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Potential of *Drosophila melanogaster* (fruit fly) as a dietary protein source for broilers

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Abstract

This study was conducted to systematically assess and compare the fluctuations in crude protein (CP), crude fat (CF), and mineral content of staged (larva to adult) *Drosophila* (fruit fly) to that of a market-purchased black soldier fly larvae (BSFL) product. Results suggested that the relative CP content by dry matter ranged from 40.11% to 53.73% during *Drosophila* development, significantly higher ($P < 0.001$) than the 36.90% in BSFL. The relative CF was higher in BSFL (39.14%) compared to that of *Drosophila* (27.03–30.10%, $P < 0.001$). Although both insects contained sufficient levels of minerals to meet the dietary requirements of most animals, *Drosophila* overall possessed a lower content of iron, sodium, and calcium ($P < 0.001$) with a higher gross energy than the BSFL ($P < 0.01$). Comparative studies of amino acid (AA) and fatty acid (FA) profiles were further carried out among *Drosophila* larva (DL), pupa, and BSFL for their economic effectiveness. The AA spectra of insect larvae generally were similar except that the DL was higher in certain AA such as lysine ($P < 0.01$), which is an essential AA often critical for chicken growth. In contrast, the BSFL included more essential FA such as linoleic (C18:2, ω-6) and linolenic (C18:3, ω-3) acids ($P < 0.01$). To follow up, a husbandry trial was performed by allotting 120, 1-d-old, weight-matched, Arbor Acres broilers at random into treatment groups consisting of a low-protein diet background that contained ~20% CP supplemented with 4% BSFL and 4% or 8% DL. The average daily growth (ADG) and average daily feed intake (ADFI) of broilers, compared to the control low-protein diet, were significantly improved by feeding DL diets ($P < 0.01$), with better live and carcass weight and higher muscle pH ($P < 0.001$), which were positively correlated with the inclusion level of DL ($P < 0.001$). However, no differences between the control and 4% BSFL diet were observed for the performance parameters mentioned above. Moreover, all birds under our experimental setting exhibited a comparable feed conversion ratio (FCR) and were in a healthy status as indicated by the meat traits and hematological indexes within normal physiological ranges. Collectively, the findings in this study provide a theoretical basis for the further exploitation of *Drosophila* as potential dietary ingredients for feed production in order to meet the food challenge in the future.

Lay Summary

Insects are regarded as one of the most promising protein sources for feed production due to its high nutritional value and low environmental cost. The objectives of this study were to analyze the dynamic nutritional composition of *Drosophila* (fruit fly) at various developmental phases in parallel with a commercial black soldier fly larvae (BSFL) meal, as well as to determine the effect of diets with their inclusion on broilers. Results showed that *Drosophila* larvae possessed a higher crude protein and a lower crude fat content when compared to the BSFL product. In the feeding trial, the performance of broilers receiving *Drosophila* diets was remarkably improved, with no significant influence on bird metabolic status and meat quality, except the pH of breast and thigh muscles in *Drosophila* diet groups being higher than that of the control group, but still in the normal range. To sum up, *Drosophila* meal evaluated herein has a good nutritional composition and thereby elicits a beneficial impact on the growth performance and meat production of broilers, making it a potential dietary protein source for poultry.

Key words: black soldier fly, broiler, dietary protein, *Drosophila*, insect larvae

Abbreviation: AA, amino acid; ADFI, average daily feed intake; ADG, average daily growth; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate amino transferase; BSF, black soldier fly; BSFL, black soldier fly larvae; CF, crude fat; CP, crude protein; DA, *Drosophila* adult; DFD, dark, firm and dry; DL, *Drosophila* larva; DP, *Drosophila* pupa; EAA, essential amino acid; FA, fatty acid; FCR, feed conversion ration; GE, gross energy; GLB, globulin; GLU, glucose; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; LW, live weight; MUFA, monounsaturated fatty acid; NEAA, nonessential amino acid; PSE, pale, soft and exudative; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TAA, total amino acid; TCHO, total cholesterol; TFA, total fatty acid; TG, triglyceride; TP, total protein; UA, uric acid

