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Original Research Article

Growth performance, welfare traits and meat characteristics of broilers fed diets partly replaced with whole *Tenebrio molitor* larvae

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ABSTRACT

The role of insects in animal nutrition has been reconsidered during recent years, paving the way for an increasing market for edible insects. Their protein and amino acid balance make them a promising source of protein for replacing high value proteins. Yellow mealworm, *Tenebrio molitor* L. (TM; Coleoptera: Tenebrionidae) larvae, have shown positive effects on broiler performance in several research studies and have a strong potential as a sustainable alternative protein source for monogastric animals. This study aimed to assess the effect of replacing various ratios of basal diets with *T. molitor* larvae on broiler performance as well as on several meat and welfare characteristics. For the study, 120 one-day-old male chicks (Ross 308) were randomly allocated in 3 treatments and 4 replications (10 birds per pen). Birds of the control group (basal diet) were fed with typical commercial maize and soymeal-based rations in mash form. The other 2 groups were treated with the same diet, after replacing 5% and 10% with dried TM larvae, respectively. On d 35 (end of trial), meat samples were collected and analysed. Body weight, feed intake, body weight gain and feed conversion ratio during the periods of 1 to 10 d (starter period), 11 to 24 d (grower period), 25 to 35 d (finisher period) and 1 to 35 d (total period) were assessed. Pododermatitis, diarrhoea, feather score and litter conditions were also assessed during the trial. The results indicated that TM larvae inclusion in the broilers' diet positively affected body weight gain values, as well as the carcass yield, the meat composition and various welfare traits. Additionally, the dietary treatments with TM larvae favourably affected meat composition and colour parameters, whereas there were also some positive effects on lipid and protein oxidation. Saturated fatty acids were decreased by the dietary supplementation whereas the polyunsaturated fatty acids to SFA ratio increased. In general, the study showed that whole TM larvae addition can provide a promising alternative to soybean meal in the diet of broilers, demonstrating a positive impact on growth, welfare and meat characteristics.

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1. Introduction

Poultry is undoubtedly the most widely consumed meat worldwide, with an average consumption of 15.1 kg/capita per year, whereas it is expected to represent almost 40% of all meat proteins by 2030 (OECD/FAO, 2021). Its low price and its leaner consistency compared to other types of meats, the modern food

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preferences in relation to fat consumption, as well as various ethical and religious barriers have all contributed to the increased consumer preference for poultry meat (de Souza-Vilela et al., 2021). Poultry meat is very rich in high biological value protein and micronutrients, the former reaching as high as 34.5% in the chicken breast (Pereira and Vicente, 2013), making it a nutritious food, particularly low in fat. Moreover, poultry meat is a significant supplier of polyunsaturated fatty acids (PUFA) (Farrell, 2013) and plays an important role in the human diet; however, this also depends on meat processing, such as preservation and cooking (Mancinelli et al., 2021). Moreover, the essential amino acid composition of poultry meat proteins, along with their high digestibility and protein efficiency ratio (PER), offer high nutritive value to human nutrition (Soriano-Santos, 2010).

Due to its importance for human nutrition, there is a strong and continuously growing demand for poultry meat. Currently, soybean meal and fishmeal are the main nutrient sources for poultry feeds, as optimal production requires specific amino acids in quantity and ratio. However, both soybean meal and fishmeal productions are related to serious drawbacks, e.g., tropical deforestation (Song et al., 2021) or overfishing (Shannon and Waller, 2021). To alleviate these issues, the poultry industry is in search of alternative poultry feed nutrient sources (Babatunde et al., 2021; Martins et al., 2021). Among the alternative feeds tested, the exploitation of insects may offer a promising alternative in poultry nutrition in view of their nutritional value, the low environmental footprint for their rearing (e.g., low GHG emission levels), as well as their ability to convert organic secondary flows into high-value commodities (van Huis and Oonincx, 2017). Insects meet the dietary requirements of several livestock animals with regard to nutritional composition (Sogari et al., 2019). It should not be disregarded that, particularly for poultry, insects form a natural food source, playing an important role in their welfare (Moula and Detilleux, 2019). The recent approval of insects as ingredients for poultry and swine diets [Commission Regulation (EU) 2021/1372] has set the basis for further exploitation of insects by the poultry industry in the coming years.

Various insect species have been evaluated as ingredients of poultry feeds, such as the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) (Maurer et al., 2016; Sypniewski et al., 2020; Tahamtani et al., 2021) or the superworm, *Zophobas morio* (F.) (Coleoptera: Tenebrionidae) (Kierończyk et al., 2018; Benzertiha et al., 2019, 2020; Józefiak et al., 2020). For instance, Benzertiha et al. (2020) reported that the inclusion of a small amount of full-fat meal from *Z. morio* in broiler chicken diets improved their growth performance. Similarly, with a partial or total (50% or 100%, respectively) replacement of the widely used soybean oil with *H. illucens* larvae fat, there was no adverse effect reported on growth attributes of young turkey poults (Sypniewski et al., 2020). One insect species that has attracted considerable interest as a poultry feed ingredient is *Tenebrio molitor* (TM) or yellow mealworm, L. (Coleoptera: Tenebrionidae) (De Marco et al., 2015; Bovera et al., 2015, 2016; Loponte et al., 2017; Biasato et al., 2019; Benzertiha et al., 2019, 2020; Elahi et al., 2020). The larvae of TM have been widely assessed in regards to their nutritional quality and functional properties in several studies; their protein, essential amino acid, vitamin and mineral content is higher than that of plants, while they have shown exceptional effects on the growth and digestibility of monogastric animals (De Marco et al., 2015; Kierończyk et al., 2018; Hong et al., 2020). However, despite the considerable number of studies that have evaluated the dietary inclusion of TM larvae in poultry feeds, most of the studies have focused on the use of meals, rather than whole larvae.

In light of the foregoing, the objective of this study was to examine the effect of the supplementation of whole TM larvae in the basal diet of broilers on their growth performance, as well as on several welfare traits and meat quality.

2. Materials and methods

2.1. Ethics and procedures

The experiment was part of the PhD thesis of Stelios Vasilopoulos and was approved by the General Assembly of the School of Veterinary Medicine, Faculty of Health Science, Aristotle University of Thessaloniki (928/17-1-2020). Husbandry, euthanasia and experimental procedures were conducted in research facilities and biosecurity precautions were taken, in line with the Greek legislative framework related to experimental animals. The local Public Veterinary Authorities have approved these procedures (Reg. 489181 (3254)/07.02.2018).

2.2. Animals, diet composition and experimental design

For the needs of the experiment, PINDOS APSI hatchery kindly donated a total of 120 one-day-old male chicks (Ross 308, 2019). These were randomly allocated into 3 groups of 10 birds each with 4 replicates. The allocated groups were housed in different pens provided with infrared lamps as a source of heat. The trial took place in the appropriately designed experimental premises of the Hellenic Agricultural Organisation-DEMETER, Research Institute of Animal Science, Giannitsa, Greece, in September and October 2020 (latitude 40.45°, longitude 22.27°). During the trial, the temperature, relative humidity and lighting were controlled according to Aviagen breeding company's recommendations. A veterinary surgeon monitored the birds' health twice daily. During the 1st day in the hatchery, spray vaccination was used to protect the birds against Newcastle Disease (ND) while subcutaneous vaccination was used against Infectious Bursal Disease (IBD). The control feed did not contain any antimicrobial or anticoccidial agent.

The 2 experimental treatments were prepared by replacing 5% (TM5) and 10% (TM10) of the basal diet with whole TM larvae (95% and 90% basal diet remained, respectively), which were previously dried. For this, the larvae were placed in thin layers on baking plates, defrosted at room temperature and dried in a static rack oven at 60 °C for 24 h. Maize and soybean meal in mash form mainly comprised the control diet, which was formulated as per the recommendations of the breeding company Aviagen (2019); the detailed composition of the basal and the 2 experimental diets tested are provided in Table 1.

