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Live yellow mealworm (*Tenebrio molitor*) larvae: a promising nutritional enrichment for laying quails

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ABSTRACT The study aimed to evaluate the effect of supplementing live *Tenebrio molitor* (**TM**) larvae to laying quails (*Coturnix japonica*) as nutritional enrichment. Live performances, apparent digestibility of nutrients (including that of sole live TM larvae), egg physicochemical quality, sensory traits, and storage stability were considered in this experiment. Sixty laying quails were divided into 2 dietary groups (6 replicated cages/group; 5 quails/cage): a Control group received a basal diet for laying quails and a **TM10** group was fed with the Control diet supplemented with live TM larvae (10% of the expected daily feed intake). For the digestibility trial, 30 laying quails were divided into 3 dietary groups: the first 2 groups were fed with the Control and TM10 diets, while the third group received *ad libitum* live TM larvae (**TM100**) as a complete replacement for the Control diet. Overall, no mortality was recorded during the trials. Quails fed TM showed a remarkable capability of digesting dietary chitin ($P < 0.0001$). TM100 quails showed the lowest digestibility for dry matter, crude protein, and energy, but that of ether extract was the highest ($P < 0.001$). The presence of live TM larvae stimulated quails' feed intake ($P < 0.0001$), but did not affect performance traits. Similarly, overall physicochemical quality attributes and storage stability were comparable in Control and TM10 eggs. The sensory features of quail eggs differed in TM10 *vs.* Control groups: TM10 eggs had the lowest overall flavor ($P < 0.01$), sulfur ($P < 0.05$) and greasy-oily ($P < 0.01$) intensities. Therefore, a 10% TM dietary supplementation is effective in stimulating feeding activity of quails, but it did not provide any productive improvement compared to a standard diet. Further studies should assess the possible beneficial effect of live TM supplementation on quail's gut health. The digestibility trial with the sole live TM larvae allowed to assess the specific nutritional value of this emerging feedstuff which is of utmost importance for future feed formulations.

Key words: *Tenebrio molitor*, yellow mealworm, feed, laying quail, egg quality

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INTRODUCTION

Insects and derived products are considered one of the possible alternative feedstuffs to improve the sustainability of the livestock sector thanks to several promising attributes: limited space requirements for farming and the possibility to exploit the vertical space, short productive cycles, limited water needs (i.e. water is often obtained directly from the feeding substrate), and the suitability of some species to mass rearing (Smetana et al., 2021). Last but not least, some insect species are perfect candidates in the perspective to set-up circular economy models (Ojha et al., 2020): insects feed on organic side-streams, including agro industrial by-products, and other feedstuffs with no commercial value and that are considered waste, they up-cycle them into high-value nutrients to be successfully included in the diets of different food-producing animals, including fish (Henry et al., 2015), poultry (Shaviklo, 2023), rabbit (Cullere et al., 2022), and pig (DiGiacomo et al., 2019). Furthermore, the residue of insect farming, that is the frass, can be used as organic fertilizer to replace the use of agrochemicals thus promoting sustainable agriculture (Poveda, 2021). From the nutritional point of view, insects are characterized by high amounts of protein with excellent biological value, lipids, minerals, water-soluble vitamins, and compounds with functional properties such as chitin and antimicrobial peptides (Koutsos et al., 2023).

In the European Union, the current legislative framework allows 8 insect species (*Hermetia illucens*, *Musca domestica*, *Tenebrio molitor*, *Alphitobius diaperinus*, *Acheta domesticus*, *Gryllodes sigillatus*, *Gryllus assimilis*, and *Bombyx mori*) which can be farmed to produce feed for food producing animals (Commission Regulation (EU), 2017; 2021) and they can also be fed alive. The

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possible use of live insects offers the perspective of using them also as environmental enrichment for food-producing animals, aiming at increasing the complexity of the captive environment. One form of environmental enrichment is foraging enrichment, which can increase bird activity, improve leg health and decrease stereotypic behaviors (Laurence et al., 2015; Ipema et al., 2020a,b). Among food-producing animals, poultry surely represents one of the most promising sectors where live insects can play a relevant role in designing novel feeding strategies, also considering that insects represent a natural food source for them (Koutsos et al., 2023).

Previous research indicated that the incorporation of low amounts (5% of the expected daily feed intake) of live *Tenebrio molitor* and *Hermetia illucens* larvae in the diet of broiler chickens (Colombino et al., 2021) can provide a slight improvement in the cecal microbiota. This was attributed to the bioactivity of chitin, an indigestible polysaccharide constituting insects' exoskeleton, which possesses antimicrobial and immunostimulant properties (Islam et al., 2017). Furthermore, when turkey poults were supplemented with live *Hermetia illucens* larvae (10% of the expected daily feed intake), feed intake and body weight gain improved, with a reduced incidence of feather pecking (Veldkamp and van Niekerk (2019).

Despite these encouraging results, further investigations into the impact of this novel feeding approach on both animal productivity and product quality (live insects are a nutrients-dense feedstuff) have been carried out to a limited extent and exclusively considering *Hermetia illucens* (Tahamtani et al., 2021). On the contrary, no studies considering the impact of live *Tenebrio molitor* (**TM**) larvae on both performance and product quality have been conducted up to now. Furthermore, it is not clear which is the apparent digestibility of live TM larvae and thus their real nutritional value, as well as if a daily provision higher than 5% of the expected daily feed intake could be feasible.

Based on these premises, the present research tested the effect of a live TM larvae supplementation (10% inclusion) as nutritional enrichment for laying quails (*Coturnix japonica*) on performance, egg physicochemical traits, and shelf-life. Furthermore, an *in vivo* digestibility trial was conducted to assess, for the first time, the nutritional value of the TM larvae as well as that of the experimental diets. The quail was chosen because it is a popular bird for egg production, and is economically interesting for many productive contexts thanks to a series of positive attributes such as early sexual maturity, rapid growth, short generation interval, limited space requirements, and high laying percentage (Dalle Zotte et al., 2019).

MATERIAL AND METHODS

Animals and Experiment Design

The research study received ethical approval from the Ethical Committee of the University of Padova under protocol number 56/2021. The trial was conducted at a farm with which the Department of Animal Medicine, Production and Health (**MAPS**) at the University of Padova has a scientific agreement. A diet, referred to as the "Control" diet (Table 1), was formulated based on the minimum requirements for laying Japanese quails (*Coturnix japonica*) as recommended by the National Research Council, Subcommittee on Poultry Nutrition (NRC, 1994).

Table 1. Ingredients of the experimental diet (g/kg as fed).

Ingredients	Control
Corn	495
Soybean meal	335
Wheat flour	30.1
Wheat bran	30.0
Soybean oil	35.0
Calcium carbonate	64.4
Dicalcium phosphate	0.70
NaCl	3.50
Methionine DL	1.30
Vitamin-Mineral premix[1]	5.00

[1]Vitamin and mineral premix provided the following per kg of diet: Vitamin A, 11500 IU; cholecalciferol, 2100 IU; vitamin E (from dl-tocopherylacetate), 22 IU; vitamin B12, 0.60 mg; riboflavin, 4.4 mg; nicotinamide, 40 mg; calcium pantothenate, 35 mg; menadione (from menadione dimethyl-pyrimidinol), 1.50 mg; folic acid, 0.80 mg; thiamine, 3 mg; pyridoxine, 10 mg; biotin, 1 mg; choline chloride, 560 mg; ethoxyquin, 125 mg; Mn (from $MnSO_4 \cdot H_2O$), 65 mg; Zn (from ZnO), 55 mg; Fe (from $FeSO_4 \cdot 7H_2O$), 50 mg; Cu (from $CuSO_4 \cdot 5H_2O$), 8 mg; I (from Ca $(IO_3)2 \cdot H_2O$), 1.8 mg; Se, 0.30 mg; Co (from Co_2O_3), 0.20 mg; Mo, 0.16 mg.

