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Dehydrated and live black soldier fly larvae as environmental enrichment in indigenous slow-growing chickens: performance, gut health, and chitinolytic enzyme activity

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ABSTRACT

The demand for sustainable and ethically farmed animal products is on the rise as consumers become more environmentally and animal welfare conscious. The need to diminish the consumption of soybean meal is urgent, and companies are looking for ways to respond to this necessity by looking for alternatives to soybean meal. This study assessed the impact of introducing whole dehydrated and live black soldier fly larvae (**BSFL**) into the diet of an indigenous chicken breed as environmental enrichment. A total of 144 39-day-old male Bianca di Saluzzo chickens were distributed among 18 pens and assigned to three different experimental groups. The control group received a diet where soybean meal was entirely replaced by alternative ingredients. The two experimental groups were given the same diet supplemented with 5% of the expected daily feed intake of whole dehydrated BSFL or whole live BSFL. Throughout the trial period (from the bird age of 39–174 days of age), live weight was recorded every 21 days, and the average daily gain, daily feed intake, and feed conversion ratio were calculated. The time required for the birds to consume the larvae was recorded three times a week. At age 147 and 174 days, 12 birds per treatment were selected based on mean live weight and slaughtered. Measurements included hot and chilled carcass weights, organ weights (spleen, liver, heart, stomach), breast and thigh muscle weights, and the corresponding yields were calculated. Acid protease activity was measured in proventriculus extract, and chitinase and chitosanase activity was calculated based on the release of reducing sugars from chitin or chitosan. The results showed little improvement in final live weights and daily feed intakes of the animals fed the insect larvae compared with control birds. Larva supplementation had no negative impact on the overall well-being of the animals assessed by blood analysis and histopathological assessment of the intestinal tract, spleen, and liver. No differences were found between the dehydrated vs live insect larvae consumption times, with all larvae being eaten up very rapidly (< 3 min). The birds fed BSFL showed an increase in chitinase activity. These findings support the potential use of whole BSFL as a form of environmental enrichment, particularly in their dehydrated form, being more convenient to use and store, which would also encourage the uptake of this practice by farmers.

Implications

This research examined the viability of using whole live and dehydrated black soldier fly larvae as environmental enrichment in an indigenous slow-growing chicken breed. The aim was to

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explore the nutritional benefits and effects of these supplementation. Key findings suggest that black soldier fly larvae have no detrimental effects on performance and health of the chickens. Moreover, dehydrated larvae offer practical advantages over live larvae, such as ease of use and storage. These attributes could contribute to more sustainable and welfare-conscious poultry farming practices, aligning with industry efforts to improve sustainability and animal welfare.

Introduction

Modern agriculture is seeing a shift towards the implementation of more sustainable practices due to consumer demand for transparency (Kaygisiz et al., 2019). Intensive livestock rearing practices are heavily dependent on soybean as a feedstuff, which in turn fosters deforestation to meet the industry's demand for this product. By consequence, the feed sector is seeking to reduce its dependency on soy as a way of combatting deforestation and restraining intensive farming practices. Insects, like black soldier flies, are emerging as a possible sustainable protein source for poultry. They are rich in nutrients and can be raised on organic waste, reducing the need for agricultural land, energy, and water resources while repurposing food industry waste (Bellezza Oddon et al., 2021; Lu et al., 2022; Martínez Marín et al., 2023).

Feeding chickens insect meal and whole larvae has been demonstrated to be safe for both the animals and the humans consuming the chicken products. Insect meal and whole un-treated larvae are now permitted within the European Union [Regulation (EU) n. 2021/1372]. Even though currently, according to the IPIFF Guide on Good Hygiene Practices for EU producers of insects as food and feed (2022), the use of treated whole insects (e.g., freeze-dried, dehydrated) is not permitted in poultry feed. Indeed, the rearing of insects for animal feed is subject to rigorous production protocols, including the evaluation of the insects' diet, hygiene, and breeding conditions (Meyer et al., 2021). Nevertheless, this practice must be continually monitored and regulated to ensure that it is carried out in compliance with food safety and health standards, enabling insect use to contribute to a more sustainable and diverse poultry industry. Although insects have been studied in the context of poultry nutrition for several years, research on the use of whole live larvae is still limited, more so than that concerning the use of dehydrated larvae. Indeed, the literature has predominantly focused on the inclusion of insects as meals, mainly focusing on the nutritional aspects and the safety evaluation of its inclusion (Dabbou et al., 2018; Schiavone et al., 2018, 2019; Bertinetti et al., 2019). However, the incorporation of whole larvae in poultry feed also offers the opportunity to consider their use in terms of animal welfare through the promotion of natural behaviours, such as foraging, considered important for psychophysical well-being in chickens (Biasato et al., 2022). Thus, a shift to the use of whole larvae in feed rather than meals is significant as it adds an element of behavioural stimulation that the latter does not offer. It is important to note that comparing the results obtained from the inclusion of meals versus whole larvae can be challenging due to the various variables involved. So far, investigations into the use of insects in poultry farming have mainly involved high−performance conventional farming hybrids, whereas little attention has been directed towards indigenous, slow−growing breeds (Leiber et al., 2017; Murawska et al., 2021). The conservation of indigenous chicken breeds and promoting their use in agriculture, especially organic systems, are important for safeguarding the future possibilities of poultry farming. The evolution of these breeds was strongly shaped by interactions between the natural environment and local communities, with the resulting birds being well adapted to the climatic, feed and management conditions of the regions in which they live (Dal Bosco et al., 2021; Franzoni et al., 2021; Castillo et al., 2021). Their genetic diversity represents a valuable resource for facing future challenges, such as climate change, emerging diseases, and changes in resource availability. The conservation of these breeds not only preserves genetic heritage (Sartore et al., 2016; Cendron et al., 2020; Soglia et al., 2021) but it could also help local agriculture remain resilient and sustainable thanks to the birds' ability to adapt to changing conditions without depending on intensive external inputs (Fiorilla et al., 2023). Furthermore, promoting the farming of native breeds could contribute to a greater awareness of local agricultural traditions and a deeper connection between producers, consumers, and the food we consume. These breeds are also usually dual-purpose breeds, meaning that the hens can be kept as layers and roosters for meat production, solving the ethical problem of killing day-old chicks (Bruijnis et al., 2015). In Italy, one such indigenous breed is the Bianca di Saluzzo, originating from Piedmont. It is known for its good egg production, of around 150–160 eggs per year, and high-quality meat in both hens and roosters. Indeed, the breed is certified as a Slow Food Presidium – a trademark recognising traditional foods of exceptional quality and cultural importance, and which aims to preserve traditional production methods (Soglia et al., 2020; Slowfood, 2023). As medium-sized bird, Bianca di Saluzzo is well-adapted to live in different kinds of environment, and it is particularly suited for small-scale, free-range farming systems (Castillo et al., 2021).

