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RESEARCH ARTICLE

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Growth performance and meat quality of medium-growing chickens fed with live black soldier fly larvae

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ABSTRACT

The study aims to evaluate live black soldier fly larvae (BSFL) supplementation in medium-growing chicken's diet, the effects on growth and slaughter performance, and quality of meat. A total of 90 male chicks, Naked Neck Label, were randomly allotted in 15 pens (5 pens/treatment) and raised until 82 days of age. Each pen was assigned to one of dietary treatments: (1) control group (CTRL) which received a commercial chicken diet, (2) BSFL10 group which received commercial feed and live BSFL at 10% of the expected weekly feed intake and (3) BSFL20 group which received commercial feed and live BSFL at 20%. As concern growth performance, the diets influenced the average daily feed intake ($p = 0.039$) and FCR, which was lower in insect-fed birds ($p < 0.001$). The slaughter weight was significantly higher ($p = 0.016$) in the BSFL10 group compared to CTRL group, while the BSFL20 group showed intermediate values. The fatty acid of the breast and thigh was influenced by the inclusion of the BSFL in the diet. The proportion of lauric acid (C12:0) and myristic acid (C14:0) were significantly higher ($p < 0.001$) in BSFL10 and BSFL20 in comparison to CTRL group. Although the nutritional value index and the total essential fatty acids did not show significant differences among the experimental groups. The results demonstrate the feasibility of supplementing commercial feed for medium-growing chickens with live BSFL, up to 20% of the expected weekly feed intake, with minimal modifications to growth performance, slaughter outcomes, and meat quality.

HIGHLIGHTS

- The study highlights the feasibility of supplementing commercial feed for medium-growing chickens with live BSFL determined minimal modifications to growth performance and slaughter outcomes.
- The FCR of the chickens improved by live BSFL supplementation.
- The supplementation of the BSFL in diet affected the fatty acid composition of the meat without compromising its quality.

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Introduction

The global human population has grown to about 8 billion people in the last decade (DESA UN 2022), and according to FAO, it is forecast to increase to 9.7 billion by 2050 (Ritchie et al. 2023). Food demand is expected to increase due to population growth and rising affluence. Indeed, it is estimated that animal product consumption will rise by 60–70% (Pulina et al. 2022), and to support this growth, it will also be necessary to increase the production of feed and raw materials. To improve production and make it more sustainable, the FAO has identified insects as an innovative, alternative, and

sustainable source of nutrients for animal feed, currently being a topic of growing interest, which is highlighted by the important increase in academic publications on this topic. The growing attention to the use of insects in animal feed is justified by their superior qualitative nutritional content and their potential to act as health promoters for livestock (Veldkamp et al. 2022; Fronickx et al. 2023; van Huis and Gasco 2023). Among the insect species used for the waste bioconversion process, the most studied and promising one to be used in livestock feed is the black soldier fly (BSF, *Hermetia illucens*), which can successfully grow on substrates consisting of various

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by-products (Meneguz et al. 2018). The BSF larvae (BSFL) contain large amounts of protein and fat, with amino acid profiles that are beneficial for poultry nutrition and represent valuable sustainable ingredients for poultry feed (Koutsos et al. 2023). Moreover, these insects are also a precious source of various bioactive compounds, which represent an important component for functional feeds. In particular, the saturated fatty acid C12:0 (lauric acid) has a beneficial effect on the immune system and acts as a virucidal and bactericidal agent reducing the inflammatory process (Veldkamp et al. 2022). Bioactive compounds, such as antimicrobial peptides, are generally sensitive to the high-temperature treatments (Nakagawa et al. 2023) used for larvae inactivation and processing (i.e. meal and oil). Utilising live larvae can help preserve these compounds by minimising denaturation, thereby enhancing their bioavailability for animals. Furthermore, the use of live larvae can simplify the production process, reducing processing costs and initial investment. This strategy also holds potential to promote the sustainable growth of the insect-based feed industry. Fiorilla et al. (2024a) recently demonstrated that supplementing the diets of slow-growing chickens with either whole live or dehydrated BSFL has positive effects on gut health. Specifically, higher propionic acid production was observed in groups fed BSFL. Additionally, gut microbiota analysis revealed an increase in beneficial bacteria, such as *Faecalibacterium*, particularly in the group supplemented with live BSFL.

It is well known that chickens with access to outdoor areas will ingest insects at all life stages, which indicates that they have evolved to include insects as a natural component of their diet (Józefiak et al. 2016). Due to their characteristics, the use of insects in chicken diets could also be a valuable tool to support and promote the welfare of birds (Bongiorno et al. 2022). In fact, live insects can be considered an environmental enrichment, as they stimulate poultry curiosity due to their motility (Star et al. 2020; Schiavone and Castillo 2024). In broiler chickens (Ross 308), the presence of live larvae was found to reduce frustration by promoting foraging behaviour and boosting activity levels (Biasato et al. 2022). Additionally, live larvae provision was observed to reduce the duration of tonic immobility, suggesting a decrease in fearfulness in broiler chickens (Ipema et al. 2020). Further welfare benefits were also observed, including improved leg health and mobility (Ipema et al. 2022). Moreover, in Muscovy ducks, dietary supplementation with 5% live BSFL has been shown to enhance birds' welfare by reducing aggressiveness, excreta corticosterone metabolites, and the heterophil-to-lymphocyte ratio (Gariglio

et al. 2023). The literature reports no significant differences or only slight improvements in growth performance between male and females medium-growing chickens supplemented with 10% live BSFL (Bongiorno et al. 2022) or slow-growing chickens fed on average 12% live BSFL (Fiorilla et al. 2024b) referred to their expected daily feed intake. Data on the impact of dietary live insects on poultry meat quality remain limited. Recently, Fiorilla et al. (2024c) were the first to report findings on the nutritional composition of breast fillets from slow-growing chickens fed either live or dehydrated BSFL.