The laying quails were divided into 2 experimental groups: the first group received the Control diet in mash form, whereas the second group received the Control diet, daily supplemented with live TM larvae (TM10: 10% of the expected daily feed intake, which was calculated based on data from previous trials (Dalle Zotte et al., 2019; Singh et al., 2023) on laying quails of the same age). The 8-wk-old live TM larvae were provided by INEF - Insect Novel Ecologic Food (Via Fossetta, 23, 35017 Piombino Dese, Padova, Italy).

A total of n = 60, 119-day-old laying Japanese quails (30 quails/treatment) were individually weighed and assigned to the 2 dietary treatments ensuring the same average live weight in each group. For each dietary treatment, a total of n = 6 replicated cages (n = 5 quails/each, with a space allowance of 0.13 m^2 per quail) were designed. Quails were housed in battery cages, fed with experimental diets for 5 wk, and with *ad libitum* access to feed and water throughout the trial. The environmental conditions of the room were monitored: the average relative humidity and temperature were 76.1% and 21.2°C respectively, and the set photoperiod was 16 h light:8 h dark.

Productive Performance

Quails were individually weighed on the first and last day of the experimental trial, to assess the live weight change along the experiment. Feed intake (**FI**) was recorded weekly on a cage basis. Also, morbidity and mortality were monitored along the trial. During the 5 wk of the experiment, the laid eggs per cage were

counted daily and individually weighed; average egg weight and egg production were then calculated. Additionally, defected eggs (i.e., broken, without solid shell, or with unusual shape meaning extremely elongated or rounded) were also daily counted and used to calculate defected egg percentage. The feed conversion ratio (**FCR**) was calculated as kg of feed consumed/kg of egg produced. At the 5th wk, the eggs were collected for 7 consecutive days, identified and analyzed for physicochemical quality, sensory profile, and storage stability. A total of n = 68 and 85 eggs/treatment (Control and TM10, respectively) were used for physicochemical analyses, n = 27 eggs/treatment for the sensory analysis, and n = 56 eggs/treatment for the storage stability trial.

Digestibility

A separate *in vivo* digestibility trial was conducted following the procedure described by Dalle Zotte et al. (2021). A total of n = 30, 85-day-old laying quails were individually housed in n = 30 digestibility cages and assigned to one of the following 3 dietary groups: the 2 diets that is, 1) Control and 2) TM10, and 3) a diet consisting of 100% live TM larvae (aiming at establishing the nutritional value of this insect species, TM100). With this purpose, quails were weighed and allocated into 3 experimental groups (10 replicates/treatment) ensuring homogenous live weight. During the trial, the environmental parameters were the same of the performance trial. The experimental diets and water were provided *ad libitum* to the quails. The digestibility trial allowed to determine the apparent nutrient digestibility of dry matter (**DM**), organic matter (**OM**), crude protein (**CP**), ether extract (**EE**), ash, starch, chitin, and energy. Subsequently, the nutritive value of the experimental diets was calculated based on the obtained digestibility data.

Physical Analyses of the Eggs

Eggs of the 5-wk performance trial were collected on a daily basis and transported to the meat and egg quality laboratory of the MAPS Department of the University of Padova (Italy) and were individually weighed to calculate the surface area. Further measurements were recorded: egg equatorial diameter (mm), and egg height (mm), using a digital caliper (Juwel Schraubtechnik, EB, Werkstraße 14, 57537 Wissen, Altenkirchen, Rheinland Palatinate, Germany) (0−150 mm—Juwel). Such measurements were used to calculate the egg shape index (%).

After physical measurements, eggs were broken for shell and interior egg quality measurements, and within 30 s albumen height was computed as the arithmetic mean of 2 measurements performed with a Haugh digital micrometer (Baxlo, Barcelona, Spain) to calculate the egg Haugh unit. The pH of the albumen (±0.1) was determined in duplicate (FG2-Five GoTM; Mettler Toledo, Greifensee, Switzerland - 3 points calibration: pH 4, 7, and 10), and yolk color was evaluated by comparison with the 15-points dsm-firmenich YolkFan^{TM} (DSM, Wurmisweg 576, CH-4303 Kaiseraugst, Switzerland). The albumen and yolk weights were determined to compute the albumen percentage and yolk percentage, as well as the yolk to albumen ratio. Furthermore, the eggshell was dried with a paper towel and weighed (±0.1 g), then eggshell thickness (mm) was measured at the equatorial level with the digital caliper. The shell percentage was calculated. The weight of the egg and of the edible portion (calculated as egg weight minus shell weight) were used to obtain the edible portion percentage.

Chemical Analyses of TM Larvae and the Experimental Diet

The chemical composition and energy content of TM larvae and experimental diet are shown in Table 2. Analyses of TM larvae and the experimental diet were carried out in duplicate following the Association of Official Analytical Chemists (AOAC, 2019) methods to determine dry matter (DM; method no. 934.01), crude protein (CP; method no. 2001.11) and ash (method no. 967.05). For the total nitrogen content of TM larvae, the Kjeldahl method was used; nitrogen content was then multiplied by 4.76 N-conversion factor to obtain the corrected crude protein content (Janssen et al., 2017). Ether extract (EE) was determined after acid hydrolysis. Gross energy (**GE**) was measured with an adiabatic bomb calorimeter. Starch (amyloglucosidase-α-amylase, method no. 996.11) content was analyzed in the experimental diet. The chitin content of TM larvae and freeze-dried excreta was analyzed according to the method described by Woods et al. (2019).

The chemical analyses of freeze-dried excreta were carried out in accordance with the same AOAC (2019) methods previously described for the experimental diet. The CP content of excreta was corrected for uric acid content, which was analyzed according to the procedure described by Cullere et al. (2016).

The fatty acid (**FA**) profile of TM larvae and experimental diet are presented in Table 3. The lipids extraction of the experimental diet and TM larvae was performed by Accelerated Solvent Extraction (**M-ASE**)

Table 2. Chemical composition (g/kg as is), mineral (mg/kg as is) and gross energy (MJ/kg) contents of Control diet and *Tenebrio molitor* (**TM**) larvae.

	Control diet	TM larvae
Dry matter	909	307
Crude protein	262	128
Ether extract	48.3	79.3
Ash	104	12.6
Starch	336	-
Chitin	-	31.0
Ca	25.9	0.13
P	3.62	2.11
Ca/P	7.15	0.06
Gross energy[1]	16.4	8.44

[1]Analyzed.

Table 3. Fatty acid profile (% of total fatty acid methyl esters, **FAME**) of Control diet and *Tenebrio molitor* (**TM**) larvae.