This study aims to study the impact of supplementing the diet of a slow−growing indigenous chicken breed, farmed under organic conditions, with dehydrated or live black soldier fly larvae (**BSFL**). A direct comparison of feeding dehydrated vs live larvae has previously only been performed in commercial broilers (Ipema et al., 2022), and never in a slow−growing chicken such as an Italian indigenous breed. This study focuses on the impact of BSFL dietary supplementation on animal growth, slaughter performance, and health status (i.e., organ histopathology). We looked for any differences between the effects of dehydrated vs live larvae provision to establish whether the easier management of dehydrated specimens could justify their use over live larvae as environmental enrichment into the diets.

Material and methods

Birds, husbandry, and diets

The trial was performed at the poultry facility of the University of Turin (Italy). The experimental protocol (No. 814715) was approved by the Bioethical Committee of the University of Turin.

The animals used in the present trial hatched from the eggs collected at the Avian Conservation Centre for Local Genetic Resources of the University of Turin (Italy). Upon hatching, the chicks were divided according to sex by an expert. Only the males were kept for this trial. All birds were subjected to the same management and environmental conditions, respecting the European Union's regulations for organic farming [Regulation (CE) n. 834/2007]. During the first 38 days of life, the chicks were housed in a brood with controlled environmental conditions: the lighting schedule was 23 h of light and 1 h of darkness on the first day, and from then on, the dark period was gradually increased by 1 h each day, resulting in 18 h of light and 6 h of darkness after 6 days. At 39 days of age, the chicks were individually labelled with a wing mark and selected on the basis of their average BW (316.8 ± 1.4 g on average). A total of 144 birds were transferred to the experimental poultry facility and divided equally between 18 pens (eight birds per pen). Each pen measured 2.0 × 3.2 m and the floor was covered with rice hulls as litter. All birds had unrestricted access to an out-

door area of the same dimensions as the indoor pen. None of the environmental conditions of the poultry house were controlled artificially: temperature, lighting, ventilation, and humidity were all natural. The animals were divided into three groups of six pens (each pen being a replicate). Each group received a different dietary treatment. The control group (**C**) was provided a basal diet characterised by the complete replacement of soybean meal with alternative ingredients (Fiorilla et al., 2024); the two experimental treatments were fed the same basal diet as C with the addition of dehydrated (**DL**) or live BSFL (**LL**) at a level equal to 5% expected daily feed intake of DM: (i) DL group: basal diet + dehydrated BSFL (ii) LL group: basal diet + live BSFL. The trial lasted from May to October 2022; the average temperatures were: May, 20.5 °C (min. 10 °C; max. 31 °C); June, 24 °C (min.15 °C; max. 33 °C); July, 26.5 °C (min. 17 °C; max. 36 °C); August, 27.5 °C (min. 18 °C; max. 37 °C); September, 20 °C (min. 10 °C; max. 30 °C); October, 17 °C (min. 9 °C; max. 25 °C). The trial lasted 135 days, from the chick age of 39 days through to 174 days when the final slaughter was conducted. An intermediate slaughtering of two birds per replicate (total of 36 birds) was also performed at 147 days of bird age.

Black soldier fly larvae management and provision

The dehydrated BSFL were provided by "Entomo Agroindustrial" (Murcia, Spain). The larvae were shipped in two different batches: a week prior to the start of the trial and mid-trial. After shipping, the larvae were stored in a cold chamber at 5 °C in airtight containers. The live black soldier fly larvae were sourced from "Inagro" (Rumbeke-Beitem, Belgium). The larvae were shipped weekly from Belgium in an insulated and protected container equipped with cool bags to maintain a chilled (<16 °C) environment and to prevent their death during the 24−h journey. Upon arrival at the experimental poultry facility, the larvae were separated from their feed substrate and cleaned, then stored in a climatic chamber set at a temperature of 12 °C to induce diapause and ensure that the larvae remained in the same instar stage throughout the entire week. Both Entomo and Inagro companies raised the insects on Gainesville substrate in order to standardise the substrate's composition. This substrate is composed of maize, wheat bran, and alfalfa, and it is widely used as a standard substrate for insect rearing (Osimani et al., 2021; Yamamoto et al., 2022).

Each week, the quantity of feed consumed by the animals was calculated, and the quantity of larvae given was set to provide 5% DM of actual feed consumed. Starting from when birds were aged 43 days, the birds' diet was supplemented with one of the two types of larvae, provided at the same DM amount, according to the larvae DM content (93.9% for dehydrated BSFL; 33.9% for live BSFL). The weekly amounts of larvae provided to the animals are reported in Table S1. The larvae were provided daily, from Monday to Saturday, at 11:00 h. in a feeder with a central bump to disperse the larvae uniformly along its edge. The feeder measured 25 cm in diameter and was used until the birds reached an age of 101 days. It was then replaced with a larger one measuring 35 cm in diameter, more appropriate for use with bigger birds, used until the end of the trial. Following the methods described by Bellezza Oddon et al. (2021), the live BSFL were reactivated before being given to the chickens. The reactivation process involved heating the larvae to a temperature of 28–30 °C for a duration of 10 min. The length of time the birds spent consuming the larvae was recorded 3 days a week using a stopwatch.

Chemical analysis of the experimental diet, the dehydrated and live black soldier fly larvae

The experimental diet, the dehydrated BSFL, and the live BSFL were ground to a particle size able to pass through a 0.5 mm sieve, and then stored in sealed plastic containers. To assess the nutritional composition of the experimental diet, different analyses were carried out following established protocols. The DM, ash, and ether extract content of the diet were determined using AOAC methods with the respective method numbers #934.01, #942.05, #984.13, and #2003.05, as specified by the International AOAC (AOAC International, 2004). The CP content was determined using the Dumas method (Dumas, 1831). The nutrient composition of the diet is presented in Table 1, while that of dehydrated BSFL and live BSFL are presented in Table 2. Finally, to determine the amino acid profile of the diet and the D-BSFL and L-BSFL, a sample was analysed using the method described by (Cohen and Michaud, 1993). The amino acid composition was measured by high-performance liquid chromatography (**HPLC**) after hydrolysis in 6 M HCl for 22 h, at 110 °C, derivatisation with 6-Aminoquinolyl-N-Hydroxy succinimidyl Carbamate and quantification with HPLC and fluorescence detection. All the analyses were performed in triplicate.

Growth performance

Throughout the trial, daily monitoring was conducted to observe any clinical signs of illness and record the mortality rates.

