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Breaking down barriers: live or dehydrated dietary whole black soldier fly larvae supplementation in slow growing chickens preserve meat quality and sensory traits

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ABSTRACT This study investigated the effects of supplementing the diet of a slow-growing autochthonous chicken breed with dehydrated or live Black Soldier Fly Larvae (**BSFL**) on meat quality and sensory attributes. The research, conducted at the University of Turin, Italy, involved 144 male birds distributed in three experimental groups. The control group (**C**) was fed a basal diet in which soybean meal was completely substituted with alternative ingredients. The 2 experimental groups were administered a diet identical to the control group but supplemented with either whole dehydrated black soldier fly larvae (**DL**) or whole live black soldier fly larvae (**LL**) at a level equal to 5% expected daily feed intake of dry matter. We evaluated the following parameters: nutrient intake, slaughtering performance, physical and nutritional meat quality, fatty acid composition, proteomics, and sensory characteristics. The results demonstrated BSFL supplementation to have no detrimental effects on overall meat quality or sensory attributes. Specifically, there were no significant differences in physical meat quality parameters, nutritional composition, lipid oxidation, or protein digestibility between control and BSFL-fed groups. Fatty acid analysis revealed higher concentrations of lauric and myristic acids in BSFL-fed chicken breast ($p < 0.005$), suggesting potential nutritional benefits from the supplement. The proteomic analysis also showed no significant differences in the expression of abundant proteins in the breast meat between groups, indicating minimal physiological impact of BSFL supplementation. Overall, this study provides reassurance to consumers and industries about the suitability of BSFL as a sustainable feed supplement for poultry that also offers potential benefits in terms of optimizing the fatty acid profile of chicken meat.

Key words: alternative farming, poultry, insect, whole larvae

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INTRODUCTION

The importance of quality production in poultry farming cannot be overstated, as it ensures the health and welfare of the animals and provides safe and nutritious food for the population. In this context, preserving the biodiversity of poultry, particularly focusing on slow-growing native breeds, is essential for maintaining agricultural ecosystems and genetic diversity, preserving advantages such as disease resistance and adaptability to local environments (Hoffmann, 2011). Furthermore products from native chicken breeds are integral to social and cultural aspects, preserving local traditions and economies (Castillo et al., 2021; Fiorilla et al., 2023). The Bianca di Saluzzo chicken is a heritage autochthonous slow-growing, dual-purpose breed renowned for its striking appearance and excellent meat qualities. Originating from the Piedmont region in Italy, this breed has an elegant white plumage and the roosters are prized for their meat quality (TuBAvI, 2024). Their inherent suitability for outdoor rearing and resilience to diverse climatic conditions make them an optimal choice for small-scale agricultural farming. It is also a Slow Food presidium and thrives in free-range environments (Slow Food, 2024).

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In the domain of meat quality, the notion of ethical quality encompasses a wide array of factors that go beyond mere taste and texture. It explores the circumstances in which the chickens are reared, placing emphasis on compassionate treatment, access to natural environments, and freedom from unnecessary distress (Prache et al., 2022). Ethical quality also encompasses considerations related to environmental sustainability, ensuring that agricultural methods minimize their ecological footprint and support biodiversity. Ultimately, ethical quality in chicken meat represents a comprehensive approach that values both animal welfare and environmental stewardship, thus contributing to a more conscientious and accountable food production system. Introducing innovative feed ingredients, like insects, aligns with sustainability goals and expands food choices (Anusha and Negi, 2023). Insects offer high protein density with minimal environmental impact, enriching nutritional profiles of poultry diets (Ordoñez-Araque et al., 2022). In particular, the use of Black soldier fly larvae (**BSFL**) in animal feed is gaining traction due to its high protein and lipid content, making it a promising alternative to traditional feed sources like soybean meal and fish meal (Schiavone and Castillo, 2024). Communicating the safety and feasibility of using BSFL in chicken nutrition involves addressing several key aspects based on recent research findings. Nutritionally, BSFL are rich in essential fatty acids, proteins, and minerals. In previous research the inclusion of BSFL meal have been shown to have no negative effects on the growth performance, meat quality, and immunity of monogastric animals when added to their diets (Muinde et al., 2023). Moreover, environmental considerations also play a significant role in consumer perspectives. BSFL can be reared on a variety of organic waste streams, converting these into high-quality biomass, which not only provides a sustainable protein source but also contributes to waste management, offering a sustainable solution to the waste-food-energy nexus (Hoffman et al., 2022). Up to today, the extensive body of research primarily focuses on the use of insect meal rather than whole larvae. The uniqueness of the present manuscript, besides the substantial amount of data, lies in the utilization of whole BSFL larvae in their live and dehydrated form. This distinctive approach could potentially open new avenues in animal feed research and the insect industry as a sustainable protein resource.

The starting point is that while adult consumers in the western world still have doubts about insects as food, younger generations exhibit greater open-mindedness on the subject (Sogari et al., 2021; Rabadán et al., 2023). Nowadays, the interest of consumers for ethical and sustainable animal products is increasingly influenced by a combination of environmental, animal welfare, and health concerns. Research indicates that consumers are more inclined towards purchasing products that are labeled with animal welfare certifications, viewing these as indicators of ethical production practices (Gorton et al., 2023). This indicates a belief that more sustainable products, particularly those with higher animal welfare standards, are worth a higher price (Garcez de Oliveira Padilha et al., 2021). The need for notion spreading among future professionals in the livestock sector suggests a gap in understanding the full scope of sustainability, including its social and economic dimensions (Damico et al., 2022). However, currently the key challenge in gaining consumer acceptance remains effective communication and education. For these resons the present study aims to address a knowledge gap between the inclusion of whole BSFL, live or dehydrated, in poultry diet, and the practical implications on chicken fillet meat quality. Moreover, the overarching objective is to provide reassurance to industries, policymakers, and consumers regarding the feasibility of incorporating whole insect larvae, live or dehydrated, in poultry feeding programs .