	Control diet	TM larvae
C6:0	0.24	0.01
C8:0	0.03	0.01
C10:0	0.00	0.01
C12:0	0.00	0.22
C14:0	0.09	3.53
C15:0	0.32	0.14
C16:0	14.0	13.9
C17:0	0.11	0.19
C18.0	3.76	2.73
C20:0	0.33	0.18
C22:0	0.26	0.07
C24:0	0.17	0.00
Total SFA	19.2	21.0
C14:1	0.02	0.12
C15:1	0.13	0.05
C16:1	0.10	1.86
C17:1	0.05	0.18
C18:1 *n*-9	23.5	43.0
C18:1 *n*-11	1.21	0.00
C20:1 *n*-9	0.09	0.09
Total MUFA	25.1	45.3
C18:2 *n*-6	49.5	30.8
C18:3 *n*-6	0.00	0.05
C18:3 *n*-3	5.38	0.90
C20:2 *n*-6	0.05	0.09
C20:3 *n*-6	0.00	0.03
C20:4 *n*-6	0.03	0.00
Total PUFA	54.9	31.9
n-6	49.6	31.0
n-3	5.38	0.90
n-6/*n*-3	9.22	34.4
Identified	99.3	98.1

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 4. Mineral profile (mg/kg, as is basis) of *Tenebrio molitor* (**TM**) larvae.

	TM larvae	EFSA reports[1]	EC No 1881/2006[2]
Macroelements:			
K	2618	-	-
P	2107	-	-
S	819	-	-
Mg	708	-	-
Na	464	-	-
Ca	128	-	-
Zn	28.4	-	-
Fe	11.6	-	-
Mn	2.61	-	-
Microelements:			
Cu	4.25	-	-
Sr	1.02	-	-
Al	1.01	-	-
Se	<1.0	-	-
Sn	<1.0	-	(inorganic)
Tl	<1.0	-	-
B	0.66	-	-
Ni	<0.5	-	-
Mo	0.28	-	-
Ag	<0.2	-	-
Ba	0.11	-	-
Cr	0.11	-	-
Be	<0.1	-	-
Co	<0.1	-	-
Li	<0.1	-	-
Sb	<0.1	-	-
V	<0.1	-	-
Ti	0.05	-	-
Contaminants:			
As	<1.0	0.00−0.29	-
Pb	<1.0	0.00−<0.08	0.50 (crustacean)
Cd	0.03	0.00−0.08	0.50 (crustacean)
Hg	0.00	0.00−0.04	0.50 (crustacean)

[1]Safety of dried yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal, 24/11/2020; Safety of frozen and dried yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal, 07/07/2021.

[2]Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L 364/5.

using petroleum ether and chloroform/methanol 1:2 as solvent binary mixtures, respectively. The fat content of the sample was determined gravimetrically after vacuum-evaporation under nitrogen stream. Samples were trans-methylated using a 4% methanolic solution of H_2SO_4 in order to determine fatty acid methyl esters (**FAME**). A biphasic separation was obtained by adding 0.5 mL of distilled water and 1.5 mL of *n*-heptane to each sample. FAME were quantified by gas chromatography (Shimadzu GC17A), equipped with an Omegawax (Sigma-Aldrich Co. LLC., Saint Louis, MO) 250 column (30 m × 0.25 μm × 0.25 μm) and flame ionization detector. Helium was used as the carrier gas at a constant flow of 0.8 mL/min. The injector and detector temperatures were 260°C. Peaks were identified based on commercially available FAME mixtures (37-Component FAME Mix; Supelco Inc., Bellefonte, PA). Results were expressed as % of total detected FAME.

The mineral profile (macro and micro elements) and heavy metals content of TM larvae are presented in Table 4. Inductively coupled plasma optical emission spectrometry (**ICP-OES**) was performed with Spectro Arcos (SPECTRO Analytical Instruments, GmbH, Kleve, Germany) after microwave digestion with Milestone rotor at 64-bar pressure, according to AOAC (2019). Data were expressed as mg/kg as is.

The amino acid (**AA**) profile of the TM larvae and experimental diet are provided in Table 5. The amino acid composition of the TM larvae and the experimental diets were determined after acid hydrolysis and pre-column derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, separated by RP-HPLC and analyzed by UV detection (Agilent 1260 Infinity, Agilent Technologies, Santa Clara, CA) following a method adapted from European Pharmacopoeia (Council of Europe, 2005). The protein content of the samples was hydrolyzed with (6M) HCl at 105°C for 24 h. Cysteine was determined as the sum of cysteine and cystine, after reaction with 3,3′-dithiodipropionic acid, producing a mixed disulfide, which then underwent acid hydrolysis accordingly. After hydrolysis, the samples were neutralized with (8M) NaOH, adjusted to volume, and filtered at 0.45 μm. Then, the derivatization step was conducted according to the manufacturer's instructions (AccQ-Tag Ultra Derivatisation Kit; Waters, Milford, MA).

Chemical Analyses of the Eggs

Eggs were collected for 2 consecutive days: 7 eggs from each cage (8 replicates/treatment) were homogenized to 1 sample (pool) and freeze-dried to guarantee enough

Table 5. Amino acid profile (g/100 g, as is basis) of Control diet and *Tenebrio molitor* (**TM**) larvae.

	Control diet	TM larvae
Essential amino acids:		
Arginine	1.34	0.64
Histidine	0.66	0.53
Isoleucine	0.60	0.41
Leucine	1.61	0.92
Lysine	1.42	0.83
Methionine	0.08	0.02
Phenylalanine	0.98	0.45
Threonine	0.78	0.50
Valine	0.66	0.61
Non-essential amino acids:		
Alanine	1.06	1.06
Aspartic acid	2.59	1.22
Cysteine	0.17	0.06
Glutamic acid	4.81	1.80
Glycine	0.95	0.80
Proline	1.25	1.05
Serine	1.20	0.69
Tryptophan	0.24	0.17
Tyrosine	0.46	0.78
Total AA	20.9	12.5

matrix to perform all the scheduled analyses. The proximate composition of egg samples was analyzed in accordance with the same AOAC (2019) method previously described for the experimental diet.

The FA profile of the eggs was analyzed following the same method previously described for the experimental diet, with the only exception that, for the fat extraction, the binary mixture of solvents hexane/Isopropanol 3:2 was used. Then, the results were expressed as % of total detected FAME. Also, the lipid peroxidability index (**PI**), atherogenic index (**AI**), thrombogenic index (**TI**), and hypocholesterolemic/Hypercholesterolemic index (**hH**) of the eggs were calculated as reported by Dalle Zotte et al. (2019). Additionally, the quantitative determination (mg/100 g egg) of FA was also obtained by using the chromatographic peak area according to the internal standard, that is nonadecylic acid (**C19:0**), and the total lipid content of the sample. Similarly, the AA profile of the eggs was analyzed following the same method previously described for the TM larvae and the experimental diet. The obtained results were expressed as mg/100 g egg.