Table 1
Ingredients and analysed chemical composition of the basal diet used to feed indigenous chicken in the trial from 39 to 174 days of age.

Item	Value
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[a]	5.90
TOTAL	1 000
AME (MJ/kg)	11.9
Chemical composition (g/100 g feed)	
DM	91.2
CP	18.1
Crude fiber	3.28
Ether extract	3.63
Amino acid composition (g/100 g CP)	
Alanine	6.53
Arginine	6.53
Aspartic Acid	9.80
Glutamic Acid	17.4
Glycine	8.17
Histidine	2.45
Isoleucine	4.19
Leucine	8.17
Lysine	6.53
Methionine	2.12
Phenylalanine	5.12
Proline	6.53
Serine	4.79
Threonine	3.76
Tyrosine	3.10
Valine	4.79

Note: AME, apparent metabolisable energy, [a]Nutritional additives: Vitamin A 8 001.60UI, Vitamin D3 3 000.60UI, Betaine anhydrous 600.48 mg, Biotin 0.04 mg, Choline chloride 333.07 mg, Folic acid 0.81 mg, Niacinamide 25.01 mg, Calcium pantothenate 7.28 mg, Vitamin B1 0.75 mg, Vitamin B12 0.02 mg, Vitamin B6 1.60 mg, Vitamin E 18.50 mg, Vitamin K3 2.50 mg, Iron (Iron-II sulfate monohydrate) 44.01 mg, Manganese (Manganese-II oxide) 62.01 mg, Selenium (Sodium Selenite) 0.25 mg, Zinc (Zinc sulfate monohydrate) 50.01 mg.

Table 2
The analysed proximate and amino acid composition of dehydrated black soldier fly larvae and live black soldier fly larvae used to feed indigenous chicken.

Item	Dehydrated BSFL	Live BSFL
DM (g/100 g)	93.9	33.9
CP (g/100 g DM)	33.9	43.3
Ether extract (g/100 g DM)	31.4	16.1
Ash (g/100 g DM)	10.8	11.7
Crude fiber (g/100 g DM)	7.98	9.09
Gross energy (MJ/kg)	17.7	14.3
Amino acid composition (g/100 g CP)		
Alanine	3.10	3.73
Arginine	1.83	2.52
Aspartic Acid	3.54	4.41
Glutamic Acid	3.87	5.46
Glycine	2.41	2.98
Histidine	0.99	1.94
Isoleucine	1.70	2.12
Leucine	2.65	3.10
Lysine	2.63	3.36
Methionine	0.76	0.88
Phenylalanine	1.71	1.97
Proline	2.04	2.73
Serine	1.53	2.05
Threonine	1.50	1.92
Tyrosine	2.67	3.21
Valine	2.44	2.97

Every 21 days, the BW of the individual birds and the feed consumption (at pen level) were recorded. The average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**) were calculated every 21 days. These parameters were also calculated for two specific age periods, 39–147 days and 39–174 days, to allow for the comparison of the two different slaughtering ages. The ADFI was calculated and expressed based on the DM content of the feed + the DM of larvae intake. The FCR was adjusted, taking into account the DM consumed by the animals, to include both the feed and larvae, following the approach outlined by Veldkamp and Niekerk (2019), Bellezza Oddon et al. (2021), and Bongiorno et al. (2022). Electronic scales (Radwag, WLC 20/A2) were used for all the measurements.

Slaughtering performance

The chickens were slaughtered at 147 and 174 days of age. The day before each slaughter, all chickens were individually weighed. Two birds (total of 36 birds/slaughter) were selected from each pen based on the pen's average BW. After a 12-h fasting period, the selected birds were re-weighed (referred to as slaughtering weight, **SW**) and then subjected to electrical stunning and bleeding, following the standard regulations of the European Union [Council Regulation (EC) No 1099/2009 of 24 September 2009]. The plucked and eviscerated carcasses were weighed to determine the weight of the hot carcass. Upon removal of the head, neck, and feet, the weight of the prepared, ready-to-cook carcass (**RTCC**) was recorded. The weights of the spleen, liver, heart, and glandular stomach were immediately measured, and their weights were expressed as a percentage of the SW. The gizzard was emptied and then weighed. The hot carcass and RTCC yields were then calculated as percentages of the SW. The chilled carcass (**CC**) weight was registered after storing it at + 4 °C for 24 h. The CC yield was calculated as a percentage of the RTCC carcass. The breasts and thighs were then separated, and their weights were expressed as percentages of the CC weight.

Blood analysis

During slaughtering at 147 and 174 days of age, blood samples were taken from the selected animals via the jugular vein. A volume of 2.5 mL of blood was collected in an EDTA tube, and an additional 2.5 mL was placed in a serum−separating tube. A blood smear was prepared using an untreated droplet of blood. The total counts of red blood cells (erythrocytes) and white blood cells (leukocytes) were determined using an improved Neubauer hemocytometer after a 1:200 dilution with a Natt-Herrick solution, following the method reported by Natt and Herrick (1952). For the analysis of cell morphology, blood smears were subjected to staining using May-Grünwald and Giemsa-Romanowski stains. The blood placed in the serum−separating tubes underwent clot formation for approximately 2 h at room temperature. Afterwards, the tubes underwent centrifugation at 700 g for 15 min, and the resulting serum was frozen at −80 °C. The serum aliquots were subjected to clinical chemistry analysis (lipid and protein profile and liver function tests) using an automated photometer (BT 1500 vet-Futurlab) following the manufacturer's instructions.

Histopathology of the intestinal tract segments and other organs

At the 147 and 174 days of age slaughtering, segments of the gut, approximately 5 cm in length, were sampled from the duodenum, jejunum, and ileum from 12 animals per group during the slaughtering process. These segments were flushed with a 0.9% saline solution to clear out their contents. The specific intestine segments collected were the duodenal loop, the tract prior to Meckel's diverticulum (jejunum), and the tract before the ileocecum junction (ileum). Moreover, samples from the spleen (the entire organ) and the left lobe of the liver were collected (0.5–1.5 g/organ). All the samples were fixed in a 10% buffered formalin solution, embedded in paraffin wax blocks, sliced into 5-µm sections, fixed onto glass slides, and stained using Haematoxylin & Eosin for histological evaluation. Histopathological changes were evaluated such as white pulp hyperplasia and depletion in the spleen, hepatocyte degeneration, and lymphoid tissue activation in the liver (Biasato et al., 2016). Regarding gut histopathological findings, the mucosa and submucosa of each gut segment were assessed for inflammatory infiltrates and gut-associated lymphoid tissue activation. All the observed histopathological alterations were evaluated using a semiquantitative scoring system as follows: absent (score = 0), mild (score = 1), moderate (score = 2), or severe (score = 3). The total score of each gut segment was obtained by adding up the mucosa and submucosa scores. All slides were evaluated blindly by three different observers, and any discrepancies were resolved through collective review using a multi-head microscope until a unanimous consensus was achieved.