2.3. Determination of total phenolic content (TPC)

The Folin-Ciocalteu method (Singleton et al., 1999) was used for the determination of the total phenolic content of the diets. A 0.01 mL aliquot of the sample was added to 0.79 mL of double distilled water, followed by the addition of 0.05 mL of Folin-Ciocalteu reagent (CHEM-LAB NV, Zedelgem, Belgium). The solution was mixed in a vortex for 10 s with the subsequent addition of 0.15 mL of 20% (wt/vol) aqueous solution of Na_2CO_3 (99.8%, CHEM-LAB NV, Zedelgem, Belgium) after 1 min. The emerging mixture was once again mixed in a vortex and kept under dark conditions for a period of 2 h. Three replicates were conducted for each sample. After the incubation period, the samples' absorbance was measured at 750 nm, with the use of a UV–Vis spectrophotometer (UV-1800, UV/Visible Scanning Spectrophotometer, SHIMADZU, Kyoto, Japan). Gallic acid was used for the creation of the standard curve (gallic acid 98+%, Alfa Aesar, Heysham, United Kingdom) and results were expressed in miligram gallic acid equivalents (GAE) per gram of feed or insect larvae material.

Table 1
Broiler chicken basal diet and experimental diets with inclusion of 5% (TM5) and 10% (TM10) dried *Tenebrio molitor* (TM) larvae (%).

Item	Basal diet			TM5			TM10		
	Starter	Grower	Finisher	Starter	Grower	Finisher	Starter	Grower	Finisher
	d 1–14	d 15–28	d 29–35	d 1–14	d 15–28	d 29–35	d 1–14	d 15–28	d 29–35
Ingredients									
Maize	55.50	60.00	61.00	52.73	57.00	57.95	49.95	54.00	54.90
Soybean meal	35.77	30.70	28.62	33.97	29.16	27.18	32.19	27.62	25.75
Soybean oil	3.50	3.50	4.50	3.33	3.33	4.28	3.15	3.15	4.05
Palm fat	0.00	1.00	1.50	0.00	0.95	1.43	0.00	0.90	1.35
Calcium phosphate	1.46	1.33	1.28	1.38	1.26	1.22	1.31	1.20	1.15
Limestone (calcium carbonate)	1.86	1.68	1.53	1.77	1.60	1.45	1.67	1.50	1.37
Salt	0.28	0.23	0.23	0.27	0.22	0.22	0.25	0.21	0.21
Sodium carbonate	0.21	0.21	0.19	0.20	0.20	0.18	0.19	0.19	0.17
Lysine	0.41	0.40	0.35	0.39	0.38	0.33	0.37	0.36	0.32
Methionine	0.39	0.35	0.31	0.37	0.33	0.29	0.35	0.32	0.28
Threonine	0.22	0.21	0.15	0.21	0.20	0.14	0.20	0.19	0.14
Valine	0.15	0.14	0.09	0.14	0.13	0.09	0.14	0.13	0.08
Vitamin and mineral premix[1]	0.25	0.25	0.25	0.24	0.24	0.24	0.23	0.23	0.23
Tenebrio molitor larvae	0.00	0.00	0.00	5.00	5.00	5.00	10.00	10.00	10.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate analysis (as fed basis)									
Moisture	10.15	10.55	11.14	9.92	10.30	10.86	10.20	10.58	11.14
Protein	22.00	21.00	20.00	23.88	22.93	21.98	26.86	25.91	24.96
Crude fiber	2.85	2.65	2.55	3.48	3.29	3.20	4.26	4.07	3.97
Crude fat	4.84	6.11	6.65	6.28	7.48	8.00	7.96	9.16	9.68
Ash	6.12	5.65	5.58	5.91	5.46	5.40	6.00	5.56	5.49
Calculated analysis (as fed basis)									
Total energy, kcal/kg	4,020.2	4,088.2	4,080.3	4,157.1	4,210.4	4,202.0	4,277.9	4,329.9	4,322.9
Lysine	1.41	1.28	1.15	1.44	1.32	1.19	1.54	1.42	1.29
Methionine + Cystine	1.08	0.99	0.92	1.05	0.96	0.90	1.07	0.98	0.92
Methionine	0.73	0.67	0.62	0.72	0.66	0.61	0.74	0.68	0.63
Threonine	0.98	0.89	0.79	1.01	0.93	0.83	1.10	1.01	0.92
Tryptophan	0.28	0.25	0.24	0.30	0.27	0.26	0.34	0.31	0.30
Valine	1.10	1.02	0.92	1.13	1.06	0.96	1.22	1.14	1.05

[1] Supplying per kilogram feed: 12,000 IU vitamin A, 5,000 IU vitamin D_3, 30 mg vitamin E, 3 mg vitamin K, 3 mg thiamine, 7 mg riboflavin, 6 mg pyridoxine, 0.035 mg vitamin B_{12}, 40 mg niacin, 13 mg pantothenic acid, 1.5 mg folic acid, 0.13 mg biotin, 340 mg choline chloride, 55 mg Zn, 155 mg Mn, 20 mg Fe, 12 mg Cu, 0.2 mg Co, 1 mg I, 0.2 mg Se, and phytase 0.01 g.

2.4. Performance parameters

Individual weighing of birds was performed at the start, before being placed into the pens, and at specific time points: d 10 to evaluate the starter period, d 24 to evaluate the grower period and d 35 to evaluate the finisher period. Feed consumption was calculated within each subgroup after withdrawing the remaining feed, 4 h prior to weighing. Based on the data acquired, the average weight gain (AWG) and the feed conversion ratio (FCR: weight of feed consumed over the lifetime of the poultry divided by the gained weight) were calculated for the starter, grower and finisher periods, accordingly. The AWG was calculated by dividing weight gain of individual birds during the corresponding period. Mortality was monitored daily and recorded for each subgroup.

2.5. Welfare status

Footpad dermatitis was assessed on d 35. Two birds per pen were randomly selected and both their footpads were simultaneously assessed after being brush cleaned. Hock burn presence and severity was assessed with a score system of 0 to 2 (0 representing no evidence of footpad dermatitis; 2 representing severe footpad dermatitis) (Butterworth et al., 2009). Similarly, the condition of the feathers was evaluated on the same day on 2 birds per pen with a scoring system of 1 to 3 (1: clean feathers; 3: very dirty feathers) (Butterworth et al., 2009). Two birds from each pen were also used to evaluate the diarrhoea score on d 35, with a scoring system of 1 to 3 (1: absence of diarrhoea; 3: severe diarrhoea) (Butterworth et al., 2009).

Faecal score was evaluated on the surface of each pen on d 10, 24 and 35; the scoring system used was 1 to 4 (1: firm and well-formed faeces; 4: watery liquid faeces) (Butterworth et al., 2009). On the same days, 2 samples were used for the evaluation of the litter score, derived from pooled samples of 3 locations from every pen; the scoring system used was 1 to 5 (1: dry, crumbly litter; 5: capped or completely wet litter). After the 21st day and on a weekly basis, equal quantities of fresh wheat straw were added to each pen. The reason for this was the potential increase of litter moisture due to water spillage from the bell drinkers.

On the same days, litter and faecal dry matter (DM) as well as litter NH_3 were analysed using the following process. Five litter samples of 100 g each were collected from every pen (4 samples were taken from the corners and 1 was taken from the centre of the pen). Before the analyses the samples underwent pooled testing twice after homogenisation (all 5 location samples were mixed together and 2 values of litter DM per pen). For the determination of litter DM, the samples were initially weighed with the use of precision scales, then dried at a temperature of 120 °C for 6 h and finally weighed once more to measure the difference in weight (Clesceri et al., 1989). For the assessment of the litter and faecal scores, a minimum of 5 subsamples of litter and a minimum of 3 subsamples of faeces were collected in order to reach a final sample of 10 g of litter and 50 g of faeces. For the determination of litter NH_3, fresh litter samples were collected from every pen. Lastly, 2

birds per replicate were picked after slaughter to measure the spleen, bursa and thymus size.