Introduction

The global need to ensure sufficient meat production for an expanding human population has led the modern poultry industry to adopt intensive indoor production systems. Under such a system, soybean meals are the major protein sources in poultry nutrition, whereas in nature birds sometimes also feed on insects in forms such as larva, pupa, and adult. Insect-derived proteins are nutritious and highly digestible and could promote the health of animals that consume them (van Huis, 2013; Makkar et al., 2014). Therefore, the use of insect meals as a protein additive in diet formulations could be potentially beneficial in poultry production (Gasco et al., 2019; Smetana et al., 2019). Moreover, insect products for chicken farming have been documented to have a lower environmental impact, and are considered to be an alternative protein and energy source that eases both competitions for food crops with humans and many ecological concerns such as water and land

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use, deforestation, and greenhouse gas emissions (Mungkung et al., 2013; Escobar et al., 2020).

In response to this situation, the utilization of insect materials in poultry diets has become a topic of growing interest. Although their nutritional composition might vary depending on the species and rearing substrates, insect meals overall are characterized by being rich in protein and fat (approximately 23–76% proteins and 10–50% lipids on a dry matter basis), which are the main sources of energy as they have the highest caloric value among all ingredients (Oonincx et al., 2015; Smetana et al., 2019; Hawkey et al., 2021). The amino acid (AA) profiles of most insect species analyzed up to now are more similar to fish meal diets, which contain substantially higher protein content but clearly are too expensive when compared to the conventional soybean-based feeds (van Huis, 2016). Given the broad distribution and abundance of insects all over the world, several insect species, either live or processed, have been tested as animal feeds (van Huis, 2013). These include the black soldier fly (BSF, *Hermetia illucens*), common housefly (*Musca domestica*), and yellow mealworm (*Tenebrio molitor*), with BSF being the most extensively studied and the most promising species for this purpose (Zuidhof et al., 2003; Wang and Shelomi, 2017; Bellezza Oddon et al., 2021).

Though not completely consistent, studies conducted on poultry have shown that including BSF in the diet improves the growth performance of broilers as well as the productive performance of laying hens (Marono et al., 2017; Bellezza Oddon et al., 2021; Tahamtani et al., 2021). Originally native to subtropical and tropical regions, the BSF is known to flourish at warm temperatures with almost all mating and oviposition occurring above 26 °C (Brammer and von Dohlen, 2007). It has been reported that the development of BSF at 27 °C from egg to prepupa lasts on average 22–24 d while from egg to adult on average 40–43 d (Li et al., 2011). During this time, the BSF larvae develop through 6 larval instars and generally grow to 18–20 mm in length by feeding on a variety of decaying organic matter (Spranghers et al., 2017). These larvae are famous for their abilities to render antimicrobial peptides and convert organic waste into high-value biomass, which allows them to be adopted for wide applications (Diener et al., 2011). Once emerged, adult flies tend to rest on vegetation without any feeding activity. However, the BSF adults are territorial and have very complex courtship and oviposition behaviors solely in a brief period of about 4 d, which still requires a better understanding if methods for continuous rearing of BSF are needed in laboratory conditions (Tomberlin and Sheppard, 2002).

In contrast, protocols for breeding fruit flies (*Drosophila melanogaster*) have been well-established as *Drosophila* has long been a prime model organism for many biological investigators and biomedical researchers over the past 100 yr (Zhang et al., 2011, 2013; Liu et al., 2017b). The fruit fly has populated scientific laboratories throughout the world because of its many advantages: it has modest dietary and spatial requirements, produces large numbers of offspring, and is robust against plagues and pathogens. In comparison to the BSF, however, a big advantage of this tiny fly (about 5 mm in length) is its rapid development. Under optimum conditions of 25 °C, the entire life cycle of the fruit flies does not take longer than 10 d. Their larvae normally experience two molts and develop to pupa 4–5 d after egg laying, followed by eclosion in another 4–5 d (Hafen, 1997). The reproduction potential of *Drosophila* is also great, with females ready for mating and egg production in less than 12 h after emerging (De Robertis and Tejeda-Munoz, 2022). Given the long duration of adults, the high reproduction rates of fruit flies, together with ease of culturing and short generation time, make it an ideal insect for mass-rearing purposes.