Activity of digestive enzymes

The following evaluations were performed on samples taken at 174 days of age. Acid protease activity was measured in proventriculus extract following the method described by Anson (1938) using haemoglobin as substrate. Protease activity was described as the micrograms of tyrosine produced per minute and per milligram of protein. Chitinase and chitosanase activity in proventriculus extract was calculated based on the release of reducing sugars from chitin or chitosan measured with 3.5-dinitrosalicylic acid assay (Miller, 1959). Endochitinase activity was obtained by incubation of 1% colloidal chitin (w/v) suspended in acetate buffer (50 mM, pH 5) with proventriculus enzyme extract for 60 min at 37 °C. Exochitinase Activity was described as the µmol of *N*-acetyl-D-glucosamine released per hour and per milligram of protein. *N*-acetyl-*β*-D-glucosaminidase activity was measured following the method described by Koh and Iwamae (2013). Activity was described as the nmol of p-nitrophenol released per minute and per milligram of protein at 37 °C. Chitosanase activity in proven-

triculus extract was measured by incubation of chitosan dissolved in a 1% acetic acid solution with proventriculus enzyme extract for 60 min at 37 °C (Doan et al., 2018).

Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics V28.0.1.0 software (IBM). Each pen (n = 6 per treatment) was treated as an experimental unit. On the contrary, the animal was treated as the experimental unit for all analyses concerning post-slaughter samples. The normality or non-normality of distribution was assessed through the Shapiro–Wilk's test, and the homogeneity of variance was determined using Levene's test. Growth performance at 147 and 174 days of age, slaughtering performance, haematological traits, and larvae consumption time were analysed using a generalised linear mixed model. This model incorporated two fixed factors: diet, time, and the interaction between diet and time. The periodic growth performance was analysed with a GLM. To account for repeated measurements within the same pen, the replicate was introduced as a random effect. Additionally, the age of the animals was included as a covariate to adjust for its potential influence on the growth performance. Liver enzymes were analysed using a generalised linear mixed model to investigate differences within treatments at 174 days of age. The scores for histopathological evaluations were analysed by fitting a generalised linear mixed model with a negative binomial distribution. The results were reported as the least square mean along with the SEM. P values $\leq$ 0.05 were considered as statistically significant.

Results

Growth performance

Birds' mortality was monitored daily throughout the trial, and only one bird died during the trial, belonging to the DL group. The overall growth performance data at 147 and 174 days of age are presented in Table 3. The results reveal differences between the groups in terms of weight (C: 2 335 g; DL: 2 440 g; LL: 2 412 g; $P < 0.05$), with the DL and LL groups having higher final

Table 3
The growth performance of an indigenous slow-growing chicken breed fed a diet supplemented with dehydrated and live black soldier fly larvae; supplementation based on the expected daily feed intake (39–147; 39–174 d) (means; n = 6).

	Diet			Age		SEM	*P*-value		
Item	C	DL	LL	147d	174d		Diet	Age	D × A
BW (g)	2 335[b]	2 440[a]	2 412[a]	2 251	2 541	20.605	0.029	<0.001	0.848
ADG (g/d)	16.6[b]	17.5[a]	17.2[a]	17.9	16.2	0.169	0.044	0.031	0.991
ADFI (g/d)	56.8[b]	58.1[a*]	58.6[a*]	57.9	57.7	0.413	0.023	0.690	0.998
FCR (g/d)	3.51	3.37	3.48	3.24	3.67	0.046	0.114	0.019	0.544

*ADFI = DL, 54.8 g (feed intake) + 3.35 (dehydrated BSFL); *LL = 55 g (feed intake) + 3.59 (live BSFL); [a], [b]: $P < 0.05$.
Abbreviations: ADG = average daily gain; ADFI = average daily feed intake (on a DM basis); FCR = feed conversion ratio (on a DM basis, including the larvae intake); C = control; DL = dehydrated larvae; LL = live larvae; DxA = interaction diet/age; [a], [b]: $P < 0.05$.

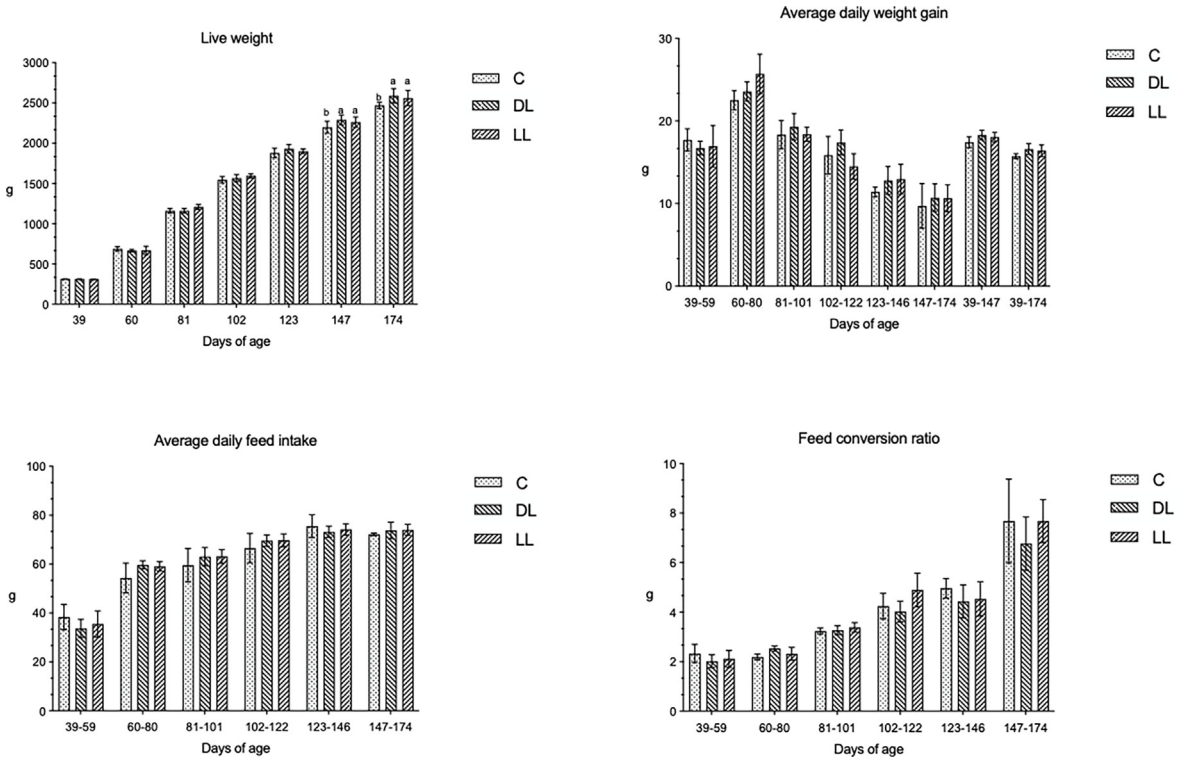

Fig. 1. Periodic growth performance of an indigenous slow-growing chicken breed fed a diet supplemented with dehydrated and live black soldier fly larvae (BSFL); supplementation based on the expected daily feed intake. C = control; DL = dehydrated larvae; LL = live larvae; a, b: $P < 0.05$.