2.6. Carcass characteristics, breast and thigh meat composition

The chemical composition of the meat was analysed for 2 birds per replicate. The process involved individually marking the birds with leg bands and transporting them to a commercial slaughterhouse for processing, according to normal practices. Carcass scalding was performed at 61 to 65 °C for a period of 60 s and defeathering was done for 25 s in a rotary drum picker. The carcasses (head, feet but no intestines) underwent air chilling at 4 °C and were weighed 24 h after slaughter. Breasts and legs of each carcass were initially removed and wooden breast and white striping scores were assessed with a scoring system of 0 to 2 (for wooden breast 0: good; 2: severe; for white striping 0: normal and without any white lines; 2: severe exhibition of white lines of >1 mm thickness and parallel to muscle fibers). Evaluation of both the above scores took place on the same birds. Similarly, breast and thigh meat derived from the same birds was carefully deskinned and deboned, ground through an industrial meat grinder and 200 g samples of minced meat were analysed for their crude protein, fat and moisture content. The analysis was done with the use of infrared spectroscopy, using a DA 7250 (PERTEN, Sweden) in transmittance mode; the reference method used was 2007.04, relevant to meat and meat products (Anderson, 2007).

2.7. Meat lipid and protein oxidation

Lipid oxidation of the broiler meat samples was evaluated through the determination of thiobarbituric acid-reactive substances (TBARS), according to the method of Ahn et al. (2004) with minor alterations. In brief, 5 g of breast and thigh meat subsamples from refrigerated carcasses (4 °C) that were kept for 1 and 3 d were collected and homogenised in an Ultra-Turrax T25 (Janke & Kunkel, IKA Labortechnik, Staufen, Germany) in 15 mL of distilled water. Next, 5 mL aliquots of the above homogenates were mixed with 50 μL of butylated hydroxyanisol (7.2%) in test tubes. Next, 5 mL of TBA (20 mM trichloroacetic acid solution in 15% trichloroacetic acid) was added to the test tubes. The final samples were mixed in a vortex and incubated in a water bath at 100 °C for 15 min. After cooling followed centrifugation of the samples at 1000 × *g* at 4 °C for 15 min. Each organic supernatant's absorbance was determined in a spectrophotometer (UV 1700 PharmaSpec, Shimadzu, Japan) for absorbance at 532 nm. Lipid oxidation was determined through the 2-thiobarbituric acid-reactive substance (TBARS) value, expressed as nanograms of malondialdehyde per gram of meat.

Furthermore, protein carbonyls were measured in samples from the same birds with the Patsoukis et al. (2004) method. In particular, a quantity of 50 μL of trichloroacetic acid (TCA) solution 20% was added to a 50 μL homogenated sample solution (diluted 1:2, vol/vol); the mixture was incubated in an ice bath and then centrifuged at 15,000 × *g* at 4 °C for 5 min. After discarding the supernatant liquid, the remaining pellet was mixed with 2,4-dinitrophenylhydrazine (DNPH) and incubated at room temperature under darkness for 1 h; then the mix was centrifuged as before. The supernatant liquid was again discarded, followed by the addition of 1 mL of 10% TCA; the mixture was again mixed in a vortex and centrifuged, as before. Once more, the supernatant liquid was discarded and a quantity of 1 mL of ethanol-ethyl acetate (1:1, vol/vol) was added, mixed in a vortex and centrifuged at 15,000 × *g* at 4 °C for 2 to 3 min. After the phase separation, the supernatant liquid was discarded and followed by the addition of 1 mL of urea solution (5 mol/L) with a pH of 2.3; the new mixture then was mixed in a vortex and incubated at 37 °C for 15 min. The final samples were centrifuged at 15,000 × *g* at 4 °C for 3 min. The analyte of the assay was carbonyl formation and it was detected by the reaction of protein carbonyls with 2,4-dinitrophenylhydrazine (DNPH), which subsequently converts to 2,4-dinitrophenylhydrazone (DNP-hydrazone); the latter is measured at 375 nm. According to the method, the calculation of the concentration of protein carbonyls was based on the molar extinction coefficient of DNPH (22×10^3 mol^{-1} cm^{-1}).

2.8. Determination of meat fatty acids

For the determination of fatty acids, samples from the same birds per subgroup were used. Aliquots of these samples underwent freeze-drying for 48 h through a HyperCOOL HC8080 freeze-dryer (Gyrozen Co., LTD, Gimbo, Korea) (−95 °C, 0.1 mbar, 1 bar = 0.1 MPa). A household blender was used to grind the freeze-dried samples and they were placed into a freezer until further analysis. The Folch method (Folch et al., 1957) was used to extract the total lipids from the samples. The procedure involved the mixing of 2 g of ground sample with 40 mL chloroform-methanol (2:1, vol/vol) solution (ChemLab, Zedelgem, Belgium), followed by homogenisation with the aid of UltraTurrax (IKA, Staufen, Germany) at 11,000 rpm for 3 min. The temperature of the samples remained stable at around 15 °C in an ice bath and the extraction was twice repeated. The extracts were filtered and phases were separated with the addition of water. After removing the upper phase, the lower chloroform phase was collected and dehydrated with anhydrous Na_2SO_4, followed by drying in a rotary evaporator. In order for the samples to undergo chromatographic analysis, transesterification was carried out. In detail, a quantity of 0.1 g of extracted lipids was weighed in a test tube, followed by the addition of 2 mL of n-hexane (ChemLab, Zedelgem, Belgium) and of 0.2 mL of a 2 mol/L methanolic solution of potassium hydroxide for the preparation of fatty acid methyl esters (FAMEs). The mixture was left to settle after being mixed in a vortex for 1 min, until a transparent upper phase containing the FAMEs became evident. This phase was collected and filtered through 0.45 μm PTFE hydrophobic filters, and then analysed by a gas chromatography apparatus (TRACE GC 2000 Series, ThermoQuest CE Instruments, Wigan, UK), equipped with a flame ionisation detector (FID) and an autosampler (TRIPLUS AS ThermoQuest CE Instruments, Wigan, UK). The column used was a BPX70 GC (30 m length, 0.32 mm i.d., 0.25 μm film thickness, SGE Analytical Science, Melbourne, Australia). The carrier gas was helium at a flow rate of 2.0 mL/min and the injector port and detector temperature were maintained at 250 °C. Split ratio was set to 1:20 and the total run time was 60 min. The oven temperature was initially set at 46 °C for 2 min and then increased to 130 °C at a stable rate of 50 °C/min for 10 min; then it was raised to 175 °C at a rate of 2 °C/min and maintained at that temperature for 2 min; then it was increased to 200 °C at a rate of 3 °C/min and maintained at that temperature for 3.5 min, before rising to a plateau of 240 °C at a rate of 10 °C/min for 5 min. FAME identification was based on the comparison of the sample retention times with those of a 37 FAME standard (AccuStandard, New Haven, USA), which was analysed under the same chromatographic conditions. The software used for the acquisition and processing of the chromatograms was ChromQuest 5.0 (ver. 3.2.1, Thermo Separation Products, Waltham, USA).

Apart from the FAMEs, other parameters that are useful for assessing the nutritional value of the fatty acid profile were also determined. These included the sum of saturated fatty acids (∑SFA), monounsaturated fatty acids (∑MUFA) and polyunsaturated fatty acids (∑PUFA); the n3 and n6 fatty acids (∑n3 and ∑n6, respectively); the PUFA to SFA, the n3 to n6 and the hypocholesterolaemic to hypercholesterolaemic (H:H) ratios. The latter was determined with the use of the equation: H:H

ratio = ∑C18:1 cis-9, C18 n-6, C20:4 n-6, C18:3 n-3, C20:3 n-6, C20:5 n-3, C22:6/∑C14:0, C16:0.