MATERIALS AND METHODS

Birds, Husbandry, and Diets

The experimental trial was carried out in the poultry facility of the Avian Conservation Centre for the Valorization of Local Genetic GENetic Resources (**CoVaGEN**) of the University of Turin (Italy) (44°50′58″ N and 7°43′13″ E). Approval for the experimental protocol (No. 814715) was granted by the Bioethical Committee at the University of Turin (Italy).

For this study we used a slow growing indigenous breed called Bianca di Saluzzo with male chickens characterized by their moderate size, with female typically weighing around 2 to 2.5 kg and males approximately 3 kg (Soglia et al., 2020; Bongiorno et al., 2022). For the first 38 d, chicks were raised under controlled environmental conditions, with a gradual adjustment to a lighting schedule composed of 18 h of light and 6 h of darkness. At 39 d of age, 144 male Bianca di Saluzzo birds with an average live weight (**LW**) of 317 ± 1.40 g were chosen, marked, and distributed across 18 pens (2.5 × 3.5 m; 8 birds/pen), with rice hulls as floor litter. The temperature, humidity, and photoperiod of the pens were not subject to any artificial control, and all animals also had access to an outdoor area (2.5 × 3.5 m). The animals were divided into 3 experimental groups of 6 pens (replicates) each. The control group (C) was provided a basal diet characterized by the complete replacement of soybean meal with alternative feed ingredients (Fiorilla et al., 2024a) (Table 1). The two experimental treatments were fed the same basal diet as C with the on top supplementation of dehydrated (**DL**) or live **(LL**) BSFL at a level equal to 5% expected daily feed intake of dry matter (**DM**), giving rise to the following experimental groups: 1) DL group: basal diet + dehydrated BSFL (**D-BSFL**); 2) LL group: basal diet + live-BSFL (**L-BSFL**). BSFL were provided every day at 11 am, placed in a feeder with a central bump to disperse the larvae uniformly around its circumference (Fiorilla et al., 2024b). Live BSFL were kept at 12°C in a state of diapause, a period of quiescence induced by unfavorable environmental conditions that allows the

Table 1. Basal diet used to feed an autochthonous slow-growing chicken breed.

Diet composition (g/kg)	
Maize meal	461
Field bean meal	110
Pea protein meal	108
Barley meal	47
Sunflower meal	95
Corn gluten meal	116
Soybean oil	16
Dicalcium phosphate	13.5
Calcium carbonate	20
Sodium chloride	1.50
Sodium bicarbonate	1.40
DL-methionine	0.70
L-lysine	4.00
Vitamin/mineral Premix[1]	5.90
TOTAL	1000
AME (MJ/kg)	11.9
Analyzed chemical composition (g/100 g feed)	
Dry matter	91.2
Crude protein	18.1
Crude fiber	3.28
Ether extract	3.63

1Nutritional additives: Vitamin A 8.001.60 UI, Vitamin D3 3.000.60 UI, Betaine anhydrous 600.48mg, Biotin 0.04mg, Choline chloride 333.07mg, Folic acid 0.81mg, Niacinamide 25.01mg, Calcium pantothenate 7.28mg, Vitamin B1 0.75mg, Vitamin B12 0.02mg, Vitamin B6 1.60mg, Vitamin E 18.50mg, Vitamin K3 2.50mg, Iron (Iron-II sulfate monohydrate) 44.01mg, Manganese (Manganese-II oxide) 62.01mg, Selenium (Sodium selenite) 0.25mg, Zinc (Zinc sulfate monohydrate) 50.01mg.

larvae to temporarily suspend metabolic development. By transferring them to room temperature prior to chicken administration, the physiological processes that end this state of quiescence are stimulated gradually resuming metabolic activity (Bellezza Oddon et al. 2021). The calculation of pen-based total feed intake was performed every three weeks. This involved computing the total weekly intake of fresh matter, including both feed and larva intake, and subsequently deriving the average dry matter intake (DMI) per bird for each pen. By analyzing the quantities of feed plus D-BSFL or L-BSFL consumed, along with the nutrient and energy compositions of all three diets, we determined the pen-based intake of nutrients (protein and ether extract) (Seyedalmoosavi et al., 2022).

Slaughtering Performance

Birds were slaughtered at either 147 or 174 d of age. The day before slaughter, all chickens were individually weighed. Two birds were selected from each pen (total of 36 birds/slaughter) based on the pen's average LW, and following a 12-h fasting period they were subjected to electrical stunning and bleeding, according to standard regulations of the European Union [Council Regulation (**EC**) No 1099/2009 of 24 September 2009]. The breast fillets were weighed, vacuum-packed, and frozen at -20°C. The caudal parts of the right-side breast fillets were analyzed to establish the proximate and fatty acid composition, whereas the cranial parts were used for sensory evaluation. The left-side breast fillets were analyzed to determine the thawing loss, in vitro protein digestibility, and the content of oxidation products by thiobarbituric acid reactive substances (**TBARS**) assay, and subjected to instrumental tenderness measurements (Warner-Bratzler) and label-free quantitative (**LFQ**) proteomics data analysis.