weights compared with C (around + 4%). A similar result was observed for ADG and ADFI, which were higher in the DL and LL treatment groups compared with C ($P < 0.05$). By contrast, no differences were observed between groups in relation to the FCR.

In the comparison of the two slaughtering ages, as expected, the final BW was higher at 174 days compared with that at 147 days of age ($P < 0.05$), although the animals showed a decrease in ADG after 147 days of age ($P < 0.05$). Moreover, the FCR also differed at the two slaughtering ages, with higher values detected at latest slaughtering age ($P < 0.05$). Statistical analysis was also conducted on the periodic measurements (taken every 21 days), and the data are visually represented in Fig. 1. A more in-depth examination of the data and the results of statistical analyses is presented in Table S1. Overall, no differences were found between the six recorded periods (39–60, 60–81, 81–102, 102–123, 123–147) for BW, ADG, ADFI, or FCR.

Larvae consumption

The exact amount of dehydrated BSFL and live BSFL provided to each chicken based on the daily feed intake and expressed as g of larvae as fed and, on a DM basis is reported in Fig. 2 and Table S2. The time the birds spent eating the dehydrated and live larvae during each of the six recording periods is reported in Fig. 3. Overall, no differences were highlighted between the two experimental treatments, with similar times spent consuming the two different types of larvae (DL: 2.17 min; LL: 2.26 min on average). On the contrary, when all six age periods are considered, the time spent eating the larvae (dehydrated and live larvae) was the greatest in the periods: 39–59 (2.57 min), 102–122 (2.39 min), and 123–146-days of age (2.44 min) ($P < 0.05$) compared to the other three recording periods.

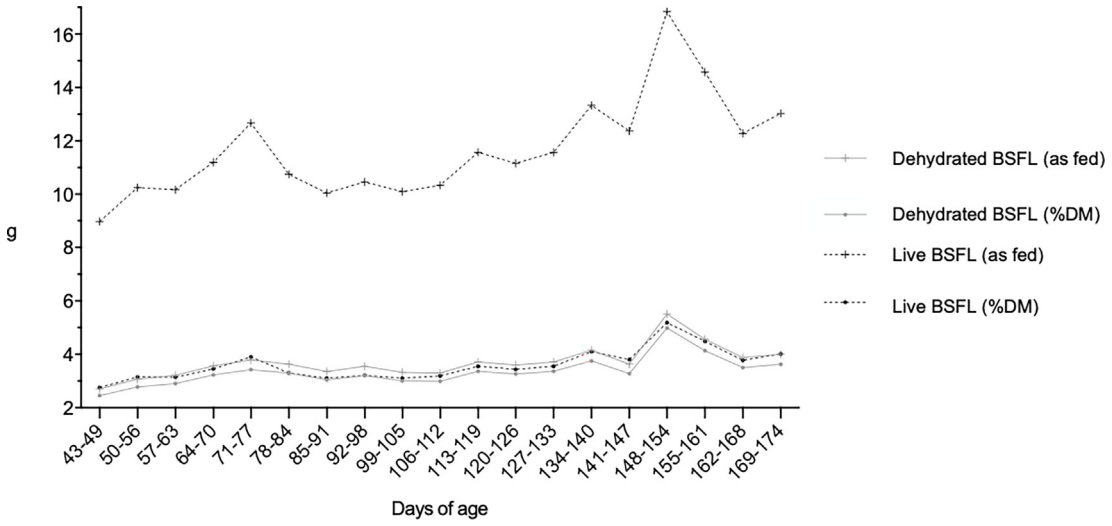

Fig. 2. Weekly amount of dehydrated and live black soldier fly larvae (BSFL) provided to an indigenous slow-growing chicken breed. Values are expressed as g of larvae as fed and g of larvae/DM.

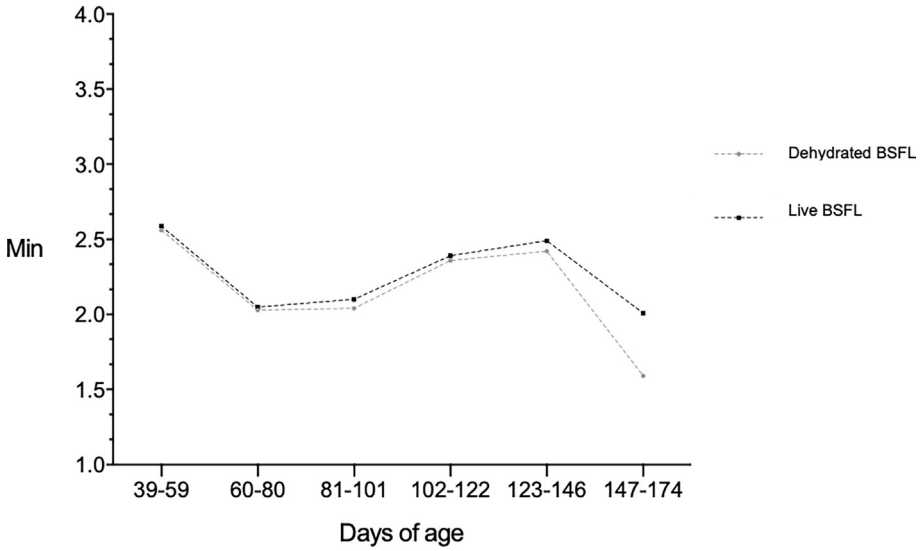

Fig. 3. Time spent by an indigenous slow-growing chicken breed eating dehydrated and live black soldier fly larvae (BSFL) (means, n = 6).

Slaughtering performance and blood analysis

The data related to slaughtering performance are reported in Table 4. 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 SW, being 11.3% higher at 174 than at 147 days of age ($P < 0.05$). Moreover, the glandular stomach and gizzard relative weights were 12.9 and 23.1% lower at 174 days than at 147 days of age, respectively ($P < 0.01$). None of the other slaughtering parameters were affected by BSFL provision (live or dehydrated).