2.9. Meat colour evaluation

A Konica Minolta CR-400/410 colorimeter (Kyoto, Japan) was used for the determination of the CIE L* (lightness), a* (redness), b* (yellowness) colour parameters of breast and thigh meat. Measurements were repeated 10 times at different points in every sample and the conditions remained unchanged during measurements. Results were expressed as the average of 10 values ± standard deviation (SD).

2.10. Statistical analysis

Data were collected according to a randomised complete block design, with pens used as the experimental units. The minimum total sample size required was defined prior to the trial initiation and was based on the "Power analysis for one-way ANOVA" methodology (Charan and Kantharia, 2013; IBM, 2021), with the G*Power 3.1.9.2 software (Faul et al. University of Kiel, Germany) and power set at ≥ 0.80. Variance analysis of the trial data (ANOVA) was done with the aid of the SPSS v27.0.1.0 statistical package (SPSS Inc./IBM Corp., Armonk, New York, USA). The level of statistical significance (*P*-value) was set at 0.05 and post-hoc comparisons were performed with the aid of Tukey's tests.

3. Results

3.1. Total phenolic content

The control feed presented a total phenolic content value of 0.51 mg gallic acid equivalents per gram of dry matter. TM5 and TM10 groups presented values of 17.6 and 20.4 mg gallic acid equivalents per gram diet, respectively for the 2 different inclusion levels of insect larvae.

3.2. Performance parameters

No mortality occurred in any of the monitored groups across the starter, grower or finisher period. Growth parameters evaluated, such as body weight gain, feed intake and FCR values calculated for the different dietary treatments tested are presented in Table 2. The 10% TM larvae addition showed a positive effect on the birds' live body weight (LBW) in comparison with the other 2 groups during the starter period, whereas during the finisher period the TM10 group was significantly higher only compared to the TM5 group. Feed intake (FI) was significantly higher in both the TM10 and the control groups (3,823 and 3,808 g, respectively) compared to the TM5 group (3,613 g) during the whole trial period. Consequently, body weight gain (BWG) was significantly higher in the TM10 group for the whole experimental period, while the TM5 exhibited the lowest performance among treatments; higher BWG was also evident during the starter period for the TM10 group, while the control group presented the lowest BWG. With regards to FCR, the experiment showed no significant differences among the different dietary treatments during the total trial period.

The consumption rate was assessed at 240, 370 and 210 g/h for the control, TM5 and TM10 groups, respectively during the first hour, and 190, 250 and 185 g/h, respectively for the first 2 h period of the 34th day.

Table 2
Effect of dietary replacement with dried *Tenebrio molitor* (TM) larvae on broiler chicken performance.

Item	Control [1]	TM5 [1]	TM10 [1]	SEM	*P*-value
Live body weight, g					
d 1	46.83	45.90	46.08	0.35	0.572
d 10	362.95	364.23	387.98	5.10	0.061
d 24	1,399.55	1,346.73	1,437.58	18.53	0.127
d 35	2,262.25 [ab]	2,208.25[b]	2,346.50[a]	22.58	0.021
Feed intake per chicken, g					
d 1–10	315.78	318.80	320.90	1.48	0.402
d 11–24	1,666.58	1,592.90	1,661.75	37.27	0.707
d 25–35	1,826.13	1,701.10	1,840.50	41.35	0.350
d 1–35	3,808.43[a]	3,612.80[b]	3,823.13[a]	36.24	0.011
Body weight gain, g					
d 1–10	316.12	318.32	341.90	5.33	0.076
d 11–24	1,036.60	982.50	1,049.60	17.67	0.281
d 25–35	862.70	861.53	908.93	12.62	0.231
d 1–35	2,215.42 [ab]	2,162.35[b]	2,300.43[a]	22.55	0.021
Feed conversion ratio, g feed/g weight gain					
d 1–10	1.000	1.007	0.939	0.018	0.244
d 11–24	1.615	1.624	1.585	0.041	0.933
d 25–35	2.115	1.979	2.023	0.038	0.349
d 1–35	1.720	1.672	1.662	0.017	0.359

[a,b] Values in the same row with no common superscript differ significantly ($P < 0.05$).
[1] Control: basal diet; TM5, TM10 diets provided with inclusion levels of dried whole *T. molitor* larvae at 5% and 10%, respectively.

3.3. Welfare status

Litter score presented no significant differences among the different treatments throughout the experimental period (Table 3). Litter moisture, however, was significantly higher for the control and the TM10 groups on d 10 compared to the TM5 group ($P < 0.05$). No significant differences were recorded among treatments on d 24 and d 35.

Pododermatitis, diarrhoea and feather scores are presented in Table 4. Pododermatitis at d 35 was significantly higher for the TM10 group, compared to the TM5 group ($P < 0.05$). Diarrhoea score showed no significant differences throughout the entire trial period.

Regarding feather score, no significant differences were found among treatments during the entire trial period, however, the 2 groups fed with insect larvae showed slightly better scores than the control group, as shown in Table 4.

Significantly higher faecal litter moisture was found in the TM10 group on d 24 in comparison with the other 2 treatments (24.28 compared to 21.54 and 22.37 for the control and TM5, respectively).

Table 3
Effect of dietary supplementation with dried *Tenebrio molitor* (TM) larvae on litter score and litter moisture.

Item	Control[1]	TM5[1]	TM10 [1]	SEM	*P*-value
Litter score[2]					
d 10	1.60	1.50	1.60	0.12	0.935
d 24	1.20	1.20	1.20	0.07	1.000
d 35	1.10	1.20	1.10	0.06	0.769
Litter moisture					
d 10	35.62[a]	32.29[b]	35.25[a]	0.61	0.044
d 24	26.37	23.91	25.19	0.49	0.122
d 35	27.34	23.39	24.35	0.72	0.059

[a,b] Values in the same row with no common superscript differ significantly ($P < 0.05$).
[1] Control: basal diet; TM5, TM10 diets provided with inclusion levels of dried whole *T. molitor* larvae at 5% and 10%, respectively.
[2] Litter score: 5-point scoring system ranging from 1 to 5 (1 illustrates dry and crumbly litter and 5 illustrates capped or completely wet litter).

Table 4
Effect of dietary replacement with *Tenebrio molitor* (TM) larvae on broilers' pododermatitis, diarrhoea and feather scores.

Item	Control [1]	TM5 [1]	TM10 [1]	SEM	*P*-value
PD [2] score					
d 35	0.80 [ab]	0.30[b]	1.20[a]	0.124	0.007
Diarrhoea score [3]					
d 35	1.70	1.70	1.50	0.131	0.785
Feather score [4]					
d 35	2.75	2.88	2.88	0.38	0.767

[a,b] Values in the same row with no common superscript differ significantly ($P < 0.05$).
[1] Control: basal diet; TM5, TM10 diets provided with inclusion levels of dried whole *T. molitor* larvae at 5% and 10%, respectively.
[2] PD: pododermatitis (footpad dermatitis).
[3] Diarrhoea score: 3-point scoring system ranging from 1 to 3 (1 indicates absence of diarrhoea and 3 indicates severe diarrhoea).
[4] Feather score: 3-point scoring system ranging from 1 to 3 (1 refers to clean feathers and 3 refers to very dirty feathers).

Table 5
Effect of dietary replacement with *Tenebrio molitor* (TM) larvae on faecal litter moisture and litter NH_3 concentration.