Building on these findings, the present study aimed to evaluate the effects of a higher supplementation level (up to 20%) on growth performance, slaughtering characteristics, and meat quality.

Materials and methods

Black soldier fly larvae rearing

The BSF colony was located at the experimental facility of the Department of Agricultural, Forest and Food Sciences (Carmagnola, TO) of the University of Turin (Italy) within two climatic chambers under controlled environmental conditions: the reproduction (12 m²; 30 ± 0.5 °C and 75 ± 5% of RH) and the production (20 m²; 28 ± 0.5 °C and 60 ± 5% of RH) areas. Both chambers were equipped with a controlled ventilation system to prevent heat stratification and ensure air circulation. The entire BSFL stage was conducted on the Gainesville diet (GA, Hogsette 1992), which was composed of a dry mix of wheat bran (50%), corn (20%) and alfalfa (30%) and diluted with tap water (70% of final moisture). The proximate composition of the Gainesville diet was as follows: 15.33% of crude protein (CP), 3.78% of ether extract (EE), 12.62% of crude fibre (CF) and 6.34% of ash – expressed on a dry matter (DM) basis. Eggs were collected once a week and hatched in an empty box. The 1-day-old larvae were harvested every 24 h, inoculated, and fed on GA (1 g of 1-day-old larvae per 1 kg of fresh diet) for 6 days. For the mass rearing, 6-day-old larvae were estimated and placed into fattening boxes (60 cm × 42 cm × 11 cm) with a feeding rate of 0.8 grams of GA per larva. At 16 days of age, when the larvae reached the maximum weight in the growth curve, larvae were sieved through a vibrating screen with a 2 mm mesh, and the fresh biomass and frass were weighted to calculate the performance parameters.

Larvae were delivered (almost 2 h of travel) every 15 days to the poultry facility of the University of Milan (Italy). The live larvae were stored in a

refrigerated room (T° : 15 °C) in order to reduce their metabolism, stop their growth, and guarantee the provision of 16-day-old live larvae to poultry feeding. A total of 200 g of BSFL was collected at each delivery and stored at -20 °C for further laboratory analyses. Prior to administration to the birds, the BSF larvae underwent a revitalisation phase, during which they were maintained at a controlled temperature of 28 °C for a duration of 10 min. This procedure served a dual purpose. First, it reactivated the natural motility of the larvae, which is a key behavioural characteristic. Larval motility plays a fundamental role in enhancing their attractiveness to the birds, as dynamic, moving prey better stimulates the birds' natural foraging instincts and feeding behaviours compared to static feed sources. Second, the revitalisation process ensured that the physical activity of the larvae was reinitiated without inducing any alteration to their chemical composition, thus preserving their nutritional integrity. This balance between biological activity and chemical stability was critical to the experimental setup, as it allowed for the assessment of the birds' response to live feed stimuli while maintaining consistency in the dietary composition of the larvae.

Birds, husbandry, and diet

The chicken trial was performed at the Poultry Unit, Animal Production Centre of the University of Milan (Lodi, Italy) and lasted 82 days. The experimental protocol was previously approved by the Italian Ministry of Health (authorization n. 659/2020_PR). A total of 90 1-day-old male chicks, Naked Neck Label (Hubbard JA57 hybrid; average initial live weight: 37.5 ± 2.75 g) were reared in a controlled environment. The animals and the environmental parameters were subjected to daily monitoring throughout the duration of the experimental period. All the chicks, at one day of age, were weighed and individually tagged with a wing mark with a unique identification number. At the age of 15 days, all the chicks were weighed again to create three experimental groups of similar weight, consisting of 30 animals/treatment, divided into 15 pens, and 5 pens/treatment (6 chicks/pen). Each pen was 1.5 m wide and 2.0 m long, and equipped with a hopper feeder, an automatic nipple drinker, and rice husk litter. The initial temperature was 30 °C and gradually decreased by 2 °C every other day, up to 18 °C when the animals reached 43 days of age; then the temperature was kept constant for the last rearing month. The light schedule was programmed as 22L: 2D at 40 lux for the first 24 h, then the light hours