As reported in Table 5, the provision of dehydrated BSFL and live BSFL had no overall impact on most of the haematological traits, on serum proteins and lipids, serum minerals, liver, or renal enzymes. Cholesterol, triglycerides, and chlorine values were affected by age only, being +18.7, + 27.2, and +6.8%, at 174 compared with 147 days of age, respectively ($P < 0.05$). Furthermore, gamma-glutamyl transferase and aspartate-aminotransferase values were also only affected by the age of the birds, being +14 and +10.4% at 174 days of age compared with 147 days, respectively ($P < 0.05$).

Histopathology of intestinal tracts and organs

Table 6 shows the histopathological alterations recorded in the main organs. Dietary inclusion of insect larvae did not affect the severity of the histopathological scores in any of the organs. Regardless of diet, the liver showed mild to moderate, multifocal to diffuse vacuolar degeneration of the hepatocytes, as well as mild to moderate, multifocal lymphoplasmacytic infiltrates. In the gut, we observed mild to moderate, multifocal to diffuse, mucosal lymphoplasmacytic infiltrates with or without gut-associated lymphoid tissue activation. The spleen showed mild multifocal white pulp hyperplasia. However, overall, there were no statistically sig-

Table 4
The slaughtering performance of an indigenous slow-growing chicken breed fed a diet supplemented with dehydrated and live black soldier fly larvae; supplementation based on the daily feed intake (means, n = 6).

	Diet			Age		SEM	*P*-value		
Item	C	DL	LL	147d	174d		Diet	Age	D × A
Slaughter Weight (SW) (g)	2 286[b]	2 413[a]	2 366[a]	2 230	2 480	33.225	0.013	<0.001	0.513
RTCC (SW %)	65.1	65.3	65.2	65.3	65.1	0.433	0.997	0.927	0.539
CC (RTCC%)	97.5	96.8	97.2	96.8	97.5	0.265	0.171	0.114	0.141
Legs (CC %)	35.5	35.6	35.2	35.1	34.7	0.647	0.756	0.158	0.598
Leg Meat (CC %)	14.1	14.2	14.3	13.9	14.4	0.138	0.729	0.112	0.093
Breast (CC %)	15.5	14.9	16.1	16.2	14.8	0.592	0.367	0.061	0.210
Spleen (SW %)	0.19	0.19	0.18	0.20	0.19	0.009	0.878	0.200	0.329
Liver (SW %)	1.55	1.56	1.57	1.53	1.59	0.060	0.975	0.354	0.655
Heart (SW %)	0.53	0.50	0.53	0.52	0.51	0.015	0.224	0.437	0.547
Gizzard (SW %)	2.07	2.16	2.06	2.24	1.95	0.077	0.559	0.004	0.120
Glandular Stomach (SW %)	0.34	0.34	0.36	0.39	0.30	0.013	0.320	0.002	0.668

Abbreviations: C = control; DL = dehydrated larvae; LL = live larvae; DxA = interaction diet/age; RTCC = ready to cook carcass; CC = cold carcass [a], [b]: $P < 0.05$.

Table 5
The haematological traits, serum proteins and lipids, minerals and parameters related to liver function of an indigenous slow-growing chicken breed fed a diet supplemented with dehydrated and live black soldier fly larvae; supplementation based on the daily feed intake (means, n = 6.).

	Diet			Age		SEM	*P*-value		
Item	C	DL	LL	147d	174d		Diet	Age	D × A
Haematological Traits									
Erythrocytes (10^6 cell/L)	3.52	3.35	3.62	4.08	2.92	0.392	0.868	0.058	0.731
Leukocytes (10^3 cell/L)	37.2	38.9	49.6	41.3	42.5	4.288	0.080	0.786	0.851
Heterophils (%)	44.3	47.0	44.4	43.1	47.5	2.668	0.668	0.122	0.338
Lymphocytes (%)	50.2	47.1	49.6	50.3	47.7	2.531	0.624	0.337	0.282
Monocytes (%)	2.45	2.59	2.32	2.88	2.03	0.399	0.874	0.068	0.209
Eosinophils (%)	0.18	0.23	0.18	0.30	0.09	0.110	0.937	0.073	0.427
Basophils (%)	2.86	2.95	3.50	3.48	3.23	0.328	0.280	0.332	0.570
Serum proteins and lipids (mg/dL)									
Cholesterol	110	113	114	102	122	3.713	0.732	<0.001	0.852
Triglycerides	40.6	47.9	42.1	38.3	48.7	4.455	0.418	0.029	0.097
Creatinine	0.11	0.11	0.11	0.12	0.11	0.005	0.485	0.092	0.517
Uric acid	4.62	4.71	5.03	4.62	4.28	0.340	0.108	0.353	0.106
Total Protein	4.45	4.40	4.34	4.21	4.58	0.083	0.603	0.062	0.425
Minerals (mg/dL)									
Phosphorus	5.68	5.43	5.74	6.04	5.89	0.197	0.212	0.131	0.334
Calcium	9.81	9.87	10.1	10.3	9.59	0.362	0.445	0.090	0.296
Chlorine	115	115	119	113	121	2.288	0.341	0.002	0.192
Magnesium	2.32	2.27	2.30	2.37	2.21	0.041	0.601	0.104	0.707
Liver function (U/L)									
GGT	32.4	33.9	33.4	31.1	35.4	8.752	0.575	0.001	0.793
AST	229	230	229	218	241	6.546	0.983	0.001	0.372
ALT	4.41	3.73	4.18	3.88	4.33	0.302	0.216	0.160	0.081

Abbreviations: C = control; DL = dehydrated larvae; LL = live larvae; DxA = interaction diet/age; GGT = gamma-glutamyl transferase; AST = aspartate-aminotransferase; ALT = alanine-aminotransferase.

Table 6
The histopathology evaluation (score 0–3*) of an indigenous slow-growing chicken breed fed a diet supplemented with dehydrated and live black soldier fly larvae; supplementation based on the daily feed intake (means, n = 6).

	Diet			Age		SEM	*P*-value		
Item	C	DL	LL	147d	174d		Diet	Age	D × A
Duodenum (Inflammation)	1.27	1.18	1.29	1.33	1.22	0.091	0.550	0.257	0.101
Jejunum (Inflammation)	1.06	0.77	0.83	1.01	0.83	0.124	0.275	0.155	0.123
Ileal (Inflammation)	1.35	1.31	1.41	1.59	1.22	0.118	0.482	0.082	0.233
Liver (Inflammation)	0.61	0.52	0.52	0.54	0.63	0.062	0.244	0.288	0.384
Liver (Degeneration)	0.14	0.21	0.18	0.23	0.12	0.080	0.953	0.193	0.427
Spleen (Hyperplasia)	0.12	0.28	0.33	0.27	0.24	0.075	0.211	0.702	0.606
Spleen (Depletion)	0.02	0.02	0.03	0.03	0.04	0.028	0.290	0.413	0.427

Abbreviations: C = control; DL = dehydrated larvae; LL = live larvae; DxA = interaction diet/age.
* score = 0; absent, score = 1; mild, score = 2; moderate, score = 3, severe.