Item	Control [1]	TM5 [1]	TM10 [1]	SEM	*P*-value
Faecal litter moisture					
d 10	23.46	22.67	22.98	0.208	0.305
d 24	21.54[b]	22.37[b]	24.28[a]	0.349	0.002
d 35	21.22	20.72	20.83	0.297	0.782
Litter NH_3 concentration					
d 10	1.21	1.19	1.20	0.010	0.804
d 24	1.16	1.13	1.17	0.007	0.052
d 35	1.24[a]	1.15[b]	1.24[a]	0.012	<0.001

[a,b] Values in the same row with no common superscript differ significantly ($P < 0.05$).
[1] Control: basal diet; TM5, TM10 diets provided with inclusion levels of dried whole *T. molitor* larvae at 5% and 10%, respectively.

Nevertheless, no other significant differences were noted for the remaining evaluation points. Additionally, litter NH_3 was found significantly higher in the control and TM10 groups in comparison with the TM5 group on d 35 ($P < 0.05$). The results are shown in Table 5.

Table 6 shows the results for the main lymphoid organs. No differences were evident for thymus or bursa weight, contrary to spleen weight, which was significantly higher for the 2 groups fed the insect larvae-based diets ($P < 0.05$).

Carcass yield after slaughter presented a significant increase in the TM10 group in comparison with the other 2 groups ($P < 0.05$). As far as wooden breast and white striping scores were concerned, the 3 groups presented no significant differences (Table 7).

Table 6
Effect of dietary replacement with dried *Tenebrio molitor* (TM) larvae on broiler lymphoid organs' weight.

Lymphoid organs	Control [1]	TM5 [1]	TM10 [1]	SEM	*P*-value
Thymus	1.43	1.58	1.38	0.044	0.147
Bursa	2.32	2.31	2.22	0.038	0.494
Spleen	1.84[b]	2.51[a]	2.48[a]	0.083	<0.001

[a,b] Values in the same row with no common superscript differ significantly ($P < 0.05$).
[1] Control: basal diet; TM5, TM10 diets provided with inclusion levels of dried whole *T. molitor* larvae at 5% and 10%, respectively.

Table 7
Effect of dietary supplementation with *Tenebrio molitor* (TM) larvae on broiler chicken carcass yield, wooden breast score and white striping score.

Item	Control [1]	TM5 [1]	TM10 [1]	SEM	*P*-value
Carcass yield, g					
d 35	1,778.11[b]	1,739.42[b]	1,937.58[a]	27.696	<0.001
Wooden breast score [2]					
d 35	0.583	0.333	0.333	0.073	0.291
White striping score [2]					
d 35	0.667	0.584	0.667	0.064	0.857

[a,b] Values in the same row with no common superscript differ significantly ($P < 0.05$).
[1] Control: basal diet; TM5, TM10 diets provided with inclusion levels of dried whole *T. molitor* larvae at 5% and 10%, respectively.
[2] Wooden breast and white striping scores, using categories from 0 to 2 (for wooden breast: 0 represents good and 2 represents severe; for white striping: 0 represents normal without any distinctive white lines and 2 represents severe, exhibiting white lines in parallel to muscle fibres, that were more than 1 mm thick).

3.4. Breast and thigh meat composition and oxidative status

The meat moisture content was found significantly higher in both breast and thigh meat of the TM5 group compared to the other 2 groups; additionally, the protein content was significantly increased in the thigh samples of both treated groups compared to the control. A significant reduction was also evident in the fat content of the thigh meat of both treated groups compared to the control (Table 8).

Determination of TBARS was performed in meat samples kept under refrigeration. On the first day of refrigeration, TBARS were significantly higher in the control group in relation to the treated groups, followed by the TM10 group and then the TM5 one, in breast and thigh meat samples alike. On d 3 however, breast samples exhibited significantly higher values in the TM10 group and lower in the control, whereas in thigh samples, the TM5 group presented significantly higher TBARS values in comparison with the other 2 groups (Table 8).

Table 8
Effect of dietary replacement with *Tenebrio molitor* (TM) larvae on broiler chicken breast and thigh meat composition, TBARS[1] values and protein carbonyls.

Item	Control [2]	TM5 [2]	TM10 [2]	SEM	*P*-value
Breast meat					
Moisture, %	73.51[b]	77.54[a]	73.98[b]	0.56	<0.001
Protein, %	23.90	24.49	24.24	0.17	0.391
Fat, %	1.68	1.24	1.29	0.10	0.147
Thigh meat					
Moisture, %	74.75[b]	76.88[a]	74.92[b]	0.309	<0.001
Protein, %	20.33[b]	21.60[a]	21.30[a]	0.176	<0.001
Fat, %	4.22[a]	2.67[b]	2.82[b]	0.223	<0.001
Breast meat TBARS, ng malondialdehyde/g meat					
d 1	0.065[a]	0.045[c]	0.050[b]	0.002	<0.001
d 3	0.030[b]	0.035[b]	0.050[a]	0.003	<0.001
Thigh meat TBARS, ng malondialdehyde/g meat					
d 1	0.090[a]	0.059[c]	0.073[b]	0.004	<0.001
d 3	0.026[b]	0.043[a]	0.024[b]	0.003	<0.001
Breast meat protein carbonyls, nmol carbonyl/mg protein	0.420[b]	0.149[c]	0.590[a]	0.040	<0.001
Thigh meat protein carbonyls, nmol carbonyl/mg protein	0.275[b]	0.213[b]	0.740[a]	0.057	<0.001

[a,b,c] Values in the same row with no common superscript differ significantly ($P < 0.05$).
[1] TBARS: thiobarbituric acid reactive substances.
[2] Control: basal diet; TM5, TM10 diets provided with inclusion levels of dried whole *T. molitor* larvae at 5% and 10%, respectively.

Dietary supplementation with insect larvae-based diets affected the protein carbonyls of both breast and thigh meat. The TM10 group displayed significantly higher values in both cases ($P < 0.05$), followed by the control group, while the TM5 group exhibited the lowest values, showing a significant decrease in the breast, as compared with the TM10 group (Table 8).

3.5. Fatty acid composition

The effect of the dietary replacement on the fatty acid composition of the breast and thigh meat of broilers are presented in Tables 9 and 10, respectively. Partial replacement of the broilers' basal diet with TM larvae resulted in a significant reduction of the total SFA in both meat types. This was mainly due to the decrease in the palmitic and stearic acids. Regarding MUFA, both treatments resulted in an increase in the breast meat, whereas only the TM10 treatment showed a positive effect on the total MUFA in the thigh meat. Specifically, the oleic acid was increased in both treatments, this being more evident in the thigh meat, while palmitoleic increased in breast meat samples but decreased in thigh meat ones. As far as PUFA are concerned, treatments showed a reduction in the breast and an increase in the thigh meat. Linoleic acid presented a significant increase in both meat types and especially with the TM10 treatment, while g-linolenic was significantly reduced with the TM5 treatment in both meat types; it was however increased with the TM10 treatment in the breast meat. Linolenic and cis-11,14-eicosadienoic acids remained unchanged; moreover, arachidonic acid was reduced whereas cis-4,7,10,13,16,19-docosahexaenoic showed a reduction in the breast meat and an increase in the thigh meat after altering the diets.

As for the total n-3 fatty acids, the TM5 treatment had a significant increase in the thigh meat but showed no significant effect on breast meat; the TM10 treatment, however, presented a significant decrease in the n-3 fatty acids in the breast and a significant increase in the thigh meat. Similarly, a significant reduction of the total n-6 fatty acids in breast meat and a significant increase in the thigh meat were observed after both treatments in comparison with the control group. As far as the n-6 to n-3 ratio was concerned, it was significantly decreased in the breast meat after 5% replacement of the basal diet with TM larvae and in the thigh meat after 10% replacement; the latter did not have an effect on the ratio in the breast meat, while the TM5 replacement significantly increased the ratio in the thigh meat.

