentage in the diets increased, while C18:1n-9, C18:1n-7, C20:4n-6 and C22:0 showed the opposite trend. A significant quadratic effect of the contrasts has been detected for C22:0 ($P < 0.5$), C22:4n-6 ($P < 0.01$) and C22:5n-6 ($P < 0.05$) and a significant effect ($P < 0.05$) of the cubic contrast for C18:1n-9 and C20:2n-6 fatty acids. The total MUFA linearly increased ($P < 0.05$) according to the increase of TML in the diets.

DISCUSSION

To our knowledge, there are very few studies about the use of insect meals in laying quails and they are divided among local insect species (Das and Mandal, 2014), black soldier fly (Dalle Zotte et al., 2019), and *T. molitor* (Shariat Zadeh et al., 2020).

The absence of mortality and clinical signs of trouble (such as diarrhea), as well as the absence of weight loss in all the groups, indicates that the inclusion of *T. molitor* in diets had no negative effects on laying

Table 7. Boiled eggs quality physical (n = 36) and chemical (n = 6 pools) traits according to quails fed control or *Tenebrio molitor* diets from 12 to 20 wk of age.

[1] Diet	CON	T5	T10	T20	[4] RMSE	Contrast *P* values Linear	Quadratic	Cubic
Yolk color parameters								
L^*	62.39	65.79	64.79	62.37	6.06	0.823	0.006	0.518
a^*	4.60	3.05	4.85	4.07	2.35	0.916	0.346	0.001
b^*	28.37	27.42	29.56	29.05	5.47	0.332	0.818	0.171
Albumen color parameters								
L^*	84.05	86.69	85.66	82.22	6.78	0.222	0.011	0.807
a^*	-5.11	-5.76	-5.46	-5.18	1.17	0.916	0.022	0.285
b^*	7.14	9.89	6.82	7.30	2.77	0.237	0.019	<0.001
Total lipids, g/100 g	10.99	11.59	10.85	12.03	0.67	0.069	0.303	0.015
CD[2], mmol Hp/kg	0.16	0.16	0.15	0.17	0.01	0.136	0.245	0.068
TBARS[3], mg MDA-eq./kg	0.040	0.032	0.024	0.022	0.02	0.058	0.678	0.904

[1]T5, T10, T20: diets in which 5, 10, and 20 % of the protein of the control diet (CON) have been replaced with the protein from defatted *Tenebrio molitor* larvae meal.
[2]CD, conjugated dienes.
[3]TBARS, thiobarbituric acid reactive substances.
[4]RMSE, root mean square error.

Table 8. Fatty acid profile (g/100 g of fatty acid methyl esters) of the raw eggs (n = 6 pools) according to quails fed control or *Tenebrio molitor* diets from 12 to 20 wk of age.