were gradually decreased until reaching, at 15 days of age, 18L: 6D at 20 lux, which was maintained until the end of the trial. A daily monitoring check was carried out to ascertain and document the mortality and health status of the birds. Each pen was assigned to one of three dietary treatments, as follows: (1) control group (CTRL) which received a commercial chicken diet [Apparent Metabolisable Energy (AME, INRA 2004): 11.7 MJ/kg, DM: 87.5%, CP: 17.5%, EE: 3.7%, CF: 4.8%, ash: 7.5%, calcium: 1.5%, phosphorus: 0.6%, sodium: 0.3%, DL-methionine: 0.085%]; (2) BSFL10 group which received the commercial feed and a supplementation of live BSFL at 10% of the expected weekly feed intake reported in the Naked Neck Label breeding guidelines (Hubbard 2021) and (3) BSFL20 group which received the commercial feed supplemented with live BSFL at 20% of the expected weekly feed intake. The commercial diet and drinking water were provided *ad libitum* throughout the trial. The daily larvae quantity was placed in one red plastic plate for each pen and distributed twice daily at 9:00 am and 3:00 p.m. The same type of plate filled with control feed was also provided to CTRL animals to create the same interaction with operators in all the treatments and avoid bias. Feed consumption (g) was recorded weekly. The birds live weight (LW, g) was measured every two weeks (Electronic scale: $d=0.1$ –Minebea Intec Miras 2, Bovenden, Germany). The average daily gain (ADG, g/d), the average daily feed intake (ADFI, g/d) and the feed conversion ratio (FCR, g/g, DM basis) were calculated for each experimental group from 15 days of age onwards. The ADFI was calculated on an as-fed basis, without considering the larvae administration. However, the average amount of provided BSFL was determined and reported as average daily larvae intake (ADLI), both on as-fed (g) and dry-matter (g) basis. The FCR was calculated taking into account the DM consumed by the animals, by including in the formula the amount (g) of larvae (DM: 23.81%) and commercial feed (DM: 87.5%) intake by the birds in accordance with the methodology described by Bongiorno et al. (2022). The effect of increased levels of live BSFL (BSFL composition = CP: 53.24%, EE: 7.19% Ash: 13.70%) on the proportional nutrient intake *via* the commercial diet and *via* larvae, in the three experimental groups, was represented (Figure 1) according to Seyedalmoavi et al. (2022).

Slaughter performance

At the end of the trial, at 82 days of age, the final LW was recorded, and three birds/pen (15 birds/dietary

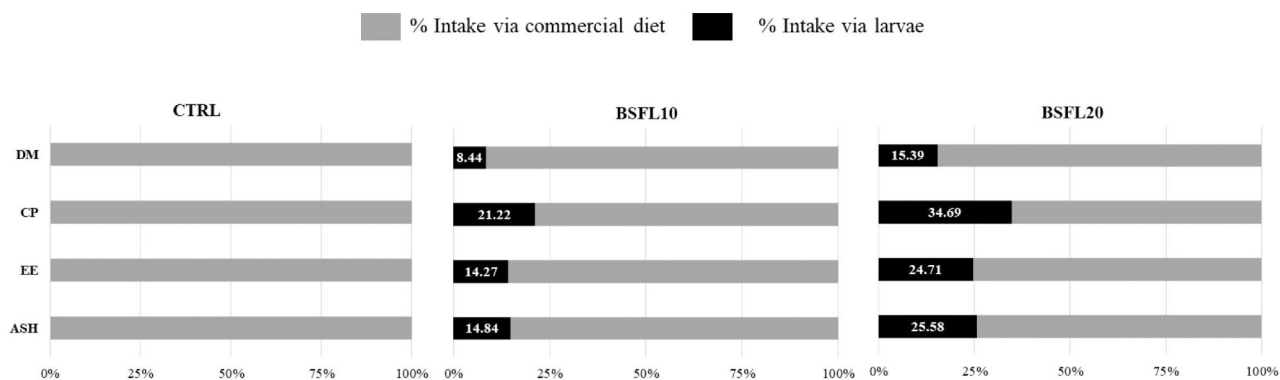


Figure 1. Effect of dietary administration of increasing levels of live black soldier fly larvae (10 and 20%) in male Label Rouge necked birds on proportional nutrient intakes via commercial diet and larvae.

Diet: CTRL = Control diet, BSFL10 = Control diet + 10% BSFL, BSFL20 = Control diet + 20% BSFL; DM = Dry matter; CP = crude protein; EE = ether extract.

treatment) were selected based on the average final LW of the pen and labelled with a shank ring. After 12 h of feed withdrawal, the selected birds were weighed again (slaughtering weight, SW) and slaughtered in a commercial abattoir (located 47 km away), according to standard procedures (electrical stunning and exsanguination). Immediately after, the weight of the carcasse (hot carcasse weight, g) was recorded. No edible viscera and abdominal fat were removed to obtain the 'ready to cook' carcasse (RTCC, g), which was cooled in a cold tunnel and refrigerated at 4 °C for 24 h. The weight of the main viscera (spleen, heart, liver, and Bursa of Fabricius) was also recorded (Electronic scale: $d = 0.01$ – KERN PLS420-3F, Balingen, Germany). On the day following slaughter, the right breast and thigh (without skin) were stored at -20 °C and then freeze-dried for subsequent laboratory analyses.

Meat quality assessment

pH and colour measurements

The pH of the right breast and thigh was determined 24 h post-mortem utilising a Crison portable pH metre (Crison Instruments, S.A., Alella, Spain) equipped with a spear-type electrode and an automated temperature compensation probe. Subsequently, the colour values of lightness (L^*), redness (a^*) and yellowness (b^*) in the CIELAB colour space (Commission Internationale de l'Éclairage; CIE 1976) were measured using a portable colorimeter, the Chroma Metre CR-400 Minolta (Minolta Sensing Inc., Osaka, Japan), with an 8 mm diameter measuring area, an illuminant D65 and a standard observer with a 2° field of view. The pH and colour values presented here are the average of two measurements taken for each sample.

Proximate composition analysis

Breast and thigh samples freeze-dried, commercial feed, and BSFL (pool freeze-dried of three different deliveries), were analysed to determine DM (method number 934.01), CP (method number 984.01), and ash (method number 942.05) contents, according to AOAC International methods (2000), and EE (method number 2003.05; AOAC 2003). Analyses were performed in duplicate.