Altogether, the PUFA to SFA ratio remained unchanged in the breast meat after both treatments. On the contrary, both treatments caused a significant increase of this ratio in the thigh meat, which was particularly evident after the TM10 treatment. Finally, the hypocholesterolaemic to hypercholesterolaemic ratio remained unchanged after part-replacement of broilers' diets with 5% TM larvae. However, the TM10 treatment showed opposite results; contrary to the breast meat where the hypocholesterolaemic to

Table 9
Effect on fatty acid composition (g/100 g of total fatty acids) of broilers' breast meat fed with *Tenebrio molitor* (TM) larvae.

Fatty acid	Control [1,2,3]	TM5 [1,2,3]	TM10 [1,2,3]
Myristic (C14:0)	0.45 ± 0.05^{c}	1.06 ± 0.02^{a}	0.59 ± 0.03^{b}
Palmitic (C16:0)	22.28 ± 0.15^{a}	21.54 ± 0.06^{b}	21.62 ± 0.24^{b}
Palmitoleic (C16:1 cis)	0.38 ± 0.02^{c}	1.55 ± 0.01^{b}	2.19 ± 0.02^{a}
Heptadecanoic (C17:0)	0.17 ± 0.03^{b}	0.29 ± 0.03^{a}	0.23 ± 0.03^{ab}
Stearic (C18:0)	11.84 ± 0.01^{a}	10.20 ± 0.05^{b}	9.96 ± 0.76^{c}
Oleic (C18:1 cis ω9)	26.36 ± 0.02^{b}	27.08 ± 0.11^{a}	26.29 ± 0.23^{b}
Linoleic (C18:2 cis ω6)	25.93 ± 0.03^{a}	26.09 ± 0.10^{b}	27.63 ± 0.32^{a}
Arachidic (C20:0)	0.11 ± 0.01^{a}	0.16 ± 0.05^{b}	0.15 ± 0.02^{a}
g-Linolenic (C18:3 cis ω6)	1.44 ± 0.03^{a}	1.08 ± 0.02^{b}	1.79 ± 0.05^{a}
Linolenic (C18:3 cis ω3)	0.35 ± 0.02	0.32 ± 0.03	0.29 ± 0.09
Heneicosanoic (C21:0)	1.02 ± 0.01^{b}	1.19 ± 0.01^{a}	1.09 ± 0.05^{b}
cis-11,14-Eicosadienoic (C20:2 cis ω6)	0.17 ± 0.02	0.15 ± 0.03	0.16 ± 0.03
Behenic (C22:0)	0.92 ± 0.03^{b}	0.88 ± 0.05^{b}	1.07 ± 0.06^{a}
cis-8,11,14-Eicosatrienoate (C20:3 cis ω6)	6.26 ± 0.09^{a}	5.80 ± 0.13^{b}	4.75 ± 0.04^{c}
Erucic (C22:1 cis ω9)	0.19 ± 0.03	0.17 ± 0.03	0.16 ± 0.03
Arachidonic (C20:4 cis ω6)	0.26 ± 0.01^{a}	0.15 ± 0.01^{b}	0.19 ± 0.03^{b}
Nervonic (C24:1 cis ω9)	1.01 ± 0.02^{a}	0.73 ± 0.05^{b}	0.79 ± 0.03^{b}
cis-4,7,10,13,16,19-Docosahexaenoic (C22:6 cis ω3)	0.64 ± 0.10^{a}	0.67 ± 0.09^{a}	0.58 ± 0.02^{b}
∑SFA [4]	37.00 ± 0.14^{a}	35.67 ± 0.06^{b}	34.88 ± 0.61^{c}
∑MUFA [5]	27.94 ± 0.15^{c}	29.82 ± 0.09^{b}	29.58 ± 0.25^{a}
∑PUFA [6]	35.05 ± 0.05^{a}	34.51 ± 0.10^{b}	35.54 ± 0.39^{a}
∑n3 [7]	0.99 ± 0.02^{a}	0.99 ± 0.07^{a}	0.88 ± 0.11^{b}
∑n6 [8]	8.13 ± 0.03^{a}	7.43 ± 0.09^{b}	7.04 ± 0.09^{c}
PUFA to SFA ratio	0.95 ± 0.07	0.97 ± 0.11	1.02 ± 0.03
n-6 to n-3 ratio	8.19 ± 0.17^{a}	7.51 ± 0.05^{b}	8.03 ± 0.05^{a}
Hypocholesterolemic to hypercholesterolemic ratio [9]	2.40 ± 0.05^{a}	2.45 ± 0.03^{a}	1.62 ± 0.04^{b}

[1] Control: basal diet; TM5, TM10 diets provided with inclusion levels of dried whole *T. molitor* larvae at 5% and 10%, respectively.
[2] Each value is the mean of triplicate determinations ± SD.
[3] Different superscripts (a, b, c) within the same row indicate significant differences according to Tukey's post-hoc test ($P < 0.05$).
[4] Saturated fatty acids.
[5] Monounsaturated fatty acids.
[6] Polyunsaturated fatty acids.
[7] Omega-3 fatty acids.
[8] Omega-6 fatty acids.
[9] Hypocholesterolemic to hypercholesterolemic fatty acid ratio = ∑C18:1cis-9, C18 n-6, C20:4 n-6, C18:3 n-3, C20:3 n-6, C20:5 n-3, C22:6/∑C14:0, C16:0.

Table 10
Effect on fatty acid composition (g/100 g of total fatty acids) of broilers' thigh meat fed with *Tenebrio molitor* (TM) larvae.

Fatty acid	Control [1,2,3]	TM5 [1,2,3]	TM10 [1,2,3]
Myristic (C14:0)	0.54 ± 0.02[b]	1.06 ± 0.03[a]	0.72 ± 0.10[b]
Myristoleic acid (C14:1)	0.13 ± 0.05	0.09 ± 0.01	0.11 ± 0.02
Palmitic (C16:0)	22.00 ± 0.38[a]	20.92 ± 0.13[b]	20.07 ± 0.15[b]
Palmitoleic (C16:1 cis)	4.31 ± 0.06[a]	2.68 ± 0.03[c]	3.22 ± 0.45[b]
Heptadecanoic (C17:0)	0.16 ± 0.03[b]	0.27 ± 0.05[ab]	0.22 ± 0.01[b]
Stearic (C18:0)	11.76 ± 0.91[a]	8.49 ± 0.44[b]	4.50 ± 0.23[c]
Oleic (C18:1 cis ω9)	30.37 ± 0.42[c]	31.60 ± 0.09[b]	32.49 ± 1.05[a]
Linoleic (C18:2 cis ω6)	25.85 ± 0.51[c]	27.79 ± 0.13[b]	32.31 ± 0.58[a]
Arachidic (C20:0)	0.19 ± 0.01	0.19 ± 0.02	0.21 ± 0.03
g-Linolenic (C18:3 cis ω6)	2.12 ± 0.04[a]	1.54 ± 0.03[b]	2.22 ± 0.04[a]
Linolenic (C18:3 cis ω3)	0.37 ± 0.04	0.29 ± 0.01	0.33 ± 0.02
Heneicosanoic (C21:0)	0.36 ± 0.03[b]	0.53 ± 0.03[a]	0.44 ± 0.01[b]
Behenic (C22:0)	0.26 ± 0.07[b]	0.42 ± 0.03[a]	0.33 ± 0.02[b]
cis-8,11,14-Eicosatrienoate (C20:3 cis ω6)	1.09 ± 0.05[b]	2.70 ± 0.11[a]	0.10 ± 0.02[c]
Nervonic (C24:1 cis ω9)	0.21 ± 0.05[b]	0.38 ± 0.07[a]	0.30 ± 0.03[a]
cis-4,7,10,13,16,19-Docosahexaenoic (C22:6 cis ω3)	0.11 ± 0.05[b]	0.23 ± 0.02[a]	0.21 ± 0.03[a]
∑SFA[4]	35.28 ± 0.64[a]	32.21 ± 0.15[a]	26.61 ± 3.17[b]
∑MUFA[5]	35.02 ± 0.39[b]	34.94 ± 0.14[c]	36.23 ± 1.76[a]
∑PUFA[6]	29.70 ± 0.55[c]	32.84 ± 0.11[b]	37.16 ± 1.41[a]
∑n3 [7]	0.48 ± 0.04[c]	0.52 ± 0.05[b]	0.61 ± 0.05[ab]
∑n6 [8]	3.36 ± 0.07[b]	4.53 ± 0.09[a]	4.24 ± 0.05[a]
PUFA to SFA ratio	0.84 ± 0.05[b]	1.02 ± 0.03[a]	1.42 ± 0.24[ab]
n6 to n3 ratio	7.05 ± 0.59[a]	8.78 ± 0.29[a]	6.96 ± 0.57[b]
Hypocholesterolemic to hypercholesterolemic ratio [9]	2.60 ± 0.05[b]	2.79 ± 0.05[b]	3.31 ± 0.69[a]