Physical Meat Quality Parameters

The breast fillet pHu and color were evaluated after 24 h of refrigeration at 4°C on the right side of the *Pectoralis major* muscle. The pHu was measured in duplicate using a pH meter (Crison, Crison Instruments, SA, Alella, Spain) with a specific electrode designed for meat penetration. The lightness (L*), redness (a*), and yellowness (b*) color indexes were determined using a portable Chroma Meter CR-400 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan). Twenty-four hours post-slaughter, breast samples were weighed, placed in a container on a supporting mesh, and sealed. The same breasts were then reweighed before cooking; following 45 min of cooking at 75°C in a waterbath, the excess fluids in the bag were blotted and the samples reweighed. The thaw loss for all fillets was determined as the percentage of weight lost during the freezing process (-20°C). The selected thawed chicken fillets were individually vacuum-sealed in plastic bags and placed in a water bath at 70.5°C for 30 min to ensure a core temperature of 70.0°C in the muscle samples. After cooling in ice-water for 45 min and additional acclimatization to room temperature, the chicken fillets were sliced into samples measuring 3 × 1 × 1 cm along the direction of the muscle fibers. Using a Warner-Bratzler device in an Instron Materials Testing Machine (Model 5944, Instron, High Wycombe, UK), 3 samples from each fillet were sheared across the fiber direction, following the method described by Hildrum et al. (2009).

Nutritional Meat Quality Parameters

In accordance with the guidelines set by the Asssociation of Offical Analytical Chemists AOAC (2004), an analysis was conducted on a freeze-dried breast segment to determine its dry matter (#950.46) and ash (#920.153) content. Crude protein (**CP**) content was determined using the Kjeldahl method (#2001.11). The estimation of ether extract (EE) content was carried out through Soxhlet extraction with petroleum ether, following the AOAC International method #991.36 (2000). Proximate composition results were expressed as grams per 100 grams of fresh matter. Each chicken fillet was divided into two portions: one portion was minced and analyzed in its raw state, while the other was vacuum-packed and subjected to heat treatment in a water bath, following the previously mentioned procedure for instrumental tenderness assessment. The heat-treated chicken fillets were also minced. For both in vitro digestion and the measurement of thiobarbituric acid reactive

substances (**TBARS**), 1 g of each minced sample was transferred into a separate tube. In vitro protein digestion followed the international consensus INFOGEST model (Brodkorb et al., 2019). After 120 min of simulated small intestinal digestion, digestive enzymes were deactivated by heating the samples at 90°C and then centrifuged. The protein content in the supernatant and pellet were determined separately, as previously outlined (Rieder et al., 2021). The supernatant was filtered through 0.45 μm syringe filters (Millipore, hydrophilic PVDF) and separated on a BioSep-*SEC*-s2000 column (Phenomenex, 300−7.8 mm) connected to a HPLC system (Dionex UltiMate 3000), according to Rieder et al. (2021). Peptides were monitored with an UV detector at 214 nm. The protein digestibility (D_{SEC}) was calculated by the percentage of small peptides (less than 1 kDa molecular weight) in the peptide size distribution of the supernatant (determined by SEC) and the amount of dissolved protein. As an indicator of lipid oxidation and oxidative stress during digestion, the TBARS content was assessed in raw chicken breast fillet and heat-treated chicken breast fillet, following the methods described by Steppeler et al. (2016); values were expressed as mg malondialdehyde (**MDA**)/kg meat.

We determined the fatty acid profile of BSFL, the diets, and breast fillet according to the approach published by O'Fallon et al. (2007). In brief, a total of 500 mg of freeze-dried meat, 140 mg of larva, or 800 mg of poultry diet were placed into a 25 mL glass tube, to which 5.3 mL of methanol and 0.7 mL of 10M KOH in acqueous solution were added. Following incubation at 55°C in a water bath for 1.5 h, the test tubes were cooled to room temperature in a water bath filled with cold tap water, then 0.58 mL of 12M H_2SO_4 in acqueous solution were added. After another incubation period of 1.5 h at 55°C, the tubes were again cooled in the same manner, adding 3 mL of hexane, then vortex-mixing for 5 min and subsequent centrifugation. From the top hexane layer, which contains the extracted fatty acid methyl esters (**FAMEs**), 40 μL were removed and diluted in a vial containing 1 mL of hexane for gas chromatographic (**GC**) analysis. The FAMEs were injected into an Agilent 7890 GC system (Palo Alto, CA) equipped with an on-column injector and a flame ionization detector. The separation was carried out on a CP-Sil88 for FAME (100 m x 0.25 mm x 0.25 μm) column, employing the conditions described in Placha et al. (2019). FAME identification was accomplished by comparing their retention times with those of reference standards (Supelco 37 Component FAME mix, 47885-U, Merck). The individual FAMEs were expressed as a percentage of the total fatty acid methyl esters. The atherogenicity (**AI**) and thrombogenicity (**TI**) indexes were calculated according to Ulbricht and Southgate (1991) as follows:

$$\mathrm{AI} = \frac{[\mathrm{C12:0} + (4 \times \mathrm{C14:0}) + \mathrm{C16:0}]}{(\sum \mathrm{MUFA} + \sum \mathrm{n-6} + \sum \mathrm{n-3})}$$

$$\mathrm{TI} = \frac{(\mathrm{C14:0} + \mathrm{C16:0} + \mathrm{C18:0})}{(0.5 \times \sum \mathrm{MUFA}) + (0.5 \times \sum \mathrm{n-6}) + (3 \times \sum \mathrm{n-3})}$$