[1]					[5]	Contrast *P* values		
Diet	CON	T5	T10	T20	RMSE	Linear	Quadratic	Cubic
C12:0	0.06	0.01	0.07	0.01	0.0047	0.793	0.444	0.139
C14:0	0.38	0.39	0.41	0.42	0.025	0.013	0.861	0.906
C14:1n-9	0.05	0.05	0.06	0.06	0.008	0.003	0.555	0.103
C15:0	0.05	0.05	0.04	0.04	0.0045	0.000	0.060	0.533
C16:0	25.73	25.94	26.27	26.06	0.68	0.301	0.481	0.006
C16:1n-9	0.78	0.73	0.67	0.73	0.2059	0.158	0.122	0.623
C16:1n-7	3.20	3.40	3.81	3.64	0.23	0.001	0.075	0.082
C17:0	0.14	0.14	0.13	0.13	0.012	0.142	0.553	0.286
C18:0	8.87	9.23	8.99	8.81	0.23	0.336	0.011	0.136
C18:1n-9	36.65	36.24	36.69	36.56	0.52	0.858	0.527	0.140
C18:1n-7	1.56	1.55	1.69	1.67	0.107	0.026	0.880	0.010
C18:2n-6	18.18	17.81	16.58	17.20	0.87	0.016	0.182	0.105
C18:3n-6	0.28	0.29	0.31	0.30	0.02	0.050	0.226	0.167
C18:3n-3	0.23	0.21	0.19	0.20	0.24	0.037	0.281	0.424
C20:0	0.02	0.03	0.03	0.03	0.004	0.007	0.690	0.074
C20:1n-11	0.04	0.03	0.04	0.04	0.006	0.218	0.390	0.502
C20:1n-9	0.12	0.12	0.12	0.13	0.008	0.224	0.114	0.904
C20:2n-6	0.09	0.08	0.08	0.09	0.067	0.697	0.013	0.452
C20:3n-6	0.15	0.13	0.18	0.16	0.033	0.163	0.911	0.026
C20:4n-6	2.38	2.51	2.60	2.56	0.072	<0.001	0.010	0.538
C20:5n-3	0.01	0.01	0.01	0.01	0.005	0.119	0.143	0.454
C22:0	0.01	0.01	0.01	0.02	0.005	0.054	0.142	0.602
C22:1n-9	0.01	0.01	0.01	0.01	0.005	0.961	0.932	0.023
C22:4n-6	0.12	0.12	0.12	0.14	0.010	0.005	0.036	0.082
C22:5n-6	0.38	0.36	0.38	0.43	0.034	0.001	0.024	0.616
C22:5n-3	0.07	0.06	0.06	0.07	0.009	0.690	0.311	0.801
C22:6n-3	0.49	0.50	0.46	0.48	0.063	0.733	0.824	0.421
ΣSFA[2]	*35.21*	*35.77*	*35.88*	*35.50*	*0.7669*	*0.486*	*0.147*	*0.988*
ΣMUFA[3]	*42.42*	*42.14*	*43.12*	*42.84*	*0.739*	*0.110*	*0.998*	*0.075*
ΣPUFA[4]*n-6*	*21.58*	*21.30*	*20.26*	*20.88*	*0.9137*	*0.074*	*0.241*	*0.161*
ΣPUFAn-3	*0.782*	*0.776*	*0.728*	*0.759*	*0.083*	*0.445*	*0.606*	*0.440*
Δ9-desaturase (C16)	11.05	11.58	12.67	12.26	0.67	0.001	0.098	0.107
Δ9-desaturase (C18)	80.51	79.70	80.31	80.57	0.50	0.391	0.018	0.067
Δ5+Δ6-desaturase (n-6)	11.96	12.70	13.90	13.39	0.58	<0.001	0.015	0.052
Δ5+Δ6-desaturase (n-3)	71.00	72.81	73.72	73.67	2.13	0.033	0.295	0.989

[1]T5, T10, T20: diets in which 5, 10, and 20% of the protein of the control diet (CON) have been replaced with the protein from defatted *Tenebrio molitor* larvae meal.
[2]SFA, saturated fatty acids.
[3]MUFA, monounsaturated fatty acids.
[4]PUFA, polyunsaturated fatty acids.
[5]RMSE, root mean square error.

quail's health status. In addition, the lack of differences in feed intake among the groups suggests an adequate palatability of the insect meal diets.

The most important difference among the groups regarding the productive performance was the laying rate which showed a progressive detriment due to the increasing inclusion levels of TML: the egg production of T20 and T10 groups was reduced by 22.14 and 13.80%, respectively, in comparison to the control group. This impairment could be attributed to the progressive reduction of dry matter (**DM**) and organic matter (**OM**) ileal apparent digestibility of the diets. On the other hand, the reduction of OM and DM digestibility is mainly due to the decrease of the crude protein digestibility when the insect diets were administered to the quails. This result was expected. It is well known that, when insect meals are included in the diet, the reduced crude protein digestibility is registered due to their chitin content, which, because of its activity of binding proteins, makes them unavailable for digestion (Longvah et al., 2011). In the present study, considering the percentage of the crude protein of the diets, the feed intake of the birds and the coefficients of the apparent ileal digestibility, the amounts of crude protein available for digestion were: 6.01, 6.14, 5.92, and 5.70 g/d for CON, T5, T10, and T20 groups, respectively.

Previous studies on laying hens showed a strong correlation between dietary crude protein (**CP**) and laying performance of hens. Keshavarz and Nakajima (1995), Liu et al. (2005) and Gunawardana et al. (2008) showed that the egg production increased due to increasing dietary CP levels although the FI was not affected by these dietary changes. The effects of dietary CP on laying productive performance were also detected for egg mass and egg weight, but with different findings. Due to its close relation to the laying rate, the egg mass also increased or decreased as the dietary CP increased or decreased (Abd El-Maksoud et al., 2011; Alagawany et al., 2016); on the contrary some authors (Meluzzi et al., 2001) found that CP at 150 g/kg in the diet induced the higher egg size compared to the other higher or lower levels of protein (170 or 130 g/kg); completely different are the

Table 9. Fatty acid profile (g/100 g of fatty acid methyl esters) of the boiled eggs (n = 6 pools) according to quails fed control or *Tenebrio molitor* diets from 12 to 20 wk of age.