[1] Control: basal diet; TM5, TM10 diets provided with inclusion levels of dried whole *T. molitor* larvae at 5% and 10%, respectively.
[2] Each value is the mean of triplicate determinations ± SD.
[3] Different superscripts (a, b, c) within the same row indicate significant differences according to Tukey's post-hoc test ($P < 0.05$).
[4] Saturated fatty acids.
[5] Monounsaturated fatty acids.
[6] Polyunsaturated fatty acids.
[7] Omega-3 fatty acids.
[8] Omega-6 fatty acids.
[9] Hypocholesterolemic to hypercholesterolemic fatty acid ratio = (∑C18:1cis-9, C18 n-6, C20:4 n-6, C18:3 n-3, C20:3 n-6, C20:5 n-3, C22:6)/(∑C14:0, C16:0).

hypercholesterolaemic ratio was decreased, the latter presented a significant increase in the thigh meat.

3.6. Meat colour evaluation

Partial replacement of broiler diet with TM larvae affected breast and thigh meat colour parameters (Table 11). Lightness (L*) was significantly higher ($P < 0.05$) in treated samples compared to the control group; the TM5 group presented the highest value in both types of meat. Redness (a*) was significantly higher in the TM10 group for both types of meat, however, the 5% TM larvae inclusion did not increase the redness of the breast meat, as was the case for the thigh meat. Similarly, yellowness (b*) was significantly increased in the TM10 group for both meat types, while the TM5 group significantly increased only the thigh meat yellowness value.

Table 11
Effect of dietary replacement of broilers' diets with whole *Tenebrio molitor* (TM) larvae on breast and thigh meat colour parameters.[1]

Parameter	Control [2]	TM5 [2]	TM10 [2]
Breast colour			
L*	41.46 ± 0.71[c]	50.63 ± 2.50[a]	49.27 ± 0.60[b]
a*	3.55 ± 0.35[b]	3.17 ± 0.37[c]	7.67 ± 0.50[a]
b*	5.81 ± 0.05[b]	4.97 ± 1.69[c]	11.35 ± 0.27[a]
Thigh colour			
L*	40.76 ± 0.19[c]	50.84 ± 1.31[a]	49.27 ± 0.60[b]
a*	3.83 ± 0.06[c]	6.38 ± 1.08[b]	7.67 ± 0.50[a]
b*	5.81 ± 0.04[c]	8.27 ± 1.73[b]	11.35 ± 0.26[a]

[a,b,c] Different superscript letters within the same row indicate statistically significant differences according to Tukey's post-hoc test ($P < 0.05$).
L* stands for lightness, a* for redness and b* for yellowness.
[1] Each value is the mean of 10 values ± SD.
[2] Control: basal diet; TM5, TM10 diets provided with inclusion levels of dried whole *T. molitor* larvae at 5% and 10%, respectively.

4. Discussion

This study aimed to evaluate the impact of TM larvae inclusion as a feed additive in the diet of broiler chickens, with minimal processing. Not surprisingly, there is disproportionately little data regarding the provision of whole larvae to chickens, as compared with the provision of processed insect meals, e.g., as pellets or flour. For instance, De Marco et al. (2015) fed broiler chickens with 2 experimental diets in which the basal diet was partially substituted by TM or *H. illucens* meal. In a similar study with broiler chickens, Pretorius (2011) replaced half of a basal diet based on maize meal with dried housefly larvae meal. Apart from insect meal, several researchers have studied the insect fat inclusion in broiler diets. Indicatively, Kierończyk et al. (2018) partially replaced soybean oil, which is commonly used in broiler chicken nutrition, with fats obtained from TM or *Z. morio* larvae. However, limited results exist on whole larvae inclusion in the diets of broiler chickens. To our knowledge, this research is the first to examine the effect of the partial replacement of a basal diet with whole TM larvae in broiler chicken nutrition. It must be noted though, that the legislative framework for the use of whole dried insect larvae varies among different countries. For instance, in Canada the use of dried whole black soldier fly larvae is approved as chicken feed (Lähteenmäki-Uutela et al., 2017), whereas in USA dried whole black soldier fly larvae are only permitted for use in aquafeeds for salmonids (Lähteenmäki-Uutela et al., 2021). As far as EU legislation is

concerned, insect proteins used for feed must be previously processed, as described in EU Regulation 142/2011.

The results of the present study showed a significantly higher live body weight of the birds in the TM10 group in comparison with the control and the TM5 group in 2 out of 3 evaluation points (d 10 and 35). Variable results have been reported so far with regard to the growth performance of broilers that were fed insect-based diets. The live weight of male broiler chickens increased linearly when the TM meal increased for young birds (d 12 and 25) and took its highest value when TM meal was included at a rate of 15% in the diet (Biasato et al., 2018). In the same study though, the highest live weight at the end of the trial was found in the 5% inclusion level group. In another recent study of Biasato et al. (2018), insect larvae meal has been included at 3 graded levels of 5%, 10% and 15% of isoproteinic and isocaloric diets. Results of this study showed that body weight response is linear between 12 and 25 d and has a maximum quadratic response to dietary TM15 inclusion level. Similarly, a linear response at 10 to 12 d and a quadratic effect between 12 and 25 d was also observed for feed intake. Moreover, FCR showed a linear response between 25 and 53 d, with a maximum 15% TM dietary inclusion.

Improved growth rates compared to the control were also noted for chickens fed with diets in which TM meal replaced almost 30% of the soybean meal (Bovera et al., 2015). However, in other studies TM meal inclusion did not present a significant effect on chicken growth (Ramos-Elorduy et al., 2002; Biasato et al., 2016). For instance, bird growth remained unaffected when TM meal was included at rates of 5% and 10% (Ramos-Elorduy et al., 2002) or 7.5% (Biasato et al., 2016) in fast and intermediate-growing chickens' diets, respectively.

The increase in the body weight of birds fed insect-based diets has been related to increased feed intake, which in turn has been attributed to the higher palatability of these diets (Biasato et al., 2018). In the present study, higher feed intake was calculated for the 10% inclusion rate in comparison to the 5% rate for the entire duration of the trial. Based on our visual observations during feeding, the birds seemed very eager to consume the larvae, picking them first and then consuming the rest of the diet. In a feed-choice test, a preference of broiler quails was evident for diets with *H. illucens* meal (Cullere et al., 2016), indicating that birds are evolutionarily adapted to this natural eating habit (Oddon et al., 2021). Similar studies indicate that 5% substitution is probably too low to make a difference to basic growth and performance indicators, and higher percentages are needed. The increase in the consumption rate is a reliable indicator that the provision of larvae is successful; however, the increase of the substitution percentage does not necessarily correspond to an analogous increase in feed intake or FCR improvement. In our study, it was found that 5% inclusion level of larvae in the diet increased the consumption rate; however, the 10% inclusion increased the feed intake. One possible explanation is that low and medium inclusion of larvae is accompanied with higher palatability of the feed, whereas, high to very high inclusion levels of larvae may lead to a feed-focus disruption, as birds may spend more time on pecking specifically insects. Eventually, chickens may also increase feed intake by spending more time on eating.