Shelf-Life Trial

The storage stability trial was carried out on 56 eggs/treatment stored for d 0 (28 eggs/treatment) and d 28 (28 eggs/treatment) with average humidity and temperature of 65±5% and 20±1°C respectively. Out of 28 eggs, 21 eggs/treatment were used to evaluate the albumen pH (FG2-Five GoTM; Mettler Toledo, Greifensee, Switzerland - 3 points calibration: pH 4, 7, and 10), Haugh unit, and yolk color (dsm-firmenich YolkFan^{TM}), whilst the other 7 eggs/treatment were assigned for thiobarbituric acid-reactive substances (**TBARs**) analysis for d 0 *vs.* d 28. The extent of egg yolk lipid oxidation (TBAR) was evaluated with a spectrophotometer (Hitachi U-2000; Hitachi, Mannheim, Germany) set at 532 nm, that measured the absorbance of TBARs and a 1,1,3,3-tetraethoxypropane calibration curve (Botsoglou et al., 1994). Oxidation products were quantified as malondialdehyde (**MDA**) equivalents (mg MDA/kg egg yolk).

Sensory Analysis

Eggs were assigned to a descriptive sensory analysis, to detect possible difference among the experiment treatments (Control *vs.* TM10). A total of 27 eggs/treatment were used and a day analysis was scheduled. Panelists participated in 2 pre-test training sessions of 1 h each to familiarize themselves with the matrix and select appropriate descriptors, which were also drawn from the literature. Additionally, panelists were provided with freeze-dried and ground *Tenebrio molitor* larvae powder to evaluate and select possible descriptors. The quail eggs used for the training sessions were obtained from quails fed with a conventional diet and eggs were processed, stored, handled and cooked in the same manner as the samples which were used for the subsequent sensory analysis. The selected descriptors were: odor intensity, sulfur, and *Tenebrio molitor*, and flavor intensity, sapidity, sulfur, greasy-oily, persistency, *Tenebrio molitor*. For TM odor and flavor, panelists were trained on a TM larvae powder. At the farm, freshly collected eggs were identified with a 3-digit random code and then transported to the MAPS Department. Once there, eggs were boiled for 5 mins, cooled under running tap water, and served to panelists in a random sequence. Each panelist evaluated a total of 8 eggs (4 eggs/treatment), except one panelist received a total of 6 eggs (3 eggs/treatment); the panel received the aforementioned list of descriptors to score on numerical and continuous 15 cm-long scales from 1 (the lowest score for each attribute) to 10 (the highest score for each attribute). All the evaluations were performed in a room where the temperature was set at 22°C. In each sensory session, unsalted crackers and still water at room temperature were available to panelists.

Statistical Analysis

Performance data and egg physicochemical traits were subjected to one-way ANOVA with the experimental diet (Control *vs.* TM10) as fixed effect following the General Linear Model (**GLM**) procedures of the SAS (SAS OnDemand for Academics-3.81 Enterprise Edition, SAS Institute Inc., Cary, NC). For digestibility, data were subjected to one-way ANOVA testing the effect of the dietary treatment (Control *vs.* TM10 *vs.* TM100) on the relevant traits. For the shelf-life trial, a 2-way ANOVA tested the effects of the dietary treatment and day of storage, and their interaction. For sensory traits, data were subjected to 1-way ANOVA with experimental diets (Control *vs.* TM10) as a fixed effect, except for the traits *Tenebrio molitor* odor and flavor.

As they were not normally distributed, percentages were compared by using a Z-test for 2 proportions and through a chi-square test. The experimental unit varied: cage for performance data, individual quail for digestibility, and single egg and pooled sample for physical and chemical characteristics of the eggs, respectively. Least square means were obtained, and post-hoc pairwise comparisons were performed using the Bonferroni correction. The significance was considered at a 5% confidence level.

RESULTS

Tenebrio molitor *Larvae*

Data concerning the chemical composition of fresh TM larvae confirmed once more the nutritional value of this feedstuff. Table 2 shows that TM larvae were rich in protein (12.8 g/100 g), and EE (7.93 g/100 g), thus resulting in a notable gross energy content (8.44 MJ/kg). The FA profile of the TM (Table 3) was characterized by a prevalence of monounsaturated fatty acids (**MUFA**; 45.3% of total FAME), followed by polyunsaturated fatty acids (**PUFA**; 31.9% of total FAME), and saturated fatty acids (**SFA**; 21% of total FAME). Oleic acid (**C18:1 *n*-9**) was the most abundant MUFA (43.0% of total FAME) while, among PUFA, the *n*-6 fraction accounted for the almost totality (31% of total FAME) and with linoleic acid accounting alone for the 30.8% of total FAME. From the analysis of the mineral profile (Table 4), it emerged that live TM larvae contain a substantial quantity of macroelements, especially potassium (2618 mg/kg), phosphorus (2107 mg/kg), and sulfur (819 mg/kg). Among microelements, copper was the most represented (4.25 mg/kg). Concerning contaminants, no particular highlights were noticed since their values were comprised within the legislative limits and values published by the European Food Safety Authority (**EFSA**) reports. The quality of the protein provided by TM larvae can be noticed by looking at their amino acid content (Table 5): among essential amino acids, leucine (0.92 g/100 g), lysine (0.83 g/100 g), and arginine (0.64 g/100 g) were the most abundant, while glutamic acid (1.80 g/100 g), aspartic acid (1.22 g/100 g), and alanine (1.06 g/100 g) were the most represented non-essential amino acids.

Productive Performance

The effect of the dietary inclusion of live TM larvae on the productive parameters of quails and egg measurements are displayed in Table 6. Over the course of the 5-wk feeding trial the final live weight, FCR, egg weight, egg production, and defected eggs did not differ between the Control and TM10 groups ($P > 0.05$), and no mortality was observed. However, the TM10 group exhibited a significantly higher ($P < 0.001$) feed intake compared to the Control group. Additionally, the TM10 group showed a lower egg shape index compared to the Control group ($P < 0.01$).

Table 6. Effect of the dietary inclusion of live *Tenebrio molitor* (TM − 10% of the daily feed intake) larvae on the productive performance of laying quails and egg measurements during a 5-wk trial.

	Control	TM10	RSD[1]	*P*-value
N. of laying quails	30	30		
Initial live weight, g	342	336	26.7	0.3726
Final live weight, g	354	347	31.1	0.4312
N. of replicated cages	6	6		
N. of total egg laid	923	950		
Feed intake (FI), g/d/quail	43.3	51.5[2]	1.55	<0.0001
Egg weight, g[3]	14.3	14.2	1.30	0.1021
Egg production, %	90.0	90.5	5.37	0.8870
Egg mass, g egg/hen/d	13.0	12.9	0.75	0.6964
FCR	3.29	3.50	0.19	0.0943
Egg shape index, %[3]	76.0	75.6	2.81	0.0100
Defected eggs, %	1.26	0.20	1.62	0.2850

[1]RSD: residual standard deviation.
[2]Mash feed + live TM larvae.
[3]Measurement performed on total eggs laid.

Digestibility

Results presented in Table 7 show the effect of the dietary supplementation of live TM larvae (10% of the expected daily feed intake - TM10 or 100% - TM100) to laying quails on the total tract apparent digestibility of nutrients and nutritive value of diets. The TM100 group exhibited the highest feed intake, followed by the TM10 group and then by the Control group (TM100 > TM10 > Control; $P < 0.0001$). Considering the DM intake, instead, the 3 treatments displayed the following rank: TM10 > Control > TM100 ($P < 0.0001$).