Fatty acid composition

Total lipids in commercial diet, BSFL (pool freeze-dried of three different deliveries) and in breast and thigh pool freeze-dried samples, were extracted with chloroform: methanol (2:1, v/v), using 2 g of sample, according to Folch et al. (1957). The preparation of fatty acid methyl esters was conducted following the methodology outlined by Christie (2003). The lipid sample (20 mg) was dissolved in Acethyl Chloride: Methanol (1:10, v/v) (methylating solution 2 mL). An internal standard was added in the form of a 1 mL solution of tricosanoic acid (C23:0) in toluene. The sample was then sealed and heated at 50 °C overnight. Then, 2 mL of a 1 M potassium carbonate solution and 5 mL of 5% NaCl were added to each sample. The fatty acids methyl esters (FAME) were extracted with 2 × 2 mL of hexane and the resulting mixture was evaporated under nitrogen. The sample was dissolved in 1 mL hexane. Fatty acid methyl esters (FAME) were separated using a TR-FAME capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness; Thermo scientific- Langerwhere, Germany) by a gas chromatograph (TRACE™ 1300; Thermo Fisher Scientific, Waltham, MA, USA), equipped with a flame ionisation detector. The temperature of the injector in split mode (ratio 1:50) was set at 250 °C and the detector at 250 °C. The carrier gas used was helium with a flow rate of 1 mL/min. In addition, the

oven temperature was set at 110°C, with an increment of 5°C/min up to 180°C then successive increments of 2.5°C/min up to 250°C for 2 min. Peaks identification was performed by comparing the retention times with those of a reference standard (Supelco 37 FAME Mix, Sigma-Aldrich, Milan, Italy). The obtained data were expressed as g/100g of the total detected FAME. Nutritional value indices were calculated using the following formulae (Daszkiewicz et al. 2022):

- Hypocholesterolemic fatty acids (DFA): $UFA + C18:0$;
- Hypercholesterolemic fatty acids (OFA): $SFA - C18:0$;
- Total Essential Fatty acids (EFA): $(C18:2 + C18:3)$;
- Nutritional Value Index (NVI): $(C18:0 + C18:1) / C16:0$;

whereas atherogenic and thrombotic indexes were calculated in accordance with the methodology proposed by Ulbricht and Southgate (1991), as outlined below:

- Atherogenicity Index (AI): $[C12:0 + (4 \times C14:0) + C16:0] / \Sigma UFA$;
- Thrombogenicity Index (TI): $(C14:0 + C16:0 + C18:0) / [(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n-6 PUFA) + (3 \times \Sigma n-3 PUFA) + (n-3/n-6)]$.

Statistical analysis

Data were subjected to statistical analysis using the SAS software, version 9.4 for Windows. The pen was considered as the experimental unit for growth performance ($n=5$ per treatment). In contrast, the individual bird was considered as the experimental unit for slaughter performance and meat quality parameters ($n=15$ per treatment). The Shapiro-Wilk's and Levene tests were carried out in order to determine whether the distribution was normal or non-normal and the homogeneity of variance, respectively. The growth performance, carcass data, organ weight, and meat quality traits were analysed by One-Way ANOVA, following verification of the assumptions. The results were expressed as the mean and the standard error of the mean (SEM) and $p < 0.05$ was declared as statistically significant.

Results and discussion

Growth performance

Non-conventional feeds, among which we find insects, are currently attracting attention due to their ability to improve sustainability and animal performance (Belhadji Slimen et al. 2023). Today's insect production offers a

variety of insect-derived products with functional and nutritional properties. These range from live larvae (Veldkamp and Van Niekerk 2019) and dried larvae (Bejaei and Cheng 2020) to various full-fat (Maurer et al. 2015), partially defatted (Maurer et al. 2015; Schiavone et al. 2017) or highly defatted insect meals (Schiavone et al. 2017) and insect oil (Kim et al. 2020).

The current state of knowledge indicates that the partial replacement of fishmeal or soybean meal in poultry feed with BSFL is an advantageous strategy, this suggests that these insects as a valuable alternative to traditional protein sources (Dörper et al. 2021).

Furthermore, the use of BSFL in research is important additionally for increasing sustainability and circularity of the value chain are the main drivers for the transition to customary insect incorporation in rations destined to farm animals (Ojha et al. 2020). In the current trial, the health status of the chickens was monitored daily, and no mortality was recorded throughout the trial. Although the supplementation of BSFL in the diet was a novelty for the animals involved in this study, they consumed the larvae consistently throughout the trial without losing interest. Concerning the growth performance of the birds (Table 1), the inclusion of live BSFL in the diet did not affect the LW and the ADG, these findings being in agreement with Star et al. (2020) and Bellezza Oddon et al. (2021) who both used live BSFL at 10% and 5% of the expected average daily feed intake in broiler diets. However, the ADFI was significantly lower for BSFL20 birds when compared to the birds belonging to the CTRL group, again regardless of the level of larvae supplementation. These findings agree with Seyedalmoosavi et al. (2022) who used 10%, 20%, and 30% inclusion of BSFL in broilers for 42 days and indicated that feed intake decreased with larvae intake. Regardless of supplementation level, the

Table 1. Growth performance of male Label Rouge Naked Neck birds ($n=5$ pens/dietary treatment) fed with increasing levels (10 and 20%) of live black soldier fly larvae (BSFL).