In light of the wide circulation of *Drosophila* in multiple areas of basic research, it is surprising that so far, no proposition has been made to systematically uncover the nutritional quality of fruit fly and explore its suitability as a protein source for poultry feed (Barker et al., 1998). By comparing with the black soldier fly larvae (BSFL) commercially available in the market, the goal of this study is to assess and monitor the dynamic changes of nutrients in different life phases of fruit fly, including *Drosophila* larvae (DL), *Drosophila* pupa (DP), female and male adult (DA female and DA male), especially the variations of amino acid and fatty acid spectra in the most important larval and pupal phase. By taking advantage of a large-scale collection of DL through a heat-shock approach, we also aim to evaluate the performance, meat traits, and blood profile of broiler chickens fed on a low-protein diet supplemented with BSFL or DL from 1 to 21 d of age.

Materials and Methods

All experimental procedures involving live birds were approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University (Nanjing, China).

Insect sample acquisition

Source of flies

The BSFL used in this study was purchased from Wuliang Biotechnology Co., Ltd (Guangdong, China), while wild-type *Drosophila* line Oregon R (BDSC #5) was from Bloomington Drosophila Stock Center (Indiana University, USA) and raised at 25 °C, 70% humidity in an environmentally controlled incubator on a 12 h light-dark schedule.

Drosophila sampling

All *Drosophila* stocks and crosses were maintained on a medium containing agar (0.59%), cornmeal (4.39%), dextrose (3.27%), Brewer's dry yeast (2.15%), Nipagin (1.46%), and propionic acid (0.35%) (You et al., 2018). Crosses were carried out with 60 adult males and 90 females in plastic bottles (height 10 cm, diameter 6 cm), and allowed to lay eggs for 12 h for sample collections. Four days after egg laying, larvae were washed and harvested from the wall of plastic bottles after heat shock in 42 °C water baths for 1–2 min. Pupa was manually removed and collected from the wall of plastic bottles 7 d after egg laying. Adult flies were collected within 24 h after eclosion. DA female and DA male were separated manually. All samples were cleaned and air dried before oven drying for the following analysis.

Nutritional determination

Prior to the analysis, samples of *Drosophila* from various phases of the life cycle, as well as the purchased BSFL, were dried at 60 °C in an oven until constant weight (dry matter) and ground to a meal a using cutting mill (Khan et al., 2018). All indexes were performed at least in triplicate and calculated by dry matter after oven drying.

Crude protein (CP)

The Kjeldahl method was used for CP determination of insect samples. Briefly, protein in the sample was disintegrated under the condition of catalytic heating to release ammonia, which then reacted with sulfuric acid and resulted in ammonium sulfate. The alkaline distillation was applied to free ammonia and subsequently absorbed by boric acid before further titration with hydrochloric acid titrant. CP content was calculated by multiplying the acid consumption by 6.25 (GB 5009.5-2010).

Crude fat (CF)

A total of 2 g insect sample was packed by filter paper and weighed after further drying at 105 °C for 120 min. The Lipid from the sample was extracted with diethyl ether using a Soxhlet system by incubation in 70–80 °C water bath for 12 h. The sample then was dried at 105 °C to a constant weight. The quantity eliminated by ether extract was the CF content (GB/T 5009.6-2003).

Ash

The insect sample (3g) was put into a crucible, and fully carbonized on a hot plate until smoke-free, then the samples were placed in a muffle furnace and burned at 550 ± 25 °C for 3 h until the ash was formed, and the ash content was calculated by weighing (GB 5009.4-2010).

Amino acid profile

The AA profile was analyzed according to methods described by GB/T 5009.124-2003. In brief, 0.1 g of sample was put into a hydrolysis tube with 10 mL of hydrochloric acid (6 mol/L), and the test tube was sealed with nitrogen. The tube then was placed in an electro-thermal constant temperature dry box at 110 ± 1 °C and was hydrolyzed for 24 h, cooled, dried, and subjected to an automatic amino-acid analyzer after being dissolved by 1 ml of buffer with pH 2.2.