Table 7
The digestive enzymes of an indigenous slow-growing chicken breed fed a diet supplemented with dehydrated and live black soldier fly larvae; supplementation based on the daily feed intake (means, n = 6).

Item	C	DL	LL	SEM	*P*-value
Acid Protease	14.9	13.3	14.0	1.022	0.586
EndoChitinase	0.49[b]	0.85[a]	1.08[a]	0.126	0.002
ExoChitinase	9.28[b]	14.6[a]	12.9[a]	1.289	0.046
Chitosanase	2.15	2.06	2.68	0.234	0.146

Abbreviations: C = control; DL = dehydrated larvae; LL = live larvae; [a], [b]: $P < 0.05$.

nificant differences between the lesions observed in the organs from birds of the three experimental treatments.

Analysis of digestive enzymes

Table 7 illustrates the activity of digestive enzymes analysed from the proventriculus of the experimental chickens at 174 days of age. Total acid protease activity remains unaltered in the experimental treatments when compared to the control. The enzymes exhibiting chitinolytic activity, namely endochitinase and exochitinase (β-N-acetylglucosaminidase), showed a difference when BSFL are incorporated into the diet, leading to increased activity for both dehydrated BSFL and live BSFL diets compared to the control ($P < 0.05$). Moreover, the inclusion of BSFL in the diet did not result in significant alterations in chitosanase activity.

Discussion

Indigenous slow-growing breeds, with their ability to adapt to the surrounding environment and their slower growth rate, represent a valuable genetic heritage that should be preserved. In this context, the inclusion of dehydrated BSFL and live BSFL in their diet is associated with various advantages, from optimising food efficiency to animal welfare and environmental sustainability. The dietary inclusion of whole insect larvae, particularly BSFL, has been widely investigated in chickens, and differences in the findings are largely dependent on the chicken genotype and variability in larvae composition. For instance, growth performance results similar to those reported here were observed in broiler chickens in a study investigating 5% inclusion of live BSFL as environmental enrichment (Bellezza Oddon et al., 2021). Moreover, in the study conducted by Bongiorno et al. (2022) on the effects of 10% inclusion of live BSFL in both male and female slow−growing (label naked neck) chickens, the authors reported no effects of the inclusion among chickens of the same sex. However, other studies identified improved growth performance in birds fed a supplement of whole BSFL. In particular, the study by Ipema et al. (2022), the only one to focus on dehydrated larvae, found significantly improved growth performance in broilers receiving an 8% inclusion of dehydrated BSFL compared with the control group. Other studies similar to ours have been conducted in other avian species to examine the effects of feeding whole BSFL. For example, Veldkamp and Niekerk (2019) investigated 10% live BSFL supplementation in turkeys and found growth performance to be significantly improved in these birds. On the contrary, in a study in ducks, (Gariglio et al., 2023) found no difference in the BW of Muscovy ducks fed live BSFL at a level of 5% supplementation. In the present study, we detected a + 3.64% difference in BW and a + 4.28% difference in ADG in DL and LL treatments compared with the C group ($P < 0.05$) when considering the whole experimental period (39–174-days of age, Table 3). The positive effect of BSFL supplementation was most evident in the last 30–40 days of the trial, at 147 and 174 days of age. This finding represents a unicum as it is the only study in the literature considering BSFL supplementation in an indigenous slow-growing chicken. To understand the overall growth performance of these birds better, in this study, the ADFI results were expressed on the basis of the DM of the feed plus the DM of the insect larvae in order to better explain and take the larvae ingested into account, and this same value was used for calculating the FCR, allowing us to better understand the role of the larvae supplementation. To conclude, the absence of any differences in the FCR between the three experimental groups of the present trial demonstrates that neither dehydrated nor live BSFL supplementation has a negative impact on animal growth.

Although insect larvae form as an integral part of the chicken's natural diet, as documented in previous studies, the results of the larvae consumption time analysis provide an intriguing contribution to our understanding of the feeding habits of these birds (Schiavone and Castillo, 2024). The studies administering larvae as environmental enrichment have predominantly focused on live larvae, presumably due to the belief that larval movement could play a crucial role in attracting the bird's attention and stimulating interest in the food (Pichova et al., 2016). However, our study found no significant differences between the consumption times of DL and LL. Until now, the only study to make a direct comparison of dehydrated BSFL and live BSFL (8% inclusion) was that by Ipema et al. (2022) in broiler chickens, in which the larvae were

either administered in the feeder or in the litter, and the time the birds spent eating the larvae was not recorded. Surprisingly, our study showed Bianca di Saluzzo chickens to spend the same short amount of time–consuming dehydrated BSFL completely as observed for the live ones, consuming them rapidly despite the absence of any movement (2.2 min DL; 2.3 min LL). This raises important questions about the traditional understanding of triggering factors for food attraction in animals and their response to sensory stimuli. In particular, faster consumption times were observed compared with the results obtained in a study on the supplementation of 5% live BSFL in broilers conducted by Bellezza Oddon et al. (2021) and those obtained by Bongiorno et al. (2022) supplementing with 10% live BSFL in label naked neck chickens. This might be attributed to the fact that the present study used an indigenous slow–growing breed, which could hypothetically retain primal instincts typical of this species. These instincts, rooted in their genetic heritage, might drive them to recognise and consume food sources more instinctively than commercial hybrids. Indeed, indigenous chicken breeds, often characterised by their adaptability and resilience, could have preserved behavioural traits refined through generations of natural selection and minimal human intervention (Mauldin, 1992; Meuser et al., 2021). This could lead to a heightened responsiveness to new foods, such as BSFL, be them alive or dehydrated. Additionally, it is worth considering that all the animals involved in the study were roosters. Indeed, also in the study conducted on label naked neck by Bongiorno et al. (2022), which considered both hens and roosters, shorter consumption times were recorded for males than for females. This is likely due to the phenomenon of pen competition exhibited by the animals. The observation that chickens rapidly and eagerly consumed both forms of larvae from the first day of supplementation (starting at 43 days of birds age) offers new perspectives for the use of insect larvae in poultry farming. These findings suggest that BSFL, independent of whether they are alive or dehydrated, can serve as a highly palatable dietary supplement for chickens (Khusro et al., 2012; Makkar et al., 2014), offering a flexible and potentially sustainable option for their integration into poultry diets, with the added benefit of the dehydrated form offering a safer and easier to manage product. Dehydrated larvae can be stored at room temperature and are ready to use, contrary to live larvae which must be reactivated to bring them out of diapause prior to use (Bellezza Oddon et al., 2021). Dehydrated larvae can also be shipped both more easily and safely than live larvae. Additionally, following the drying process, they are completely separated from the rearing substrate and produce no additional organic material, allowing for more convenient and safer storage and utilisation. The key difference lies in the water content of the larvae themselves: while live larvae are composed of approximately 70% moisture (Arabzadeh et al., 2022), the dehydration process reduces this to less than 10%, facilitating their long-term storage and transportation without the risk of deterioration or degradation. Reducing the moisture content also helps to preserve the nutritional quality over time, allowing for more flexible use in multiple contexts (Prescott, 1920). Moreover, biosecurity is a crucial aspect to consider when dealing with both live and dehydrated larvae. Live larvae, being living organisms, may pose a challenge from a biological risk management perspective. By contrast, dehydrated larvae offer a significant biosecurity advantage because before being processed into this form, they can undergo processes of sterilisation and quality control to eliminate or significantly reduce risks associated with pathogens (Chitrakar et al., 2019). This pre-market screening capability makes them a safer choice for many applications. Therefore, in the context of biosecurity, dried larvae emerge as the preferable option to harness the benefits of insects without compromising safety.