Item	Age (days)	DIET ¹			SEM	<i>p</i> -value
		Control	BSFL 10	BSFL 20		
LW ² , g	15	200.6	200.9	200.8	3.14	0.998
	82	2554	2623	2659	21.7	0.138
ADG ² , g/d	15–82	36.81	37.82	38.41	0.37	0.220
ADFI ² , g/d as fed	15–82	97.81 ^a	93.78 ^{ab}	93.06 ^b	0.88	<0.039
ADLI ² , g/d as fed	15–82	–	7.41	14.81	–	–
ADLI ² , g DM/d	15–82	–	1.76	3.53	–	–
FCR ² , g DM/d	15–82	2.33 ^a	2.22 ^b	2.21 ^b	0.02	0.001

¹Diet: CTRL = Control diet, BSFL10 = Control diet + 10% BSFL, BSFL20 = Control diet + 20% BSFL.

²LW = live weight; ADG = average daily gain; ADFI = average daily feed intake; ADLI = average daily larvae intake; FCR = feed conversion ratio, corrected considering the total amount of DM from both the commercial diet and BSFL.

^{a,b}Different superscript letters within a row indicate significant differences among the treatments ($p < 0.05$).

experimental groups that received live BSFL in the diet also showed significantly lower FCR (adjusted for BSFL consumption) than those of the CTRL group chicks. Our outcomes agree with Dörper et al. (2024) who used diets based on BSF products (such as BSF larvae meal and oil), also live BSF larvae, and reported significantly lower FCR in chickens eating live larvae compared with the control and those eating the other diets. Several previous studies used live, dehydrated frozen, or defrozen BSFL, in poultry diets as a replacement or supplement to traditional protein sources (Makkar et al. 2014; Veldkamp and Van Niekerk 2019; de Souza Vilela, Adronicos et al. 2021; Ipema et al. 2022; Seyedalmoosavi et al. 2022). The growth performance we obtained are in agreement with the results of these studies, suggesting that the inclusion of insects in the chicken diet improves their growth performance.

Slaughter performance

Live BSFL supplementation did not exert any effect on most of the evaluated slaughter performance parameters (Table 2). Exceptions regarded the SW, which was significantly higher ($p = 0.016$) for the birds belonging to the BSFL10 group when compared to the ones belonging to the CTRL group, while the birds belonging to the BSFL20 group showed intermediate values. The SW reflects the LW reported in Table 1, revealing some statistical differences that were not observed for LW, likely due to reduced variability among the selected birds. Unexpectedly, the SW in the BSFL20 group did not differ from the control group, despite the numerical difference, but was similar to that of the BSFL10 group, possibly due to random effects.

Table 2. Slaughter performance of male Label Rouge Naked Neck birds ($n = 15$ birds/dietary treatment) fed with increasing levels (10 and 20%) of live black soldier fly larvae (BSFL).

Item	DIET ¹			SEM	<i>p</i> -value
	Control	BSFL10	BSFL20		
SW ² , g	2490 ^a	2583 ^b	2549 ^{ab}	22.09	0.016
Hot carcass weight, g	1983 ^a	2069 ^b	2049 ^b	21.14	0.016
RTCC ² yield, %SW	69.00	69.32	69.80	0.288	0.156
CC ² yield, %SW	68.10	68.41	68.96	0.278	0.098
Breast yield, %CC	21.61	21.94	21.78	0.264	0.678
Thigh yield, %CC	35.73	36.23	35.84	0.289	0.449
Spleen, %SW	0.15	0.14	0.15	0.006	0.862
Heart, %SW	0.50	0.50	0.49	0.016	0.864
Liver, %SW	1.50	1.45	1.43	0.028	0.251
Bursa of Fabricius, %SW	0.08	0.08	0.10	0.008	0.111
Abdominal fat, %SW	1.73	1.71	1.69	0.107	0.971

¹Diet: CTRL = Control diet, BSFL10 = Control diet + 10% BSFL, BSFL20 = Control diet + 20% BSFL.

²SW = slaughter weight; RTCC = ready to cook carcass; CC = cold carcass.

^{a,b}Different superscript letters within a row indicate significant differences among the treatments ($p < 0.05$).

However, concerning hot carcass weight, both BSFL supplemented groups showed significantly higher values ($p = 0.016$) when compared to CTRL birds. Such findings confirm previous results obtained by Bellezza Oddon et al. (2021) and Bongiorno et al. (2022). According to Moula et al. (2018) we observed no significant difference in organ weights in birds fed with or without BSFL.

Meat quality parameters

The pH, colour and proximate composition of both breast and thigh meat were not affected by treatment (Table 3), confirming the results previously obtained by de Souza Vilela et al. (2021) and Fiorilla et al. (2024c).

Fatty acid composition

Fatty acids analysis results are reported in Table 4. The fatty acid composition of the total lipids in the commercial diet was mainly characterised by a higher proportion of UFA, specifically oleic (C18:1 *c*9) and linoleic (C18:2 *n*-6) acids, which together comprised approximately 77% of the total detected fatty acids (50% MUFA + 27% PUFA). In contrast, SFA comprised around 22% of total detected fatty acids, with palmitic acid (C16:0) as the primary component. Confirming previous findings (Barragan-Fonseca et al. 2017; Fiorilla et al. 2024c), the fatty acid profile of the BSFL used in the current study was characterised by a high content of saturated fatty acids (SFA), mainly lauric (C12:0) and

Table 3. Colour, pH, and proximate composition of breast meat and thigh meat from male Label Rouge Naked Neck birds ($n = 15$ birds/dietary treatment) fed with increasing levels (10 and 20%) of live black soldier fly larvae (BSFL).