The overall bird health was evaluated through the assessment of various welfare characteristics. Addition of whole larvae in the birds' normal diets showed minimal effect on footpad dermatitis (FPD), while litter moisture, litter and diarrhoea scores were unaffected. The most important factor affecting FPD is the litter condition in the pen (Bessei, 2006), which in turn is partly affected by diet composition (Jones et al., 2005). This casts an additional indication that whole TM larvae do not cause any disorders to these characteristics and the results stand in accordance with those of Ramos-Elorduy et al. (2002) and Biasato et al. (2016). Thus, values for litter NH_3 concentration for treated birds were found to be similar to the control group, while faecal litter moisture was negatively affected only during the grower phase. As mentioned above, there are earlier studies that illustrate that the substitution of meals with insects may provide contradicting results in the above characteristics, either positive or negative. Moreover, the inclusion of insect larvae positively affected the carcass yield without affecting the visual meat quality. Lipid peroxidation through TBARS evaluation showed lower values, especially evident in the thigh meat on d 1 and for the highest inclusion level; on the contrary, protein carbonyls were found to be higher in the TM10 group. A possible explanation for the delay in lipid oxidation could be the higher TPC content of the TM supplemented diets, whereas higher protein inclusion of the diet might be associated with higher values of protein oxidation.

Insect larvae can act as a sustainable addition to animal feeds, providing valuable amounts of energy, protein and fat to their diets (de Souza-Vilela et al., 2019). Sogari et al. (2019) refers to their role as novel feed additives, since they contain immune-boosting bioactive components (such as antimicrobial agents, chitin etc.) and can improve animal gut health. It is a source of feed rich in proteins and fats but still requires further technological evolution and research in order to make it affordable in comparison to current proteinaceous commodities. Moreover, insect farming will need to provide evident proof for the avoidance of heavy metals and other toxic substances that may pass through the insects' and consequently the broilers' intestinal path; and also, the effect of any allergies caused to the animals. Interestingly, our results show that the increase of larval containment from 5% to 10% does not necessarily mean a further improvement of all characteristics, at least in the case of the ones where the inclusion of larvae had a positive effect. In fact, there were cases where 5% replacement performed better, compared with 10% replacement, suggesting that there is a "cutline" in this substitution. Hence, prioritisation of the characteristics that need to be improved should be one of the key elements in calculating the percentage of the inclusion of insects in the standard diet.

Insects are an important source of fat and fatty acids. Dietary replacement with TM larvae significantly affected the fatty acid composition of meat in the treated groups. According to Soriano-Santos (2010), the low-fat content of chicken meat, cholesterol, the total PUFA content and particularly the beneficial n-3 PUFA as well as the atherogenic myristic acid (C14:0) contribute to a healthy diet. In this trial, the total amount of SFA was reduced in both treated groups while thigh meat presented significantly increased amounts of PUFA and the PUFA to SFA, n6/n3 and H/H ratios. It seems that thigh meat, which naturally contains a higher amount of fat compared to breast meat, is more prone enrichment with PUFA originating from insect larvae; especially when supplemented at the highest inclusion level. Dietary replacement with TM larvae also displayed a positive impact on both breast and thigh meat fat content (Table 5), whereas myristic acid increased after both treatments. An increased MUFA percentage was observed, which was mainly attributed to the higher content of palmitoleic and oleic acids in breast and thigh meat, respectively, which is in accordance with other trials with HI (Schiavone et al., 2019). Additionally, the role of n-3 and n-6 fatty acids in human nutrition is crucial due to their regulatory role in the cardiovascular system. Both fatty acid families compete with each other for the same enzymes and an increase in one may cause a decrease in the other. Despite the important changes monitored during this trial, the n-6 to n-3 ratio remained largely unaffected, with a significant increase only in the thigh meat of birds fed with TM5. Lastly, the nutritional value of fats can be assessed by the PUFA to SFA and hypocholesterolemic to

hypercholesterolemic ratios, with low values being unfavourable due to a potential cholesterolemia increase. In our study, the PUFA to SFA ratio was slightly increased in the breast meat but significantly higher in the thigh meat of treated birds; on the other hand, the hypocholesterolaemic to hypercholesterolaemic ratio was significantly higher in the thigh meat, but reduced in the breast meat of treated birds compared to the control group. These values may indicate a positive effect on broiler meat but further analysis of fatty compounds would be needed in order to provide more detailed information. It was also noted that the fat content of the treated diets was substantially increased, in proportion to the inclusion level of insect larvae. Moreover, it was evident that both treated groups presented increased PUFA and decreased SFA; these changes however, were more evident in the thigh meat. The above findings of higher fat content and higher unsaturated fatty acid composition should make the treated breast and thigh meat more vulnerable to lipid oxidation, however TM larvae inclusion seems to play a role in delaying it.

Poultry meat colour may be affected by several factors and can be interpreted by consumers in different ways, according to their preferences (Wideman et al., 2016). Colour results of this experiment indicate that L*, a* and b* values were elevated, particularly in the TM10 group; this could be due to a change of the pH of the meat, as mentioned by Pieterse et al. (2013). Increased lightness (L*) can also be linked with the oxidation of myoglobin to metmyoglobin, displaying pale coloration in poultry meat (De Avila Souza et al., 2022); however, since TBARS values were found to be mostly lower in the treated groups, increased L* values are not expected to be associated with oxidative reactions. Even so, L* values of samples were within the optimal lightness range, that is between 49 and 50 (Garcia et al., 2010) for both types of meat and both treatments. Additionally, the a* to b* ratio was found to be higher in both types of meat for both treatments. This indicates higher a* values (redness) compared to the paler control samples. These results do not agree with a 1% TM diet replacement in slough form by Kim et al. (2014), but are in accordance with broiler trials with a higher feed replacement % by *H. illucens* (HI) larvae (Schiavone et al., 2019). Panel tests for colour, texture and flavour of the meat produced from broilers fed with partly replaced diets with TM larvae would be an important addition to future work.

Several studies have shown the significant potential of TM larvae in partially replacing current protein sources in animal feeds. Their high nutritional value, especially for monogastric animals, and the low strain on the environment for breeding them has piqued the poultry industry's interest in finding optimal inclusion levels in animal diets. In this study, the partial replacement of a basal diet with TM larvae had a positive impact on bird growth, without affecting the welfare traits examined. In this regard, the inclusion of whole TM larvae in standard diets for poultry could be further implemented to alleviate cost shortcomings in insect-based meals at industrial scale, where insect meals are still too expensive to be utilised in large scale poultry production protocols. However, several challenges still need to be addressed before TM can be used as a partial replacement of current feeds in poultry, including consumers' acceptance of meat products fed with insects in the western world and product palatability, while satisfying the requirements of the relevant legislation (EU Regulations 142/2011, 68/2013 and 2015/2283).

5. Conclusions

Our findings showed that dietary replacement of broiler diets with whole TM larvae at 5% or 10% inclusion can positively influence broiler growth without negatively affecting the welfare traits of the birds or the quality characteristics of the produced meat. The evaluation of whole TM larvae under the light of either feed additive (inclusion up to 5%) or feed raw material (inclusion 10%) warrants further research exploration.

Author contributions

S. Vasilopoulos: Writing - Original Draft, Formal analysis, Visualization, Resources. **I. Giannenas:** Conceptualization, Methodology, Project administration. **S. Savvidou**: Resources, Data Curation. **E. Bonos**: Formal analysis, Visualization. **C. I. Rumbos**: Writing - Review & Editing. **E. Papadopoulos**: Supervision. **P. Fortomaris**: Supervision. **C. G. Athanassiou**: Writing - Review & Editing, Resources.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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