Proteomics

Breast samples were thawed then cut into small pieces for protein extraction. A total of 100 mg meat were homogenized in 1,000 μL extraction buffer [100mM Tris-HCl (pH 7.6), 4% SDS, 0.1M DTT], using a Precellys®24 (Bertin Technologies) and applying two homogenization steps at 6,000 rpm with a 5 s pause between each step. Samples were then centrifuged at 16,000 *g* for 15 min. The protein concentration was determined using a Bio-Rad DC RCTM protein assay kit (Bio-Rad Laboratories, Hercules, CA). Sixty μg of protein were then alkylated with 55 mM 2-iodoacetamide and digested with Trypsin/Lys-C (Promega, Madison, WI) at an enzyme-to-protein ratio of 1:30 (w/w) on a Microcon-10YM (Merck Millipore, Burlington, MA) centrifugal filter unit at 37°C. From the resulting peptide solution, 20 μg were purified and concentrated using a StageTip according to the protocols by Rappsilber et al. (2007). Peptides were mixed with a loading buffer [2% (v/v) ACN and 0.05% (v/v) trifluoroacetic acid], and 1 μg of peptides analyzed using a nano-UHPLC coupled with a Q-Exactive Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) at the MS/Proteomics Core Facility at Campus Ås (Norway). Detailed LC-MS/MS settings can be found in Koga et al. (2019). Peptides from the 12 most intense peaks obtained during the 120-min elution were fragmented, and the mass-to-charge ratios of these fragmented ions measured (tandem mass spectrometry, MS/MS). Mass spectral data were processed using MaxQuant ver. 2.3.1.0 (Cox and Mann, 2008). The parameters for protein identification included trypsin/Lys-C specificity with reference to the proteome database for the broiler chicken, Gallus gallus (Entry nr. UP000000539) downloaded from UniProt (43710 entries). Other parameters were maintained as defaults, and the label-free quantification (**LFQ**) algorithm was employed for protein quantification. LFQ intensity values from MaxQuant were imported into Perseus software version 2.0.6.0 (Tyanova et al., 2016) for data analysis and visualization. LFQ intensities were log2-transformed, and proteins present in 70% of biological replicates for at least one of the feed groups were retained. Missing values were thereafter imputed from a normal distribution (width 0.3 and downshift 1.8) and principal component analysis carried out. Partial least squares analysis was also performed in MATLAB.

Sensorial Meat Quality

Refrigerated chicken breasts, received in vacuum-packed PA/PE bags, were stored overnight at 4°C. The vacuum-packed breasts were then cooked in a water

bath at 75°C for 45 min, using induction plates, then cooled in ice water for 20 min. Ten trained judges (age range, 30−50 yr), expert in sensory descriptive evaluation, performed the sensory descriptive analysis (**DA**). Attributes for this study were compiled from the existing literature on poultry meat and presented to judges for familiarization. The descriptive analysis aimed to define the sensory profile of each type and identify potential differences among the dietary treatments and the 2 slaughter ages. Judges conducted the sensory analysis, following the standard protocol UNI 8589:1990, in individual booths equipped with notebooks and specific software for sensory data acquisition (FIZZ Biosystèmes, France). The test was conducted in duplicate, adhering to the conditions outlined in the standard UNI 13299:2016 for descriptive analysis, using intensity scales (ISO 8586:2012). Samples were coded with three-digit numbers and presented randomly to the judges. To ensure a neutral palate between samples, judges were provided with mineral water to cleanse their mouths. For the DA test, judges were instructed to indicate the relative intensity of sensory attributes for each sample using a nine-point scale (where 1 = hardly perceptible; 9 = very intense).

Statistical Analysis

Statistical analysis was carried out using R studio software (V.4.1.1). The homogeneity of variance was determined using Levene's test, and the normality or non-normality of distribution was assessed through the Shapiro−Wilk's test. Meat quality parameters, nutrient composition, and sensorial analysis of the meat (n = 12) were analyzed using a generalized linear model (**GLM**). This model incorporated three fixed factors: diet, time, and the interaction between diet and time.

To compare the proteomic data obtained for the control and 2 larvae feed (DL and LL) groups, we conducted Welch's T-tests for all identified proteins, considering a 95% confidence limit and taking the false discovery rate (**FDR**) into account. Protein covariances were extracted using partial least squares regression (**PLS**). The results were reported as the least square mean plus the standard error of the mean (**SEM**). P values ≤ 0.05 were considered statistically significant. A statistical trend was considered for P values ≤ 0.1.

RESULTS

Nutrient Intake and Slaughtering Performance

Figure 1 reports the total CP and EE intake for the three experimental groups during the experimental period (39−174 d of bird age). The results reflect the proximate composition of the D-BSFL and L-BSFL larvae (Table 2), with the L-BSFL-supplemented diet conferring a higher nutritional intake of CP compared with that of the D-BSFL-supplemented diet ($p < 0.05$). By contrast, the nutritional intake of EE was higher in DL compared with LL ($P < 0.01$) (Figure 1). No significant differences were observed in the overall slaughtering performance in relation to the dietary treatment, and no interaction was detected between diet and bird age. As expected, age affected the slaughter weight, being 11.3% higher at 174 than at 147 d of age (Table 3) ($p < 0.05$).

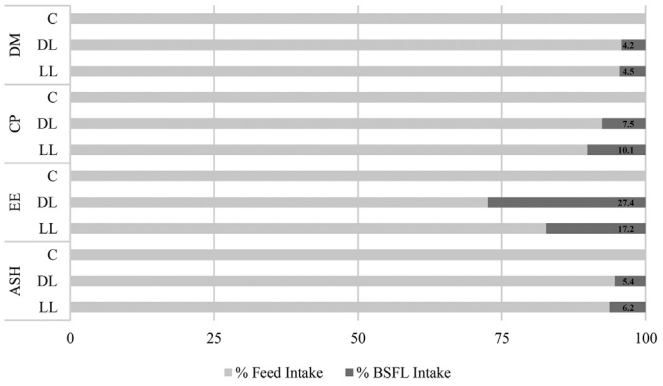

Figure 1. Nutrient intake of an autochthonous slow-growing chicken breed fed a basal diet (C) supplemented with dehydrated (**DL**) or live (**LL**) black soldier fly larvae. C: control; DL: dehydrated larvae; LL: live larvae; DM: dry matter; CP: crude protein; EE: ether extract.