Colour and pH	DIET ¹				<i>p</i> -value	
	CTRL	BSFL10	BSFL20	SEM		
L* ²	Breast	53.51	52.93	54.33	0.49	0.143
	Thigh	53.36	52.82	53.60	0.65	0.688
a* ²	Breast	-1.84	-1.42	-1.73	0.22	0.384
	Thigh	4.64	4.41	4.92	0.35	0.659
b* ²	Breast	8.23	8.28	8.19	0.36	0.984
	Thigh	6.13	6.75	6.57	0.49	0.659
pH	Breast	5.66	5.65	5.64	0.01	0.802
	Thigh	6.28	6.32	6.27	0.03	0.368
Proximate composition (%)						
DM ²	Breast	26.55	26.44	26.19	0.16	0.270
	Thigh	25.56	26.18	25.93	0.25	0.242
CP ²	Breast	24.12	24.16	23.93	0.14	0.485
	Thigh	19.58	19.64	19.63	0.16	0.968
EE ²	Breast	0.90	0.80	0.78	0.05	0.242
	Thigh	4.68	5.05	4.99	0.19	0.383
Ash	Breast	1.40	1.36	1.35	0.02	0.354
	Thigh	1.18	1.25	1.18	0.03	0.131

¹Diet: CTRL = Control diet, BSFL10 = Control diet + 10% BSFL, BSFL20 = Control diet + 20% BSFL; ²Colour values of lightness (L*), redness (a*) and yellowness (b*); DM = dry matter; CP = crude protein; EE = ether extract.

Table 4. Fatty acid profile (% of total detected FAME) of total lipids of the black soldier fly larvae (BSFL) and commercial diet.

Fatty Acid	BSFL	Commercial diet
C10:0	0.38	–
C12:0	22.9	0.13
C14:0	6.76	0.42
C14:1 n-9	0.09	0.03
C15:0	0.33	0.08
C16:0	22.1	16.7
C16:1 n-9	2.67	0.51
C17:0	0.49	0.16
C17:1 n-10	0.13	–
C18:0	6.41	4.20
C18:1 c9	16.9	26.7
C18:2 n-6	18.6	47.4
C18:3 n-3	1.66	2.62
C20:0	0.36	0.32
C20:4 n-6	0.05	–
C22:0	0.09	0.18
C20:1 n-9	–	0.26
C20:2	–	0.06
C24:0	–	0.19
Σ SFA ¹	59.9	22.4
Σ MUFA ¹	19.8	27.0
Σ PUFA ¹	20.3	50.1
UFA ¹	40.1	77.1
n-3PUFA	1.66	2.62
n-6 PUFA	18.6	47.4
Σ n-6 PUFA / Σ n-3 PUFA	11.1	18.1
Σ UFA / Σ SFA	0.72	3.45
Σ MUFA / ΣSFA	0.35	1.21
Σ PUFA / Σ SFA	0.37	2.24

¹SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids.

palmitic (C16:0) acids. In BSFL, total SFA represented about 60% of the total detected fatty acids, while monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were almost equally represented, reaching about 20% of total detected fatty acids each. The most abundant individual MUFA and PUFA were oleic (C18:1 c9) and linoleic (C18:2 n-6) acids, respectively. The BSFL has a high content of unsaturated fatty acids (UFAs), around 40%, and a high n-6/n-3 ratio due to the predominance of n-6 series PUFAs over n-3 series PUFAs (Spranghers et al. 2017). These findings are in agreement with the fatty acid profile of BSFL presented by Ewald et al. (2020) and Fiorilla et al. (2024c). The fatty acid profiles of BSFL could be modified according to the rearing substrate (Makkar et al. 2014), to improve the percentages of the healthy EPA and DHA (Barroso et al. 2017).

As expected, (Daszkiewicz et al. 2022; Fiorilla et al. 2024c), the supplementation of live BSFL in the diet affected the fatty acid profile of the chicken’s breast and thigh (Table 5). In particular, a significant increase in total SFA and a decrease in total UFA was observed in both breast and thigh when using both BSFL10 and BSFL20 diets compared to the administration of the CTRL diet alone. However, the extent of this difference is

Table 5. Fatty acid profile (% of total detected FAME) of total lipids and nutritional quality indices assessed in the breast meat and thigh meat of male Label Rouge Naked Neck birds (n = 15 birds/dietary treatment) fed with increasing levels (10 and 20%) of live black soldier fly larvae (BSFL).