The TM10 group showed an apparent digestibility of nutrients comparable to that of the Control diet. Conversely, TM100 group displayed the lowest digestibility for DM ($P < 0.001$), organic matter ($P < 0.001$), and protein ($P < 0.001$), while ether extract was better digested in TM100 than in groups Control and TM10 ($P < 0.0001$). As a result, energy digestibility was similar in the 3 treatment groups. Starch digestibility was not affected by the presence of TM10. For what concerns chitin, TM10 showed a 100% digestibility which was higher than the 88.7% of the TM100 group ($P < 0.0001$).

The supplementation of 10% live TM larvae to the diet of laying quail did not alter the nutritive value of diets, whereas the nutritive value of 100% live TM larvae was lower than that of Control and TM10 diets ($P < 0.0001$).

Egg Physical Traits and Proximate Composition

The inclusion of live TM larvae into laying quails' diet (TM10) did not affect the physical traits of the whole egg and of its parts, including albumen, yolk and shell weight, as well as their proportion to the total egg weight, albumen pH and Haugh index, yolk color, and shell thickness (Table 8). As it was observed for the physical traits of eggs, also proximate composition was

Table 7. Effect of the dietary inclusion of live *Tenebrio molitor* (**TM** − 10% of the daily feed intake or 100%) larvae on the apparent nutrient digestibility in laying quails and nutritive value of the diets.

	Control	TM10	TM100	RSD[1]	*P*-value
N.	10	10	10		
Live weight, g	337	337	339	26.2	0.9817
Feed intake, g	68.6[B]	82.3[B]	157[A]	20.8	<0.0001
Dry matter (DM) intake, g	62.3[B]	73.4[A]	48.9[C]	7.73	<0.0001
DM excreted, g	26.8	29.2	29.5	5.08	0.4412
Apparent digestibility, %:					
DM	57.0[A]	60.1[A]	44.0[B]	5.35	<0.0001
Organic matter	59.7[A]	62.6[A]	47.1[B]	4.96	<0.0001
Protein	50.8[A]	50.8[A]	24.0[B]	10.7	<0.0001
Ether extract	83.9[B]	85.9[B]	94.0[A]	3.60	<0.0001
Starch	96.5	97.0	-	0.83	0.1625
Chitin	-	100[A]	88.7[B]	2.37	<0.0001
Energy	65.3	67.9	65.4	3.67	0.2460
Nutritive value of diets:					
Metabolizable protein (MP: g/kg diet)	133[A]	132[A]	31.2[B]	16.3	<0.0001
Metabolizable energy (ME: MJ/kg diet)	10.7[A]	11.0[A]	5.38[B]	0.47	<0.0001
MP/ME	12.4[A]	12.0[A]	5.75[B]	3.35	0.0016

[1]Residual standard deviation.
[A-C]Means in the same row with different superscript letters significantly differ for $P < 0.01$.

not influenced when live TM larvae (TM10) were supplemented to the laying quails' diet (Table 9).

Egg Fatty Acid and Amino Acid Profiles

The effect of the dietary inclusion of live TM larvae into laying quails' diet on the FA profile (% of total FAME) of eggs collected at wk 5 is depicted in Table 10. The main fatty acid classes that is, SFA, MUFA, and PUFA remained unaffected by the dietary inclusion of live TM. However, some individual SFAs, such as myristic acid (**C14:0**), pentadecylic acid (**C15:0**) and margaric acid (**C17:0**) were higher ($P < 0.001$) in TM10 group than in Control group. In the case of individual MUFAs, the proportion of myristoleic acid (**C14:1**) and heptadecenoic acid (**C17:1**) was significantly higher in TM10 than the Control group ($P < 0.05$). For the vaccenic acid (**C18:1 *n*-11**), Control eggs showed a higher percentage than the TM10 ones ($P < 0.05$). As for individual PUFAs, the TM10 diet increased the proportion of dihomo-γ-linolenic acid (**C20:3 *n*-6**) in quail eggs compared to the Control ($P < 0.05$). As a result, the omega-6/omega-3 (***n*-6/*n*-3**) ratio increased in eggs belonging to the TM10 treatment compared to the Control ones ($P < 0.05$). As a consequence of the limited

Table 8. Effect of the dietary inclusion of live *Tenebrio molitor* (**TM** − 10% of the daily feed intake) larvae on eggs physical traits.

	Control	TM10	RSD[1]	*P*-value
N.	68	85		
Egg weight, g	14.6	14.6	1.13	0.8150
Edible portion, %	88.8	88.5	1.12	0.1611
Surface area, cm^2	26.3	26.4	1.45	0.8220
Yolk weight, g	4.37	4.36	0.50	0.9223
Yolk, %	30.0	29.7	2.34	0.4308
Yolk color	5.41	5.53	0.80	0.3650
Albumen weight, g	8.57	8.60	0.77	0.7928
Albumen, %	58.8	58.9	2.90	0.9182
Yolk to Albumen ratio	0.51	0.51	0.06	0.5724
Albumen pH	8.62	8.68	0.19	0.0891
Haugh unit	95.8	96.3	4.46	0.5180
Shell weight, g	1.63	1.67	0.17	0.1488
Shell thickness, mm	0.25	0.25	0.03	0.5401
Shell, %	11.2	11.5	1.12	0.1611

[1]RSD: Residual standard deviation.

Table 9. Effect of the dietary inclusion of live *Tenebrio molitor* (**TM** − 10% of the daily feed intake) larvae on the proximate composition of quail eggs (g/100 g egg).

	Control	TM10	RSD[1]	*P*-value
N.	8	8		
Water	72.7	71.3	3.27	0.4231
Protein	13.2	13.6	1.42	0.5424
Lipids	10.6	11.4	1.36	0.2597
Ash	1.01	1.07	0.13	0.3321

[1]RSD: residual standard deviation.

Table 10. Effect of the dietary inclusion of live *Tenebrio molitor* (**TM** −10% of the daily feed intake) larvae on the fatty acid profile of quail eggs (% of total FAME).

	Control	TM10	RSD[1]	*P*-value
N.	8	8		
C14:0	0.35	0.41	0.02	0.0002
C15:0	0.04	0.05	0.03	0.0013
C16:0	24.8	25.2	0.38	0.1035
C17:0	0.15	0.17	0.01	<0.0001
C18:0	10.7	10.8	0.40	0.8045
C20:0	0.10	0.09	0.01	0.3587
C24:0	1.41	1.33	0.11	0.1433
Total SFA	37.6	38.0	0.53	0.1682
C14:1	0.06	0.07	0.01	0.0483
C16:1	3.21	3.18	0.29	0.8310
C17:1	0.07	0.08	0.03	0.0004
C18:1 *n*-9	33.5	32.7	1.08	0.1811
C18:1 *n*-11	1.60	1.41	0.13	0.0148
Total MUFA	38.4	37.5	1.25	0.1485
C18:2 *n*-6	18.4	18.9	1.08	0.3684
C18:3 *n*-6	0.28	0.26	0.03	0.3208
C18:3 *n*-3	0.90	0.83	0.09	0.1513
C20:2 *n*-6	0.09	0.09	0.03	0.6008
C20:3 *n*-6	0.12	0.16	0.04	0.0285
C20:4 *n*-6	2.81	2.73	0.12	0.2589
Total PUFA	22.6	23.0	1.23	0.5332
n-6	21.7	22.1	1.14	0.4379
n-3	0.91	0.85	0.11	0.2464
n-6/*n*-3	23.9	26.2	2.10	0.0483
AI	0.43	0.44	0.01	0.0089
TI	0.35	0.34	0.02	0.1119
PI	32.1	32.4	1.43	0.7495
hH	2.21	2.16	0.05	0.0686
Identified	98.6	98.5		

[1]RSD: residual standard deviation.
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AI, atherogenicity index = (C12:0 + 4 x C14:0 + C16:0)/[Total MUFA + Total (*n*-6) + total (*n*-3)]. TI: Thrombogenicity index = (C14:0 + C16:0 + C18:0)/[(0.5 x total MUFA) + 0.5 x (*n*-6) + 3 x (*n*-3/*n*-6)]; PI: Peroxidability index = (% monoenoic x 0.025) + (% dienoic x 1) + (% trienoic x 2) + (% tetraenoic x 4) + (% pentaenoic x 6) + (% hexaenoic x 8); hH: Hypocholesterolemic/Hypercholesterolemic index = (C18:1 *n*-9 + C18:2 *n*-6 + C20:4 *n*-6 + C18:3 *n*-3 + C20:5 *n*-3 + C22:5 *n*-3 + C22:6 *n*-3)/(C14:0 + C16:0).