Physical Meat Quality Parameters

The physical meat quality parameters are detailed in Table 4. The assessment of pHu_{24} in breast fillets produced similar results for the different dietary treatments and slaughter ages. Regarding breast color, neither larval supplementation nor age impacted the values of L*, a* or b*. Regarding thaw losses, no significant differences were observed between the dietary treatments and the 2 distinct slaughter ages. Likewise, there were no noticeable variations in terms of meat shear force

Table 2. Fatty acids and aminoacids composition of the basal diet and dehydrated (**DL**) and live (**LL**) black soldier fly larvae used to feed an autochthonous slow-growing chicken breed.

Fatty acids (%)	C	DL	LL
C12:0	0.12	33.2	43.6
C14:0	0.10	7.75	8.54
C16:0	12.9	16.1	12.4
C16:1n7	0.20	3.44	2.64
C17:1	0.05	0.83	0.26
C18:0	2.89	3.09	2.56
C18:1n9+n7	26.7	10.9	13.1
C18:2n6	51.7	13.5	7.59
C18:3n3	3.74	1.25	0.99
C20:2n6	0.08	0.17	0.16
C20:3n6	0.18	0.03	0.02
C20:4n6	0.03	0.04	0.01
SFA	15.9	60.8	68.1
MUFA	26.9	15.1	16.0
PUFA	55.7	14.9	8.76
Analized aminoacid composition (g/100 g CP)			
Lysine	7.00	2.63	3.36
Methionine	2.05	0.76	0.88
Threonine	3.44	1.50	1.92

C: control; DL: dehydrated larvae; LL: live larvae; SFA: saturated fatty acids; MUFA: mono-unsaturated fatty acids; PUFA: poly-unsaturated fatty acids; CP: crude protein.

Table 3. Slaughter performance of an autochthonous slow-growing chicken breed fed a basal diet (C) supplemented with dehydrated (**DL**) or live (**LL**) black soldier fly larvae slaughtered at 147 and 174 d of age (means, n = 12).

	Diet			Age			*p*-value		
	C	DL	LL	147d	174d	SEM	Diet	Age	D×A
Slaughter Weight (g)	2286[b]	2413[a]	2366[a]	2230	2480	33.22	0.013	<0.001	0.513
Ready to cook carcass (SW %)	65.1	65.3	65.2	65.3	65.1	0.433	0.997	0.927	0.539
Cold carcass (RTCC%)	97.5	96.8	97.2	96.8	97.5	0.265	0.171	0.114	0.141
Breast fillet (CC %)	15.5	14.9	16.1	16.2	14.8	0.592	0.367	0.061	0.210

C: control; DL: dehydrated larvae; LL: live larvae; SEM: standard error of the mean; SW: slaughter weight RTCC: ready to cook carcass; CC: cold carcass
[a, b]: $P < 0.05$.

between the three dietary treatments and 2 slaughter ages.

Nutritional Meat Quality Parameters

Table 5 reports the results of the proximate composition of breast fillet. Protein content was similar among treatments and the two slaughtering ages. By contrast, while EE content did not vary between the dietary treatments, it was significantly influenced by age, being 66% higher at 147 compared with at 174 d of age ($p < 0.05$).

The analysis of lipid oxidation products in chicken fillets involved the assessment of thiobarbituric acid reactive substances (**TBARS**). As shown in Table 5, the raw fillets contained low levels of MDA, but this value increased significantly following heat treatment. Notably, there were no significant differences in TBARS between dietary treatments in either the raw or heated samples. Furthermore, the heat-treated samples exhibited higher levels at 147 d of age with respect to the later slaughtering time at 174 d of age ($p < 0.05$). The in vitro protein digestibility analysis of chicken breast fillets revealed no significant differences among treatments and slaughter ages (Table 5).

The fatty acid composition of the breast fillets (Table 6) evidenced higher concentrations of lauric acid (C12:0) in DL and LL compared with the C group ($p < 0.005$). Moreover, a statistical trend was also present for myristic acid (C14:0), with higher values in DL (+72%) and LL (+ 50%) with respect to the C group ($p = 0.078$). Additionally, a notable difference emerged between the two slaughter ages for both lauric and myristic acid, with lower results at 174 d (- 46%) compared with 147 d of age. Despite these variations, the saturated fatty acid (**SFA**), monounsaturated fatty acid (**MUFA**), and polyunsaturated fatty acid (**PUFA**) content of the breast fillet remained unaffected by the inclusion of BSFL. Finally, no differences were found in the atherogenic and thrombogenic indices between the experimental groups.

Proteomics

Proteomic analysis identified a total of 447 proteins. Principal component analysis (**PCA**) revealed no discernible separation between the 3 dietary and the 2 age groups. Additionally, Welch's t-test indicated no significant differences in protein expression between the D-BSFL and L-BSFL groups. Even when the FDR was not taken into account, the significant differences in protein expression were only marginally distinct from those of the control group. Partial least squares (**PLS**) analysis showed grouping for the different feeds, which were, nonetheless, very close to each other. Examination of the proteomics data revealed a noticeable convergence in the distributions of individual proteins. Moreover, PLS analysis unveiled a close clustering of data points, indicating a cohesive response among the proteins examined (Figure 2). Taken together, these observations suggest that despite the differences between diets, the overall physiological impact of the larval supplementation on the chickens was minimal or non-significant.

Sensorial Meat Quality

Table 7 summarizes the results of the sensory evaluation of the breast meat. No significant differences were found between the different dietary treatments and the

Table 4. Physical meat quality of breast fillet of an autochthonous slow-growing chicken breed fed a basal diet (**C**) supplemented with dehydrated (**DL**) or live (**LL**) black soldier fly larvae slaughtered at 147 and 174 d of age (means, n = 12).