Fatty acid		DIET ¹			SEM	p-value
		CTRL	BSFL10	BSFL20		
C10:0	Breast	0.05	0.07	0.06	0.007	0.408
	Thigh	0.01 ^a	0.02 ^a	0.03 ^b	0.003	0.008
C12:0	Breast	0.12 ^a	0.41 ^b	0.84 ^c	0.065	<0.001
	Thigh	0.07 ^a	0.50 ^b	1.07 ^c	0.113	<0.001
C14:0	Breast	0.57 ^a	0.73 ^b	0.92 ^c	0.048	<0.001
	Thigh	0.57 ^a	0.83 ^b	1.06 ^c	0.060	<0.001
C14:1 n-9	Breast	0.09 ^a	0.10 ^a	0.12 ^b	0.005	0.006
	Thigh	0.11 ^a	0.14 ^b	0.16 ^b	0.009	0.001
C15:0	Breast	0.09	0.09	0.10	0.005	0.609
	Thigh	0.08	0.09	0.09	0.004	0.125
C16:0	Breast	24.8	25.0	24.7	0.02	0.707
	Thigh	23.6	24.1	23.8	0.279	0.490
C16:1 n-9	Breast	3.32	3.08	3.17	0.176	0.603
	Thigh	4.55	4.53	4.40	0.221	0.876
C17:0	Breast	0.14	0.13	0.15	0.009	0.368
	Thigh	0.12 ^a	0.14 ^{ab}	0.15 ^b	0.005	0.007
C17:1 n-10	Breast	0.04	0.04	0.06	0.006	0.073
	Thigh	0.06 ^a	0.06 ^{ab}	0.07 ^b	0.002	0.012
C18:0	Breast	9.36	9.70	9.70	0.194	0.299
	Thigh	7.67	7.67	8.11	0.164	0.110
C18:1 c9	Breast	31.9 ^a	30.7 ^{ab}	29.9 ^b	0.518	0.037
	Thigh	35.2 ^a	34.5 ^a	33.0 ^b	0.445	0.005
C18:2 n-6	Breast	19.6	19.5	19.6	0.327	0.953
	Thigh	22.8	22.7	22.9	0.332	0.935
C18:3 n-6	Breast	0.15	0.15	0.15	0.009	0.944
	Thigh	0.18	0.17	0.16	0.010	0.399
C18:3 n-3	Breast	0.89	0.89	0.90	0.029	0.990
	Thigh	1.21	1.22	1.22	0.025	0.971
C20:0	Breast	0.10	0.10	0.10	0.004	0.335
	Thigh	0.08	0.09	0.10	0.006	0.210
C20:1 n-9	Breast	0.23 ^a	0.22 ^{ab}	0.19 ^b	0.009	0.024
	Thigh	0.26	0.23	0.25	0.011	0.346
C20:2 n-6	Breast	0.29	0.29	0.30	0.019	0.982
	Thigh	0.19	0.20	0.22	0.013	0.268
C20:3 n-6	Breast	0.47	0.45	0.48	0.034	0.741
	Thigh	0.26 ^a	0.22 ^b	0.25 ^a	0.008	0.011
C20:4 n-6	Breast	5.81	6.16	6.22	0.408	0.743
	Thigh	2.29 ^a	1.96 ^b	2.25 ^a	0.099	0.045
C22:0	Breast	0.10	0.08	0.11	0.011	0.132
	Thigh	0.03	0.03	0.05	0.008	0.121
C24:0	Breast	0.74	0.82	0.80	0.055	0.558
	Thigh	0.27	0.22	0.19	0.024	0.121
C22:6 n-3	Breast	0.98	1.05	1.13	0.092	0.512
	Thigh	0.27	0.20	0.25	0.039	0.470
Σ n-3PUFA	Breast	1.87	1.94	2.03	0.088	0.465
	Thigh	1.49	1.42	1.47	0.048	0.660
Σ n-6PUFA	Breast	26.4	26.6	26.8	0.415	0.808
	Thigh	25.7	25.3	25.8	0.374	0.605
Σn-6PUFA/Σn-3PUFA	Breast	14.3	13.8	13.4	0.473	0.467
	Thigh	17.4	17.9	17.7	0.537	0.825
Σ SFA ²	Breast	36.1 ^a	37.2 ^b	37.6 ^b	0.342	0.010
	Thigh	32.5 ^a	33.7 ^b	34.7 ^b	0.391	0.001
Σ MUFA ²	Breast	35.6	34.2	33.5	0.591	0.056
	Thigh	40.2 ^a	39.5 ^a	37.9 ^b	0.513	0.012
Σ PUFA ²	Breast	28.2	28.5	28.8	0.465	0.715
	Thigh	27.2	26.7	27.2	0.401	0.589
Σ UFA ²	Breast	63.9 ^a	62.7 ^b	62.3 ^b	0.342	0.010
	Thigh	67.4 ^a	66.2 ^b	65.2 ^b	0.391	0.001
Σ MUFA/Σ SFA	Breast	0.98 ^a	0.92 ^b	0.89 ^b	0.022	0.016
	Thigh	1.23 ^a	1.17 ^a	1.09 ^b	0.025	0.001
Σ PUFA/Σ SFA	Breast	0.78	0.76	0.76	0.014	0.639
	Thigh	0.83	0.79	0.78	0.016	0.063
Σ UFA/Σ SFA	Breast	1.77 ^a	1.68 ^b	1.65 ^b	0.024	0.009
	Thigh	2.07 ^a	1.97 ^b	1.88 ^b	0.034	0.001

(continued)

Table 5. Continued.

Fatty acid		DIET ¹			SEM	p-value
		CTRL	BSFL10	BSFL20		
DFA / OFA	Breast	2.74 ^a	2.63 ^b	2.59 ^b	0.033	0.012
	Thigh	3.02 ^a	2.84 ^b	2.76 ^b	0.048	0.001
EFA ²	Breast	20.7	20.5	20.6	0.344	0.958
	Thigh	24.2	24.1	24.3	0.351	0.950
AI ²	Breast	0.42 ^a	0.45 ^b	0.47 ^b	0.007	0.001
	Thigh	0.38 ^a	0.42 ^b	0.44 ^b	0.009	0.004
TI ²	Breast	0.95	0.98	0.98	0.01	0.264
	Thigh	0.85 ^a	0.88 ^{ab}	0.90 ^b	0.01	0.027
NVI ²	Breast	1.66	1.62	1.60	0.025	0.189
	Thigh	1.82	1.75	1.72	0.031	0.124

¹Diet: CTRL = Control diet, BSFL10 = Control diet + 10% BSFL, BSFL20 = Control diet + 20% BSFL; ²SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids; DFA/OFA = UFA + C18:0/SFA-C18:0; EFA = total essential fatty acids = C18:2 + C18:3; AI = atherogenicity index; TI = thrombogenicity index; NVI = Nutritional Value Index = (C18:0 + C18:1)/C16:0.