Table 11. Effect of the dietary inclusion of live *Tenebrio molitor* (**TM** − 10% of the daily feed intake) larvae on the fatty acid content of quail eggs (mg/100 g egg).

	Control	TM10	RSD[1]	*P*-value
N.	8	8		
C6:0	2.26	2.94	0.68	0.0855
C14:0	31.1	38.4	3.99	0.0044
C15:0	4.26	5.10	0.50	0.0081
C16:0	2171	2353	289	0.2674
C17:0	12.7	16.2	1.64	0.0016
C18:0	938	1012	141	0.3541
C20:0	8.60	8.59	1.54	0.9756
C24:0	124	124	19.2	0.9792
Total SFA	3292	3560	451	0.2934
C14:1	5.12	6.33	0.77	0.0115
C16:1	281	296	36.4	0.4452
C17:1	6.45	7.72	0.73	0.0060
C18:1 *n*-9	2925	3060	372	0.5198
C18:1 *n*-11	139	132	18.0	0.4506
Total MUFA	3356	3502	418	0.5364
C18:2 *n*-6	1608	1759	206	0.2001
C18:3 *n*-6	24.2	24.5	3.58	0.9029
C18:3 *n*-3	79.3	77.3	11.4	0.7364
C20:2 *n*-6	8.23	8.06	2.00	0.8675
C20:3 *n*-6	10.3	15.3	3.51	0.0188
C20:4 *n*-6	245	256	33.3	0.5653
Total PUFA	1976	2142	252	0.2475
n-6	1896	2063	242	0.2254
n-3	80.2	78.7	11.6	0.8029

[1]RSD: residual standard deviation.
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 12. Effect of the dietary inclusion of live *Tenebrio molitor* (**TM** − 10% of the daily feed intake) larvae on the amino acid (**AA**) profile of quail eggs (g/100 g egg).

	Control	TM10	RSD[1]	*P*-value
N.	8	8		
Essential amino acids				
Arginine	0.54	0.50	0.06	0.1981
Histidine	0.34	0.32	0.04	0.2494
Isoleucine	0.35	0.33	0.04	0.4571
Leucine	0.87	0.79	0.10	0.1749
Lysine	0.91	0.81	0.04	0.1189
Methionine	0.14	0.13	0.02	0.1486
Phenylalanine	0.53	0.49	0.06	0.2503
Threonine	0.55	0.50	0.07	0.1514
Valine	0.45	0.41	0.05	0.1097
Non-essential amino acids				
Alanine	0.60	0.55	0.07	0.2075
Aspartic acid	1.19	1.08	0.15	0.1967
Cysteine	0.13	0.12	0.02	0.2230
Glutamic acid	1.58	1.44	0.19	0.1612
Glycine	0.43	0.40	0.05	0.2715
Proline	0.39	0.82	0.05	0.2119
Serine	0.89	0.36	0.10	0.2222
Tryptophan	0.13	0.14	0.02	0.4660
Tyrosine	0.23	0.22	0.03	0.3131
Sum AA	10.3	9.42	1.22	0.1949

[1]RSD: residual standard deviation.

effect of the dietary supplementation of live TM larvae on quails' egg FA profile, also the health indexes were comparable in Control and TM10 eggs. The sole exception was the AI, which exhibited the highest value in TM10 eggs ($P < 0.01$).

Looking at the amounts of FAs (Table 11), a similar outcome than that reported for the FA proportions (Table 10) was observed: main FA classes were comparable in eggs of the 2 dietary treatments, while the same individual SFA, MUFA, and PUFA for which a treatment effect was observed with percentage data, were influenced also in the case of quantitative data. The only exception was the C18:1 *n*-11 whose amount was similar in Control and TM10 eggs.

Also, the AA contents of eggs did not change as a result of the live TM larvae supplementation to the laying quails' diet (Table 12). Control and TM10 eggs displayed similar amounts for each of the essential and non-essential amino acids, as well as the same sum of amino acids.

Egg Shelf-Life

Table 13 shows the effect of the dietary supplementation of live TM larvae into the diet of laying quails on the storage stability of eggs over a 28-d retail display, as well as the effect of storage time on the considered traits within the treatment group, including their interaction. The qualitative parameters monitored along the trial were albumen pH, yolk color, Haugh unit and oxidative status of lipids (TBARs). As expected, results highlighted that albumen pH increased in eggs of both Control and TM10 groups at d 28 of storage compared to those at d 0 ($P < 0.0001$), and that, conversely, Haugh unit (freshness) and yolk color decreased ($P < 0.001$). However, albumen pH, Haugh unit and yolk color showed similar values for both Control and TM10 eggs. In contrast to other traits, the initial oxidative rate of

Table 13. Effect of the dietary inclusion of live *Tenebrio molitor* (**TM** − 10% of the daily feed intake) larvae on the physical traits (albumen pH, yolk color, Haugh unit) and oxidative status (TBARs[1], mg MDA[2]/kg egg yolk) of quail eggs, assessed over a 28-d display trial.

	Control	TM10	RSD[3]	*P*-diet	*P*-diet*day
N.	21	21			
Albumen pH					
D 0	8.64	8.67	0.19	0.5513	0.5002
D 28	9.59	9.58	0.05	0.6366	
RSD[3]	0.17	0.12			
P-day	<0.0001	<0.0001			
Yolk color					
D 0	5.67	6.00	0.60	0.0750	0.1838
D 28	4.84	4.81	0.64	0.8739	
RSD[3]	0.71	0.52			
P-day	0.0008	<0.0001			
Haugh unit					
D 0	95.9	96.3	4.85	0.7848	0.5510
D 28	81.5	83.0	2.74	0.1022	
RSD[3]	4.35	3.60			
P-day	<0.0001	<0.0001			
N.	7	7			
TBARs					
D 0	1.56[A]	1.21[B]	0.12	0.0004	0.0001
D 28	1.14[B]	1.26[B]	0.13	0.1210	
RSD[3]	0.11	0.14			
P-day	<0.0001	0.5338			

[1]MDA: malondialdehyde.
[2]TBARs: thiobarbituric acid-reactive substances.
[3]RSD: residual standard deviation.
[A-B]Means within the same row and column with superscripts is for significant interaction $P < 0.001$.