	Diet (D)			Age (A)			*p*-value		
	C	DL	LL	147 d	174 d	SEM	Diet	Age	D×A
pHu_{24}	5.74	5.71	5.70	5.70	5.72	0.027	0.376	0.409	0.749
L*	51.2	51.8	51.9	52.8	51.9	0.927	0.639	0.099	0.406
a*	-0.45	-0.46	-0.42	0.13	0.14	0.181	0.144	0.258	0.806
b*	8.68	9.50	8.84	8.46	7.47	0.654	0.301	0.132	0.578
Thaw loss (%)	2.74	2.80	3.09	3.07	2.66	0.234	0.179	0.182	0.444
Shear force (N)	19.0	17.5	18.1	17.8	19.3	1.040	0.237	0.294	0.455

C: control; DL: dehydrated larvae; LL: live larvae; pHu: ultimate pH; L*: lightness; a*: redness; b*: yellowness; SEM: standard error of the mean of the model; N: Newton.

Table 5. Proximate composition (% fresh matter), lipid oxidation (TBARS, mg malonaldehyde/g fresh meat) and protein digestibility (%) of breast fillet of an autochthonous slow-growing chicken breed fed a basal diet (C) supplemented with dehydrated (**DL**) or live (**LL**) black soldier fly larvae slaughtered at 147 and 174 d of age (means, n = 12).

	Diet (D)			Age (A)			*p*-value		
	C	DL	LL	147 d	174 d	SEM	Diet	Age	D×A
Dry matter	33.2	33.9	32.8	33.4	33.8	0.054	0.719	0.624	0.557
Crude protein	24.8	24.5	25.1	25.3	24.9	0.181	0.559	0.691	0.802
Ether extract	2.04	2.43	1.97	2.68	1.61	0.048	0.396	<0.001	0.924
Ash	1.04	1.11	1.07	1.05	1.02	0.011	0.532	0.609	0.448
TBARS (raw)	0.123	0.119	0.120	0.135	0.108	0.023	0.760	0.032	0.103
TBARS (heat treated)	1.97	1.89	2.02	2.58	1.59	0.450	0.181	<0.001	0.073
Protein digestibility	91.5	91.8	91.7	91.6	91.7	0.319	0.862	0.958	0.888

C: control; DL: dehydrated larvae; LL: live larvae; SEM: standard error of the mean of the model.

control group fed the alternative, soybean meal-free, diet, indicating that dietary supplementation with BSFL, be it with dehydrated or live larvae, had no impact on the sensory properties of the chicken meat. Age did impact the meat's consistency however, with the panelists reporting higher values for the meat obtained at the younger slaughter age (147 d). This was the only sensorial difference reported by the trained panelists. Furthermore, we found no significant interaction between diet and age.

DISCUSSION

This research investigated the effects of supplementing the diet of a slow-growing autochthonous chicken breed with same amount (5% DM) of dehydrated or live BSFL. The data demonstrate the absence of any detrimental effect on the slaughtering performance, physical and nutritional quality of the fillet meat, and on its sensory traits. This result may help to reassure both consumers and the sector industries on the use of BSFL. To understand the context better, the results from this trial must be compared with previous research using insect meals included in chicken feed, as the literature currently lacks studies specifically examining the effects of incorporating whole live *vs* dehydrated BSFL. First of all, we calculated the contribution of the feed and the insect larvae to the birds' dietary intake of protein and fat to clarify the nutritional impact of the supplements. This approach has been previously described and used by other authors in studies focused on layers (Tahamtani et al., 2021) and broilers (Seyedalmoosavi et al., 2022). In this context, the analysis of lipid oxidation products, namely thiobarbituric acid reactive substances (**TBARS**), plays a crucial role in evaluating the

Table 6. Fatty acids composition of breast fillet of an autochthonous slow-growing chicken breed fed a basal diet (C) supplemented with dehydrated (DL) or live (LL) black soldier fly larvae slaughtered at 147 and 174 d of age (means, n = 12).

	Diet (D)			Age (A)			*p*-value		
	C	DL	LL	147 d	174 d	SEM	Diet	Age	D×A
C12:0	0.04[b]	0.77[a]	0.53[a]	0.47	0.25	0.101	<0.001	0.010	0.105
C14:0	0.47	0.81	0.70	0.70	0.58	0.043	0.078	0.092	0.639
C16:0	19.5	19.4	20.1	20.0	19.5	0.249	0.260	0.118	0.455
C16:1n9+n7	1.16	1.40	1.36	1.36	1.16	0.204	0.271	0.135	0.372
C17:1	0.35	0.31	0.32	0.34	0.34	0.021	0.092	0.601	0.203
C18:0	10.6	10.4	10.8	10.8	11.0	0.192	0.128	0.115	0.515
C18:1n9+n7	23.9	23.8	23.4	23.6	23.2	0.317	0.497	0.624	0.459
C18:2n6_LA	19.9	19.8	19.2	20.4	18.1	0.281	0.099	0.005	0.149
C18:3n3_ALA	0.78	0.79	0.74	0.91	0.56	0.055	0.175	0.011	0.391
C20:2n6	0.35	0.31	0.29	0.33	0.32	0.017	0.094	0.329	0.855
C20:4n6	10.3	9.10	10.13	9.17	11.2	0.205	0.127	0.002	0.518
C22:4n6	1.58	1.53	1.68	1.61	1.67	0.188	0.324	0.587	0.141
C22:5n6	0.53	0.47	0.55	0.49	0.57	0.057	0.449	0.128	0.385
C22:5n3	1.24	1.14	1.21	1.18	1.25	0.144	0.734	0.401	0.303
C22:6n3_DHA	1.29	1.14	1.25	1.09	1.43	0.180	0.192	0.021	0.640
$\sum$ SFA	31.6	31.9	31.7	31.6	31.4	0.518	0.202	0.254	0.904
$\sum$ MUFA	25.3	25.5	25.1	25.3	24.7	0.396	0.503	0.521	0.446
$\sum$ PUFA	36.7	35.1	35.8	35.9	35.9	0.633	0.230	0.920	0.435
$\sum$ n-6	3.38	3.11	3.26	3.24	3.30	0.118	0.313	0.568	0.621
$\sum$ n-3	33.3	32.0	32.6	32.6	32.6	0.495	0.256	0.988	0.398
$\sum$ n-6/$\sum$ n-3	10.0	10.4	10.2	10.3	10.2	0.258	0.835	0.726	0.570
$\sum$ PUFA/$\sum$ SFA	1.20	1.10	3.31	1.14	2.23	1.130	0.412	0.327	0.401
AI	0.35	0.40	0.37	0.38	0.36	0.065	0.101	0.138	0.795
TI	0.77	0.81	0.81	0.80	0.81	0.048	0.128	0.586	0.919