[1]					[5]	Contrast *P* values		
Diet	CON	T5	T10	T20	RMSE	Linear	Quadratic	Cubic
C12:0	0.01	0.01	0.01	-	0.003	0.007	0.768	0.225
C14:0	0.40	0.40	0.40	0.42	0.031	0.369	0.474	0.788
C14:1n-9	0.06	0.05	0.06	0.06	0.0004	0.253	0.466	0.469
C15:0	0.05	0.04	0.04	0.04	0.005	0.008	0.858	0.856
C16:0	26.26	26.16	26.35	26.28	0.7366	0.857	0.978	0.682
C16:1n-9	0.76	0.76	0.76	0.72	0.1033	0.487	0.567	0.781
C16:1n-7	3.45	3.26	3.93	3.81	0.6001	0.126	0.878	0.150
C17:0	0.14	0.14	0.13	0.12	0.015	0.118	0.269	0.444
C18:0	9.02	9.25	8.80	8.72	0.274	0.012	0.176	0.048
C18:1n-9	36.61	36.23	36.94	37.12	0.580	0.046	0.261	0.140
C18:1n-7	1.58	1.54	1.69	1.75	0.193	0.008	0.534	0.388
C18:2n-6	17.24	17.78	16.36	16.44	1.58	0.201	0.724	0.242
C18:3n-6	0.28	0.28	0.30	0.30	0.030	0.254	0.818	0.421
C18:3n-3	0.23	0.22	0.18	0.19	0.037	0.025	0.402	0.226
C20:0	0.02	0.03	0.03	0.03	0.004	0.354	0.429	0.806
C20:1n-11	0.03	0.03	0.04	0.04	0.0084	0.101	0.828	0.427
C20:1n-9	0.11	0.11	0.12	0.12	0.010	0.167	0.331	0.879
C20:2n-6	0.08	0.08	0.07	0.08	0.010	0.589	0.653	0.027
C20:3n-6	0.15	0.16	0.17	0.16	0.0168	0.082	0.223	0.450
C20:4n-6	2.35	2.40	2.56	2.52	0.094	0.001	0.318	0.091
C20:5n-3	0.01	0.01	0.01	0.01	0.006	0.549	0.722	0.641
C22:0	0.02	0.02	0.01	0.02	0.004	0.008	0.016	0.217
C22:1n-9	0.01	0.01	0.01	0.01	0.004	0.987	0.587	0.362
C22:4n-6	0.13	0.11	0.12	0.14	0.0139	0.178	0.003	0.675
C22:5n-6	0.42	0.35	0.36	0.41	0.058	0.916	0.022	0.680
C22:5n-3	0.07	0.06	0.06	0.07	0.013	0.414	0.090	0.566
C22:6n-3	0.51	0.50	0.48	0.44	0.095	0.202	0.717	0.812
ΣSFA[2]	*35.90*	*36.03*	*35.76*	*35.60*	*0.644*	*0.335*	*0.578*	*0.660*
ΣMUFA[3]	*42.61*	*42.00*	*43.56*	*43.62*	*1.195*	*0.048*	*0.495*	*0.110*
ΣPUFA[4]*n-6*	*20.64*	*21.14*	*19.94*	*20.05*	*1.638*	*0.327*	*0.766*	*0.313*
ΣPUFAn-3	*0.83*	*0.78*	*0.73*	*0.70*	*0.138*	*0.114*	*0.904*	*0.930*

[1]T5, T10, T20: diets in which 5, 10, and 20% of the protein of the control diet (CON) have been replaced with the protein from defatted *Tenebrio molitor* larvae meal.
[2]SFA, saturated fatty acids.
[3]MUFA, monounsaturated fatty acids.
[4]PUFA, polyunsaturated fatty acids.
[5]RMSE, root mean square error.

findings by Summers et al. (1991) and Lopez and Leeson (1995) who reported that the egg weight is strongly related to the content of CP in the diet. However, Summers and Leeson (1983) found that early egg size was not affected by the increase in dietary CP. This agrees with Khajali et al. (2008), Alagawany and Abou-Kassem (2014), and Alagawany et al. (2016), who indicated that low dietary crude protein has a negative effect on hens' performance, especially during the late stage of production.