Analysing the *in vivo* performance and the adaptation of chickens to insect larvae is important, but their slaughtering performance and overall blood traits offer other equally important data that enable conclusions to be drawn on as many aspects as possible. In this study, no significant differences were identified between the three dietary treatments in slaughtering performance and blood analysis results. Whilst we observed no significant influence of experimental treatment, differences in the assessed traits were closely related to the age of the animals. In agreement with a previous study (Soglia et al., 2020), the birds of the present trial reached the growth plateau phase at around 150 days of age, at which point their rate of growth reduced significantly. This finding occurred across all the animals in the trial and was not attributable to the provision of BSFL. Overall, the decrease of 22.5% in glandular stomach yields and the 12.9% reduction in gizzard yields observed between 147 and 174 days of age can be attributed to the typical physiological processes that occur during the final stages of organ growth. During this period, internal organs complete their growth phase while the growth of muscle and overall body mass continues along their allometric trajectory (Alshamy et al., 2018). Moreover, the results of organ histopathology showed no differences between the birds belonging to the control or experimental groups. In particular, no lesions were found in the different segments of the intestinal tract, liver, or spleen, in line with previous studies on the safety of the insect larvae inclusion at percentages lower than 20% (Neumann et al., 2018). Moreover, in accordance with previous studies, the supplementation of whole dehydrated and live BSFL did not influence the blood parameters in terms of haematological traits, serum proteins and lipids, minerals, liver function, or immunoglobulins (Bellezza Oddon et al., 2021). Moreover, the results of this study demonstrate that in indigenous slow–growing male chickens, there are no significant differences between DL and LL groups; thus dehydrated BSFL, which are associated with more favourable practical and management aspects, provide an equally valid alternative to live larvae. With regards to the serum protein and lipids observed at the two slaughter ages assessed in the present trial, the higher levels of cholesterol, gamma-glutamyl transferase, and alanine-aminotransferase found at 174 days of age with respect to 147 days of age are probably typical of their stage of physiological growth (Rezende et al., 2021). These values tend to rise with age, once muscle development and hormonal fluctuations have reached a stable point (Bueno et al., 2017). Furthermore, all the animals of this trial were roosters, which in this breed reach peak sexual maturity around the time of the later slaughtering age assessed in this study (174 days of age). As a matter of fact, this hormonal phenomenon could greatly impact physiological processes in these birds (Grosse and Craig, 1960; Odula Olwande et al., 2010). Finally, the activity of proventriculus enzymes demonstrates a significant impact from the incorporation of both dehydrated and live BSFL on enzymes possessing chitinolytic capabilities, while protease activity was not affected by dietary treatments. The presence of chitinases in the avian proventriculus has been documented in prior studies (Jackson et al., 1992; Han et al., 1997; Koh and Iwamae, 2013; Tabata et al., 2018). Our findings further indicate that the inclusion of dehydrated or live BSFL, known for their chitin-rich exoskeletons (Triunfo et al., 2022), enhances the activity of chitinolytic enzymes.

While there is currently no research specifically examining how the inclusion of chitin affects chitinolytic enzyme activity in chickens fed insect larvae, the natural response of the animal of this trial allowed them to develop chitinolytic enzymes, ultimately playing a role in improving insect digestibility. This phenomenon could facilitate the access of proteolytic enzymes to the protein within the chitin-protein complex (Hossain and Blair, 2007; Sangkaew and Koh, 2021) showcasing an extraordinary example of these birds'

natural response. Although the use of dehydrated larvae is not currently permitted within the European Union, studies like ours are fundamental to provide the scientific data that enable policymakers to encourage legislative changes to allow dehydrated larvae to be used in animal nutrition, as was also the case for bringing about Regulations (EU) 2017/893 and 2021/1372.

Conclusions

The overall results of this study emphasise how BSFL, whether in the live or dehydrated form, may be suitable to apply in slow-growing chicken farming. The integration of whole insects' larvae is being widely addressed for their potential to enhance the nutritional composition of feed. Such studies contribute to a growing body of evidence in favour of the integration of insects into poultry feed. The birds of this trial showed an incredible adaptability to the supplementation of black soldier fly larvae, even developing specific enzymes to better digest the larvae. Our results also validate the importance of future research into the use of dehydrated larvae over live larvae as they have the potential to overcome numerous biosafety-related barriers, which consider introducing live organisms into poultry farms risky. The recently approved EU Regulation on the use of insect proteins in poultry and pig feed (EU Regulation No. 2021/1372) is expected to generate a strong demand for insect-derived ingredients in animal nutrition and lead to an increase in the production capacity of the EU insect sector, which will greatly expand the potential market for insect-derived products in poultry feeding. As research in this field continues to expand, the broader implications on poultry nutrition and production will also require further investigation to shed even more light on the possibilities that insect-derived nutrition offers to the poultry industry.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2024.101239.

Ethics approval

The animal study was reviewed and approved by the Bioethical Committee of the University of Turin (Italy) Via Verdi 8, 10124, Turin (Italy) (Prot. No. 814715).

Data and model availability statement

The authors declare that data are not deposited in an official repository. The data that support the study findings are available upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

The authors declare that they have no conflicts of interest.

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