C: control; DL: dehydrated larvae; LL: live larvae; SEM: standard error of the mean of the model; SFA: saturated fatty acids; MUFA: mono-unsaturated fatty acids; PUFA: poly-unsaturated fatty acids AME: apparent metabolizable energy; AI: atherogenicity index; TI: thrombogenicity index.
[a,b]: $p < 0.05$.

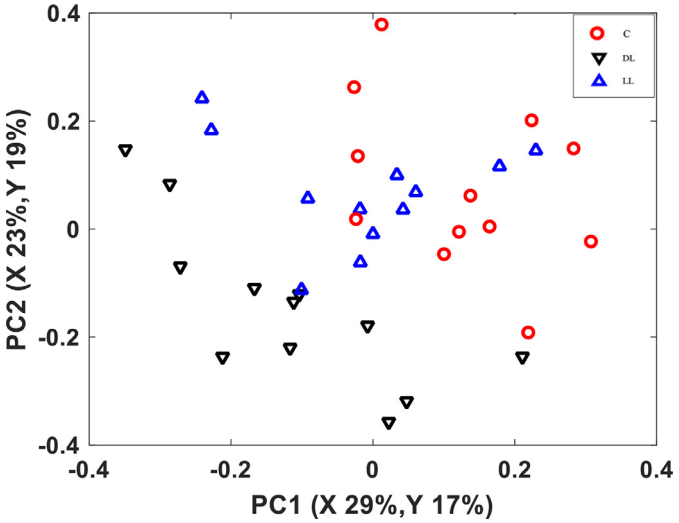

Figure 2. Score plot of partial least squares analysis based on label-free quantification intensities of identified proteins of breast fillet of an autochthonous slow-growing chicken breed fed a basal diet (C) supplemented with dehydrated (**DL**) or live (**LL**) black soldier fly larvae. C: control; DL: dehydrated larvae; LL: live larvae;.

oxidative stability and overall quality of meat products. The decrease in TBARS with age is consistent with the observed decline in lipid content over time. However, it is important to note that TBARS are not solely dependent on lipid content but also on lipid composition (i.e., the fatty acid profile) and the presence of antioxidants (Schiavone et al., 2010). Therefore, in addition to lipid quantity, the quality and presence of antioxidant molecules also influence TBARS levels. Moreover, while raw fillets exhibited low levels of TBARS, indicating minimal lipid oxidation, heat treatment resulted in an increase in MDA values, suggesting oxidative changes induced by cooking. The results of the study by Hoac et al. (2006), in which TBARS levels were increased in heat-treated muscle samples, are in line with ours and suggest that the heat treatment of chicken meat plays a crucial role in lipid oxidation, with varying effects on TBARS levels depending on the specific cooking conditions and temperatures applied. Notably, despite variations in the processing treatments and slaughter age, no significant differences were observed in TBARS levels between the different dietary treatments. This finding is promising from a quality assurance perspective, indicating that BSFL supplementation did not compromise the oxidative stability of the meat. Moreover, the observation that MDA levels were 62% higher in heat-treated samples at 147 d compared with 174 d of age might have been influenced by differences in muscle composition, metabolism, or antioxidant capacity (Malila et al., 2022), emphasizing the need to consider age-related factors in meat quality assessments. Indeed, the fact that the birds were approaching sexual maturity (around 5 mo of age), which correlates with rises in testosterone production, may have increased energy metabolism and consequently affected lipid consumption, possibly providing an explanation for the reduction in lipids observed at 174 d of age compared with the earlier slaughter age of 147 d (Leszczynski et al., 2008).

Consistent with prior research, we obtained the reassuring result that supplementing the chickens' diet with whole BSFL had no effect on the atherogenic and thrombogenic indices (Lu et al., 2022). Moreover, the lack of any differences among the experimental treatments in meat color, pH, shear force, thaw losses, and protein

Table 7. Sensorial analysis of breast fillet of an autochthonous slow-growing chicken breed fed a basal diet (C) supplemented with dehydrated (**DL**) or live (**LL**) black soldier fly larvae slaughtered at 147 and 174 d of age (means, n = 12).