In our trial, performed in an early phase of laying, the progressive decrease of laying rate was accompanied by a progressive increase in the weight of the eggs. This result should not be surprising. Unfortunately, in this trial we could not measure the amino-acid digestibility, but it is logical to assume that the reduction of proteins available for digestion also implies a reduction of the relative amino acid availability. Our hypothesis is that the strong reduction in eggs number laid by quails in T10 and T20 groups made the amount of amino acids available with the respective diets adequate to support a higher egg weight. These considerations are mainly for methionine and lysine which, as it is well known, are, respectively, the first and the second limiting AA in poultry and involve the regulation of egg weight (methionine) and egg mass (lysine; Fakhraei et al., 2010). However, as the laying rate strongly decreased from the control to T20 group, the egg mass linearly decreased accordingly. The FCR, which is tied to the egg mass, showed the same trend. According to our results, Shariat Zadeh et al. (2020) found a progressive decrease of egg mass of quails when *T. molitor* larvae meal increased from 25 to 100% in replacement of fishmeal and significant effects were already evident in the 25% group. In the study by Shariat Zadeh et al. (2020) the FCR values in the groups fed the insect meal diets were higher than in the control group.

According to Iqbal et al. (2017), increased egg weight resulted in the shell percentage significantly decreased, while the eggshell thickness was not influenced by the egg weight, corroborating the findings by Wolanski et al. (2007) and Crosara et al. (2019). The lack of differences among the groups for eggshell weights confirms the findings by Jonchère et al. (2012) who underlined that egg-laying birds have a limited amount of calcium available to produce the shell, approximately 2.0 to 2.5 g Ca^{2+}, irrespective of the egg size. However, the amount of Ca available for quails is sufficient in order not to cause significant changes in the shell thickness. The increased weight of eggs is accompanied by an increase in the circumference value.

The increase of the albumen and yolk weights from the control to T20 groups are in line with the increase of the egg weight according to Tebesi et al. (2012), Alkan et al. (2013), and Khawaja et al. (2013). However, when expressed as percentage of the egg weight, only the albumen showed a proportion progressive increase.

No difference in the yolk color of the fresh eggs was detected among the groups. This is not in line with previous findings, in which the addition in the diet of insect meal from *Hermetia illucens* modified the yolk color of hen (Secci et al., 2018) and quail eggs (Dalle Zotte et al., 2020). However, it must be considered the use of different insect species as ingredient in the diets, the lower level of insect meal inclusion in the diets of the present trial, as well as the overall feed formulation of the mentioned articles, which contained different amounts of carotenoid sources, as vegetable oils and maize. It is widely established that carotenoids are important not only for coloring but also for their protection against oxidative damages, even though we found these last slightly affected by the diet. Indeed, the overall content of the secondary oxidation products, that we observed, agreed with the values found by Ren et al. (2017) in raw yolk from laying hens.

The oleic (C18:1), linoleic (C18:2n-6), and palmitic (C16:0) fatty acids characterized the lipid fraction of *T. molitor* larvae with minor influence of their relative abundance in relation to the rearing substrates (Ruschioni et al., 2020). Despite the fatty acid profile of the control diet differed from the other ones containing the insect meal, it is of relevance that egg yolks showed only few modifications. First, birds can synthesise ex novo C16 by the acetate/malonate way (Klasing, 2000), then can convert this fatty acid into C18:0 and desaturate into both in C16:1n-7 and C18:1n-9. This fact explains the highest values of these FAs found in the yolks despite their relative low amount in the feeds administered. However, the egg yolk from quails fed graded inclusions of TM meal showed significantly higher content of both C16:0 and C16:1n-7 than the control group, as a different enzymatic activity occurred, as supported by the significant linear increase in Δ9-desaturase activity on C16:0. In addition, some authors have previously observed that the overall fatty acid profile of yolk was only partially affected by the dietary intervention with insect meal (Secci et al., 2018; Dalle Zotte et al., 2020), probably due to the remarkable activity of desaturase and elongase on saturated fatty acids (as C14:0 and C16:0) for providing C14:1n-9, C17:1n-10, C16:1n-9, C18:1n-9, and C18:1n-11 fatty acids. In the present case, the retrieving in quail eggs of C14:1n-9 and C16:1n-9, not detected or scarcely contained in the feeds, might support this hypothesis. Interestingly, the groups fed the diets containing the insect meal showed a level of MUFA higher than the control group, thus possibly supporting the hypothesis that insect might affect the gene expression or the activities of these fundamental enzymes. In this regard, Nguyen et al. (2017) highlighted that the organ more affected by dietary TM was the liver, which is the main site for lipid metabolism and fatty acid synthesis and where the gene related to the elongation of fatty acids are expressed, especially after the sexual maturity of laying birds (Zhang et al., 2017).