^{a,b,c}different letters show a significant difference between treatments and significantly different values ($p < 0.05$).

relatively modest, with increases of +4.1% and +6.8% for breast and thigh meat, respectively, at the highest BSFL supplementation rate, compared to control group. As live BSFL supplementation result, the MUFA/SFA and UFA/SFA ratios decreased in the groups with the inclusion of BSFL compared to the CTRL group. The reductions were -9.2% (breast) and -11.4% (thigh) for MUFA/SFA, and -6.8% (breast) and -9.2% (thigh) for UFA/SFA at the highest BSFL supplementation rate. Among individual SFA, the proportions of lauric (C12:0) and myristic (C14:0) acids noticeably increased in the breast meat from BSFL-fed chickens, when compared to CTRL-fed birds; the meat from birds fed the BSFL20 diet even showed significantly higher ($p < 0.001$) lauric and myristic acids proportions when compared to the meat of birds fed the BSFL10 diet. This may be influenced by the intake of EE, as shown in Figure 1, which differs between the larvae groups. The SFA increase, although limited in magnitude in this study, could represent a negative aspect for human health (e.g. cardiovascular diseases) but, as reported by different authors (Alves et al. 2017; Xia et al. 2021), medium chain fatty acids (MCFAs), such as lauric and myristic acids, may show beneficial effects as well (i.e. antibacterial, anti-inflammatory and antioxidant properties). In addition, MCFAs, have been shown to increase the expression of genes involved in cholesterol elimination (CYP7A1) (Aprianto et al. 2023) and may contribute to reducing cholesterol level (Osuch et al. 2024). Furthermore, the amount of MUFA n-9, specifically oleic acid (C18:1) and eicosanoic acid (C20:1n-9), was considerably lower in the BSFL20 group in comparison to the CTRL group. These results are in accordance with the breast fatty acid profile presented by de Souza Vilela et al. (2021), who used four different inclusions (5, 10, 15 and 20%) of BSFL in broiler diet, and by Fiorilla et al.

(2024c), who fed slow-growing chickens by supplementing approximately 5% dry matter by live or dehydrate BSFL.

As presented in Table 5, for the thigh, the SFAs capric acid (C10:0), lauric acid (C12:0) and myristic acid (C14:0) increased significantly in BSFL10 and BSFL20 groups as the percentage of live larvae inclusion in the diet augmented. Furthermore, a significant decrease in MUFA ($p = 0.012$) was observed only in the BSFL20 group compared with the other two experimental groups. This reduction is revealed by the significant ($p = 0.005$) in oleic acid (C18:1n-9). Total PUFAs showed no differences within the experimental groups in both breast and thigh. The hypocholesterolemic fatty acids and hypercholesterolemic fatty acids ratio (DFA/OFA) was significantly lower in the BSFL10 and BSFL20 groups compared to the CTRL group, this finding was mainly linked to the increase in SFAs (Daszkiewicz et al. 2022). Moreover, the significant increase in SFAs influenced the Atherogenic Index (AI) which was considerably higher in the BSFL10 and BSFL20 groups than in the CTRL group. On the other hand, the Thrombogenic Index (TI) was significantly higher only in the thigh of the BSFL20 group ($p = 0.027$). These findings differ from the study by de Souza Vilela et al. (2021), which indicated a significant increase in both the AI and the TI in the breast. Despite these observed differences among the experimental groups, the NVI, the PUFA/SFA ratio and the total essential fatty acids did not show significant differences among the experimental groups. This contrasts with the findings of Daszkiewicz et al. (2022), who reported that BSFL meal in broiler chicken diets can have a significant negative effect on NVI and total essential fatty acids. Importantly, the fat composition of the larvae, which directly influences the fatty acid profile of animal products derived from chickens consuming them, can be manipulated through the choice of substrate (Schreven et al. 2021). Therefore, further research is needed to optimise the fatty acid profile of the larvae and, consequently, the quality of derived animal products.

Conclusion

From a practical perspective, the use of live larvae is viable and could promote new feeding practice in poultry production. However, additional studies focusing on life cycle assessment and economic analysis are essential to comprehensively evaluate the advantages of integrating this system into current production practices. The results of this study demonstrate the feasibility of supplementing commercial feed for medium-growing

chickens with live BSFL, up to 20% of the expected weekly feed intake, with minimal modifications to growth performance, slaughter outcomes, and meat quality. Moreover, the FCR of the chickens improved with live BSFL supplementation. Regarding meat quality parameters, the inclusion of live larvae in the diet led to a slight increase in SFA as the supplementation level increased, without altering the nutritional value index or the essential fatty acids profile in the meat.

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Authors' contributions

CT, LZ, LG, MR and AS conceived and designed the experiment. CT, LZ, NGZ, SBO, and IB performed the trial and collected the experimental data. CT, SPM, SBO and LZ analysed the data. CT, AL and SBO carried out the chemical analyses. CT and AS wrote the first draft of the manuscript. All the authors critically reviewed the manuscript for its intellectual content and gave their approval for the final version to be published.

Disclosure statement

The authors declare that they have no competing interests.

Ethical approval

The experimental protocol was approved by the Italian Ministry of Health (authorization n. 659/2020_PR).

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Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

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