	Diet (D)			Age (A)			*p*-value		
	C	DL	LL	147 d	174 d	SEM	Diet	Age	D×A
Overall odor	5.97	5.70	5.54	6.57	5.98	0.276	0.881	0.089	0.954
Typical odor	5.77	5.57	5.30	5.70	5.36	0.370	0.361	0.835	0.982
Plant odor	2.50	2.47	2.36	2.56	2.32	0.179	0.331	0.982	0.949
Off-odor	1.32	1.45	1.40	1.36	1.40	0.184	0.832	0.967	0.979
Consistency	4.09	4.27	5.02	5.70	4.34	0.320	0.293	0.003	0.929
Fibrousness	3.70	3.90	4.35	4.08	3.89	0.317	0.524	0.538	0.981
Floury texture	2.75	2.87	2.92	2.80	2.80	0.187	0.942	0.887	0.956
Greasiness	2.77	2.81	2.60	2.65	2.76	0.139	0.418	0.418	0.740
Adhesiveness	3.57	3.47	3.59	3.65	3.46	0.214	0.992	0.537	0.842
Juiciness	3.33	3.27	3.20	3.33	3.25	0.136	0.872	0.582	0.743
Chewiness	5.17	4.80	4.45	4.76	4.79	0.245	0.530	0.925	0.803
Astringency	3.45	3.34	3.30	3.54	3.28	0.210	0.930	0.386	0.940
Pungency	1.89	1.84	1.83	2.02	1.65	0.144	0.968	0.118	0.986
Sweet	2.77	2.78	2.90	3.05	2.53	0.178	0.950	0.109	0.879
Salty	3.57	3.47	3.50	3.45	3.65	0.208	0.979	0.508	0.954
Sour	1.87	1.81	1.75	1.66	1.94	0.214	0.972	0.197	0.974
Bitter	1.72	1.69	1.70	1.59	1.77	0.141	0.976	0.367	0.925
Umami	3.82	3.87	3.85	3.67	4.04	0.214	0.999	0.243	0.994
Overall flavor	5.72	5.57	5.40	5.76	5.37	0.259	0.944	0.299	0.996
Typical flavor	5.42	5.27	5.25	5.39	5.22	0.260	0.986	0.653	0.940
Plant flavor	2.52	2.55	2.31	2.57	2.35	0.172	0.902	0.374	0.813
Metallic flavor	2.35	2.45	2.32	2.31	2.47	0.176	0.975	0.508	0.985
Wild flavor	2.24	2.32	2.25	2.39	2.11	0.173	0.976	0.244	0.954
Off-flavor	1.27	1.27	1.27	1.42	1.31	0.133	0.950	0.553	0.896
Freshness	5.02	5.17	4.90	4.99	5.02	0.251	0.946	0.920	0.959

C: control; DL: dehydrated larvae; LL: live larvae; SEM: standard error of the mean of the model.

digestibility is consistent with previous studies focusing on BSFL fat and meal inclusion (Kierończyk et al., 2023), and further support the industrial potential of BSFL supplementation. Furthermore, the findings of this study on unaltered quality traits are reinforced by the favorable outcome of the assessment of sensory traits. Indeed, the trained panelists did not express any preference for the BSFL-fed animals or the C group. This result addresses another common concern in the industry about the potential for changes in meat taste, and it agrees with previous studies indicating no notable effect of the dietary inclusion of BSFL fat on meat sensory characteristics (Kierończyk et al., 2023). Additionally, prior studies investigating the incorporation of BSFL meal and fat revealed significant impacts on the fatty acid composition of poultry meat, leading to higher levels of SFA, MUFA and PUFA within the meat from chickens (Schiavone et al., 2017), ducks (Gariglio et al., 2021) and partridges (Secci et al., 2018). Specifically, diets enriched with BSFL have been associated with an increase in the concentration of certain SFA, such as C12:0 and C14:0, in breast muscle (Schiavone et al., 2018; Dabbou et al., 2021; Lu et al., 2022). In this study, the higher level of lauric and myristic acid found in the breast of chickens fed BSFL aligns with previously described studies and could be explained by looking at the fatty acid composition of the dehydrated and live larvae, which are rich in this SFA. The production of chicken breast with higher levels of lauric acid is desirable due to the beneficial effects of this fatty acid. Lauric acid has been shown to possess antimicrobial properties, particularly against pathogens like Campylobacter spp., which are a major concern for food safety and consumer protection (Hankel et al., 2018). Moreover, dietary supplementation with lauric acid enhances the meat's nutritional value, and some research suggests that lauric acid may have positive effects on cholesterol levels, potentially raising HDL cholesterol whilst lowering LDL cholesterol (McCarty and Dinicolantonio, 2016).

Despite the considerable research focus over recent years on the possibility of including insect larvae in animal diets, some reservations about their use have remained, which we sought to dispel in this work, particularly by reassuring the industry and consumers about the positive effects of supplementing chicken diets with BSFL larvae. The unaffected, and in some cases even improved, physical properties and nutritional quality of the meat align perfectly with the absence of differences in the results from the sensory trait analysis, providing a comprehensive picture of the effects of BSFL supplementation.

In conclusion, this study demonstrated that integrating 5% (on a DM basis) dehydrated or live BSFL into the diet of a slow-growing autochthonous chicken breed had no adverse effects on breast fillet quality or sensory attributes. The consistent results indicate that incorporating BSFL into chicken diets is both a promising and feasible strategy. Additionally, the insights provided on the fatty acid composition of the chicken breast meat highlight potential benefits of BSFL supplementation, offering a way to optimize the fatty acid profile of chicken meat through dietary supplementation. Furthermore, the positive outcomes of meat sensory evaluations reinforce the notion that BSFL supplementation does not compromise the meat's taste. Overall, this research provides data to reassure both consumers and the sector industry about the suitability of BSFL as a sustainable feed ingredient for poultry, paving the way for further exploration and integration into poultry production systems.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Edoardo Fiorilla: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing − original draft, Writing − review & editing. **Marta Gariglio:** Conceptualization, Investigation, Methodology, Writing − review & editing. **Francesco Gai:** Conceptualization, Investigation, Methodology, Writing − review & editing, Funding acquisition. **Valeria Zambotto:** Investigation. **Valentina Bongiorno:** Investigation. **Eleonora Erika Cappone:** Investigation. **Rune Rødbotten:** Investigation, Writing − review & editing. **Shiori Koga:** Investigation, Writing − review & editing. **Anne Rieder:** Investigation, Writing − review & editing. **Erik Tengstrand:** Investigation. **Sara Pozzo:** Investigation. **Giulia Maria Daniele:** Investigation. **Marta Cianciabella:** Investigation. **Stefano Predieri:** Investigation. **Claudio Forte:** Investigation. **Achille Schiavone:** Conceptualization, Investigation, Methodology, Writing − original draft, Writing − review & editing, Funding acquisition.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

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