Desaturase (Δ5 and Δ6) and elongase enzymes are even able to catalyse the synthesis of long chain fatty acids, as PUFAn-3 and PUFAn-6, starting from C18:3n-3 and C18:2n-6, respectively (Güçlü et al., 2008). Our results highlighted this pathway in all the considered groups, since the FAs produced during the desaturation/elongation process have been detected in raw yolks (C22:6n-3, C20:3n-6, C20:4n-6, C22:4n-6, C22:5n-6), irrespective their scarce amount or the fully absence in the administered diets. As expected, since the content of the dietary precursors affects the enzymatic activity, the TM10 yolks were the richest in C20:3n-6 and C20:4n-6, while the TM20 yolks contained the highest amount of C22:4n-6 and C22:5n-6 together with a lower content of their precursor (*i.e.*, the linoleic acid), abundantly contained in the feeds including TML. Noteworthy, the increase in C20 and C22 PUFAn-6 and the Δ5-Δ6 desaturase activity on PUFAn-6 was linear in the yolks of quails fed the diets including TML until the inclusion rate of 5.6%, while the enzymatic activity esteemed was contracted at the highest inclusion, despite the huge difference in dietary C18:2n-6 content (see Table 1). In addition, the predominance of PUFAn-6 on PUFAn-3 in the diet seemed to be responsible for the PUFAn-6 prevalence on the n-3 fraction, as previously noted by Dalle Zotte et al. (2020).

Quail eggs are commonly consumed as boiled, so the physicochemical characteristics of the boiled eggs were evaluated. Yolk thermal processing, as pasteurization (de Souza and Fernández, 2011 or water-bath treatment (Llave et al., 2018) increased yolk color, especially the b^* value, due to the disruption of the fat-soluble carotenoids as lutein, zeaxanthin and beta-carotene in a minor extent and the subsequent formation of Maillard' products. Besides, Llave et al. (2018) showed a high correlation between color changes and the non-denaturation ratio profile of high-density lipoproteins (**α-HDL**) and ovalbumin in yolk and albumen, respectively. Specifically, authors observed that increasing the thermal denaturation rate of these proteins augmented the color difference. Hence, considering the significant quadratic and cubic contrasts emerged for the boiled eggs and the relation between color and proteins, it could be presumed that a lower inclusion level of TM might affect in some extent at least the ovalbumin fraction of quail egg whites. Despite the studies on this field are still scarce and difficult comparisons can be done to debate the present data, we can mention that ovalbumin, whose content might slightly vary in egg white, contributes to the stability and the final volume of a batter in which the egg albumen is added (Lomakina & Míková, 2006). In this regard, Secci et al. (2020) found that the Angel cake made with the egg white from laying hens fed diet including 7.3% of *Hermetia illucens* larvae meal raised a significant lower final height than the control group,

being 15.95 and 20.25 mm, respectively. Furthermore, Ko et al. (2020) noted that the concentration of yolk histidine linearly increased as the TM larvae meal inclusion increased in laying hens' diet (1, 2, and 3%). Despite the authors did not explain their findings, they supported the hypothesis that TML might in some extent interact with protein metabolism. This topic warrants further investigations.

From a nutritional point of view, the consumption of boiled eggs from quail fed TM meal included at 2.4, 2.8, or 5.6% in the diet brings the same quantity and quality of lipids than the eggs from quail fed the conventional protein sources. If we looked at the present results, as suggested by Sanders (2010), who highlighted the importance to consider food not only as a nutrient but also for its integrated role on environmental sustainability, it would seem reasonable to introduce TML meal in laying quails' diet.

CONCLUSIONS

Our results indicated that the inclusion of a defatted *Tenebrio molitor* larvae meal at 2.8 and 5.6% in laying quail diets negatively affected the laying performance and some physical characteristics of eggs, due to the impairment of nutrients digestibility, in particular of crude protein. In addition, TML inclusion in quail diets partly modified the fatty acid profile of the yolk, increasing the levels of C16:0 and C16:1n-7 fatty acids, possibly due to the increase in Δ9-desaturase activity. Thus, the best inclusion level of defatted TML meal for laying quails seemed to be 1.4% of diet. However, further efforts of research should be done to reduce the FCR, ameliorate digestibility and to increase PUFAn-3 deposition in egg yolks when the insect-based diets are utilized.

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DISCLOSURES

The authors declare that there are no conflicts of interest.

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