Table 14. Effect of the dietary inclusion of live *Tenebrio molitor* (**TM** − 10% of the daily feed intake) larvae on the sensory traits of quail eggs.

	Control	TM10	RSD[1]	*P*-value
N.	27	27		
Odor:				
Intensity	59.0	54.0	9.50	0.0634
Sulfur	44.0	36.9	13.0	0.0512
Tenebrio molitor[2]	0.22	0.30	−0.31	0.7562
Flavor:				
Intensity	44.8	35.4	10.9	0.0030
Sapidity	30.2	28.5	6.58	0.3435
Sulfur	28.0	22.7	9.12	0.0405
Greasy-oily	59.0	50.9	10.2	0.0061
Persistency	34.9	32.6	9.76	0.4095
Tenebrio molitor[2]	0.22	0.15	0.35	0.7261

[1]RSD: residual standard deviation.
[2]For *Tenebrio molitor* a Z-test for 2 proportions was conducted, as the trait was not normally distributed.

TM10 egg lipids on d 0 of storage was lower compared to Control egg lipids ($P < 0.001$). However, after 28 d of retail display storage, eggs from both Control and TM10 groups showed comparable oxidative status. Notably, within the Control group a significant day effect was evident, with TBARs values on d 28 being lower than those on d 0 ($P < 0.0001$), whereas TM10 eggs had similar oxidative status at both d 0 and 28 ($P > 0.05$).

Egg Sensory Traits

The dietary supplementation of live TM larvae to laying quails' diet affected the sensory traits of eggs (Table 14). While odor attributes were similar in eggs of the 2 treatment groups, eggs of the TM10 group had an overall milder flavor than Control eggs. In fact, overall flavor intensity ($P < 0.01$), sulfur ($P < 0.05$), and greasy-oily ($P < 0.01$) flavors scored lower values in TM10 than in Control eggs.

DISCUSSION

Tenebrio molitor is surely one of the most interesting insect species from the perspective of providing alternative and sustainable feedstuffs for food-producing animals, including poultry. This is testified by the number of research articles that have been published in the last decade, assessing the possible inclusion of its protein (i.e., meal) or fat fractions into the diet of different animal species, including poultry and swine (Hong et al., 2020), fish (Henry et al., 2015), and rabbit (Volek et al., 2021). Despite this, the use of live larvae occupies a marginal part of this literature. Bellezza Oddon et al. (2021) observed that the provision of live *Tenebrio molitor* larvae to broiler chickens (5% of the expected feed intake) had no effect on growth and carcass traits, hematological and serum parameters, as well as gut morphometric indexes and histopathological alterations. The same research group observed that this feeding treatment did not alter the mucin composition or local immune response of chickens too, while slightly improved cecal microbiota by enhancing a minor fraction of short chain fatty acid-producing taxa (Colombino et al., 2021). Considering other insect species, live *Hermetia illucens* larvae were tested as a possible tool to limit feather pecking in laying hens (Star et al., 2020): a larvae dispenser provided a daily amount of 12 g live larvae/hen (10% of daily feed intake), and this resulted in hens (65 wk of age) with better feather condition than control ones, as a result of less pecking. Then, the same insect species was studied by Veldkamp and van Niekerk (2019) on turkey poults and by Ipema et al. (2020a,b) on broiler chickens. In all trials, however, the possible effect of live *Hermetia illucens* on product quality was not assessed.

This research represents the first study exploring the impact of live *Tenebrio molitor* larvae as nutritional enrichment for laying quails. In a research conducted on laying hens, feeding live black soldier fly larvae at 0, 10, and 20% of their daily expected DM intake or *ad libitum* access to live BSF larvae for 12 wk, stimulated hen's interest and feed intake (Tahamtani et al., 2021), with the whole daily portion of live larvae being consumed in approximately 5 min. This was confirmed also in the present research; in fact, a first subjective aspect that was observed during both the performance and the digestibility trials on laying quails, but which is of importance in explaining the experimental results, was the remarkable interest and extremely excited response of laying quails when seeing the live larvae in their feeder. Feeding stimulus, constant during the 5-wk performance trial, was so strong that they consumed the whole portion of larvae within 2 to 5 min from the administration. Scientifically, this was confirmed and highlighted by the results on the feed intake, both during the performance and during the digestibility trials. Despite the Control diet was nutritionally complete, and this was testified by the satisfactory laying performance of Control quails (Table 6), TM10 quails exhibited a higher feed consumption that was almost exclusively attributable to the presence of larvae. The average feed consumption of live TM larvae was 4.84 g/quail/d (data not shown). Also, during the digestibility trial, no statistical difference was observed for feed intake, but Control and TM10 quails consumed 68.6 *vs.* 82.3 g feed/d, respectively. Quails of the treatment TM100 displayed a particular outcome in terms of feed intake, since it was more than double that of the other 2 groups (157 g/d; $P < 0.0001$): on the one hand, this extreme result could be attributable to the inadequate nutritional profile of the TM larvae compared to the Control diet (Table 2), thus triggering quails to maximize feed intake, as well as to the lower DM content of TM100 *vs.* the Control feed. In fact, the DM intake of TM100 quails was only 48.9 g/d, *vs.* 62.3 and 73.4 of Control and TM10 quails, respectively.

The digestibility trial allowed to observe that TM larvae were overall less digestible than the Control diet which was expected, since they contain chitin which can act as an antinutritional factor mainly reducing the digestibility of the protein faction (Kroeckel et al., 2012): in the present digestibility trial, TM100 quails had had an apparent CP digestibility of 24.0%. The

antinutritional activity of chitin, however, is not always observed in poultry trials, since the effect is dose-dependent and poultry species possess enzymes that can hydrolyze the β-1, 4 glycoside bonds of chitin thus allowing its digestion (Tabata et al., 2018). This was practically demonstrated in the present digestibility trial, where TM10 quails displayed a complete digestion (100%) of chitin, while it decreased in *ad libitum* TM larvae feeding (TM100). Despite this, a remarkable 88.7% chitin digestibility was observed. Low chitin amounts can have a probiotic effect on poultry gut health (Bovera et al., 2015) and, in general, no particular drawbacks on productive outcomes have been observed in previous researches on laying hens (Star et al., 2020; Tahamtani et al., 2021), quails (Dalle Zotte et al., 2019), as well as considering meat-producing poultry species (Loponte et al., 2017; Biasato et al., 2018; Zsedely et al., 2022) fed with different insect meals or live larvae. Interestingly, the sole provision of live TM larvae (TM100) provided the best ether extract digestibility, thus emphasizing that the lipid fraction should not negatively be affected by the presence of chitin in the diet.

Results dealing with the physical egg traits provided evidence that the live TM larvae can be fed to laying quails, together with a standard diet, without impairing egg quality. Despite no other research studied the impact of the dietary inclusion of live TM larvae into laying quails or hens' diet, feeding hens with 10, 20% or ad libitum live HI larvae (Star et al., 2020; Tahamtani et al., 2021) did not affect the egg physical quality parameters, except the yolk color, which was paler in the *ad libitum* group. The latter result was attributed to the reduced intake of the control diet, thus determining a reduced uptake of carotenoids. In studies where TM meal was included in the diets for laying hens (Ko et al., 2020) or quails (Secci et al., 2021) at different inclusion levels (5−20% dietary inclusion), no drawbacks on egg physical traits were detected.

Previous research on the use of insect meals into laying quails' diet highlighted that the dietary treatments had a marginal effect on the FA profile and content of egg lipids (Secci et al., 2018; Dalle Zotte et al., 2019). This was attributable to a noticeable activity of desaturase and elongase enzymatic patterns on SFA, as well as on the synthesis of long chain FA (Güçlü et al., 2008; Secci et al., 2021). Furthermore, birds can exploit the acetate/malonate way to synthesize ex novo palmitic acid (**C16:0**), partly convert it into stearic acid (**C18:0**) and desaturate both into palmitoleic acid (**C16:1 *n*-7**) and C18:1 *n*-9 (Klasing, 1998). This is coherent with the results observed in the present research, as the lipid fraction of TM larvae was richer in some individual FAs compared to the Control diet, particularly C14:0, myristoleic acid (C14:1), palmitoleic acid (**C16:1**), C17:1, C18:1 *n*-9. Conversely, TM larvae displayed a remarkably lower amount of linoleic acid (**C18:2 *n*-6**) compared to the Control diet. Despite this, the FA profile and content of egg lipids was only marginally affected by the inclusion of live TM larvae into the quails' diet. Above all, C18:1 *n*-9, total MUFA and overall *n*-6 were not different in the eggs of the 2 dietary treatments, despite a notable difference for this FA was noticed in the TM larvae *vs.* the Control diet. In addition to the metabolic pathways previously discussed, it must be highlighted that quails of the present experiment ingested 4.84 g/larvae/d, roughly corresponding to 0.38 g lipids/d. Conversely, the Control diet contributed to about 2.1 g lipids/d. Therefore, the relative contribution of TM lipids to the overall dietary lipids was relatively low.

Eggs contain high quality proteins and are known to be good source of essential AA. Literature data (Genchev, 2012) indicate about 49.5% essential amino acids, with lysine and leucine being the main AA. This is in line with the results of the present study as quail eggs provided about 45% essential AA, and lysine and leucine were the most abundant ones. The comparable results of Control and TM10 quail eggs in terms of AA contents was expected, since *Tenebrio molitor* is known to be an excellent AA source (Makkar et al., 2014).

Coherently to previous research on eggs obtained from quails fed with different insect sources (Dalle Zotte et al., 2019; Singh et al., 2023), also in the present trial no particular effects linked to the dietary treatment on eggs' shelf-life were observed. The pH values of quail eggs (average: 8.66) at d 0 of shelf-life are typical for this species (Dalle Zotte et al., 2019) and are indicative of freshness. Similarly, also the high initial Haugh unit of both groups (average: 96.1) is a further indication of egg freshness. In fact, at d 28 of shelf-life, pH increased and the Haugh unit decreased along with storage duration. This trend is naturally attributable to the progressive loss of carbon dioxide, and the disruption of the ovomucin-lysozyme complex, which is known to negatively affect albumen consistency (Doğan et al., 2018), ultimately leading to a reduction in egg quality. Despite this, eggs at 28 d of storage quail eggs could be categorized within the best grade, AA, in accordance with the classification proposed for hen eggs (Caner and Yüceer, 2015), where Haugh unit >72 is categorized as AA grade, 72−60 as A grade, 59−31 as B grade, and <30 as C grade. The progressive attenuation of yolk color traits during storage is also a natural result of chemical, enzymatic and physical degradation processes for which also xanthophylls and carotenoids, the main yolk pigments naturally contained into some feed ingredients (i.e. corn and *Tenebrio molitor*), are progressively degraded (Omri et al., 2019).

The supplementation of antioxidant-rich feeding substrates during the laying period reduces egg susceptibility to the oxidative phenomenon, thus offering the potential to increase the oxidative stability of poultry products.

In the present study, at d 0 storage, eggs of the TM group displayed lower TBARs values than Control ones, which was unexpected. These differences, however, disappeared at d 28 of storage as the oxidative degree of the Control eggs decreased with storage time, which was also unexpected. As highlighted in previous studies (Finke, 2002; Secci et al., 2018), insect larvae contain tocopherols

and β-carotene, which are known for their antioxidant activity and could have potentially improved to oxidative status of TM eggs compared to Control ones along the storage period. This would have also been coherent with the result of Dalle Zotte et al. (2019), where eggs of quails fed with black soldier fly larvae meal exhibited a lower oxidative rate compared to Control ones at the end of a 28-d storage trial. A hypothesis to explain why eggs of the Control group at d 0 of storage displayed the highest TBARs value could be attributed to a limitation of the TBARs test. Specifically, pigments (insect-derived, but more in general derived from feed ingredients) could have interacted with the chromatic reaction resulting from the TBA and the oxidation products, therefore altering the reading at the spectrophotometer. This interference had already been observed when a rooibos (*Aspalathus linearis*) extract was added in the manufacturing of rabbit meat patties (Cullere et al., 2019), but in that case, a *post-mortem* additive was considered, and an alteration of the color of the product was visually evident, which was not the case of the present study. For these reasons, further investigations in to explain present findings about the TBARs values are necessary and caution is suggested to infer on present results.

The sensory profile of a food product is a key aspect to ensure consumer liking and thus a successful market placement. In the present research it was observed that TM10 eggs had an overall milder sensory profile than Control ones, mainly due to lower sulfur and greasy-oily flavors perceptions. This being the first study considering the possible effects of live TM larvae as feed for laying quails on the sensory traits of their eggs, no direct literature comparisons can be performed. A recent research analyzing the sensory characteristics of raw and cooked *Tenebrio molitor* larvae (Seo et al., 2020), depicted that the aroma of raw mealworms is primarily composed of hydrocarbons (50.11%) and aldehydes (37.14%), with 4-methylbenzaldehyde being the prevalent individual component. These corresponded to a strong wet-soil-like notes but mild oily, shrimp-like, and sweet-corn-like notes, thus possibly explaining why the overall sensory traits of TM10 eggs of the present trial did not display peculiar sensory attributes compared to the Control ones. In another study considering the effect of increasing dietary inclusion levels of *Hermetia illucens* meal (10 and 15%) on the sensory characteristics of quail eggs (Dalle Zotte et al., 2019), no relevant changes in the overall sensory profile were observed as well as on specific off-odors and off-flavors perception, with the sole exception of the "feed" off-flavor, which linearly increased with the *Hermetia illucens* meal inclusion level.

CONCLUSIONS

Results of the present experiment indicated that supplementing live TM larvae to laying quails at 10% of their daily feed intake stimulated feeding activity and did not pose any drawback on productive performance and mortality rate, as a result of a satisfactory nutritive value of the TM10 diet. Also, egg physicochemical quality, sensory characteristics and shelf-life were comparable with conventional eggs, which is a key factor to ensure both health and consumer expectations. The sole administration of live TM larvae allowed the quantification of the specific nutritive value of this emerging feedstuff, which is of pivotal importance to optimize feed formulations for poultry. To complete the scientific evaluation of this emerging nutritional enrichment for quails, future research could be directed in assessing the potential beneficial effect of TM10 supplementation on quail's gut health, similarly to what it was conducted on broiler chickens, to understand if a 10% supplementation level is adequate or if lower levels provide similar outcomes. In addition, a deeper evaluation of the value of TM as an enrichment tool should be considered.

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DISCLOSURES

The authors declare no conflicts of interest.

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