Fatty acid (FA) composition

The FA spectra were analyzed by taking a 0.2 g sample. The sample was put into a 50 mL of the flask and 6 mL of sodium hydroxide methanol solution was added. The sample was saponified and then 7 mL of boron trifluoride was added. After extraction for 3 min, a methyl ester solution was obtained. The 0.2 μL of methyl ester solution was taken into the syringe and subjected to gas chromatography analysis to determine the FA composition (GB/T 17376-2008).

Mineral composition

The procedure to estimate various mineral elements refers to the method mentioned by Rezic et al. (2011). In short, a 0.3–0.5 g sample was soaked overnight in 10 mL nitric acid in a polytetrafluoroethylene inner tank secured by a safety valve. Then the digestion tube was heated by a microwave digestion furnace until the reaction was completed, and allowed to cool down to 70 °C to drive off the acid. The digestive juice was transferred to a 50 mL volumetric flask and fixed to the scale for tests. The contents of Ca, Fe, K, Na, P, and Zn were determined by the Inductively Coupled Plasma Optical Emission Spectrometer (7400 ICP-OES, Thermo Fisher Scientific, USA).

Gross Energy (GE)

The GE was determined using an adiabatic calorimeter (Tianyu Instrument Manufacturing Co Ltd, China). A total of 1 g sample was used for measurement and burned in the presence of oxygen in the cartridge of the calorimeter. The heat released from the cartridge was absorbed by the water surrounding outside. Gross energy was generated according to the alteration of water temperature.

Density

The density was calculated based on the ratio of mass to volume, both of which were obtained by measuring a group of insects. For example, the volume of the *Drosophila* sample was estimated by the space occupied by ~100 flies when they were covered by water in a tube with constant volume.

Birds and experimental design

A total of 120, 1-d-old male Arbor Acres broiler chicks (average live weight: 45.68 ± 0.21 g) were purchased from Jinghai Poultry Industry Group Co., Ltd. (Jiangsu, China) and randomly allotted to 20 pens with 6 birds per pen. Each pen was 90 cm long × 60 cm wide × 40 cm high, and assigned by completely randomized design to four dietary treatments (five replicate pens per treatment) as follows: corn-soybean low-protein diet (Control) and diets in which soybean meals were partly substituted with 4% BSFL, 4% or 8% DL. All diets were isonitrogenous and isocaloric for GE. The composition and nutrient levels of the four experimental diets (Table 1) are formulated to meet or exceed the nutrient requirements (except the CP content) for broiler chickens recommended by (NRC., 1994) and adjusted according to broiler nutrition specifications (Liu et al., 2020).

The experiment period lasted for 21 d, covering two feeding phases: starter and grower. All birds were given free access to feed and water in an environmentally controlled room on a 23 h light–1 h dark schedule. The temperature of the chicken coop was set at 33 °C for the first 7 d and then reduced by 1 °C per day to a final temperature of around 26 °C for the rest of the experimental duration.

Growth performance

The birds were group weighed by pen at 1 and 21 d of age to obtain the live weight (LW) and calculate the average daily growth (ADG). The feed consumption of 1–21 d was recorded by replicating to compute average daily feed intake (ADFI) and feed conversion ratio (FCR).

Slaughtering procedure and recording

At 21 d of age, after 12 h of feed withdrawal, one bird per pen (five birds per diet) with an LW close to the average weight of that pen was marked, weighed, and slaughtered according to standard procedures (electrical stunning and exsanguination). The feather, foot cuticle, and beak shell were removed to obtain the plucked carcass, which was weighed immediately for calculating the dressing percentage by LW. Then the heart, liver, kidney, intestine, breast, and thigh were further dissected according to the previous study. The organ index (%) was calculated as per the following formula: organ index (g/kg) = the weight of organ (g)/LW (kg).

Table 1. Ingredient composition of the experimental diets based on a low-protein diet

Items	Diets (d 1–21)[1]				*P*-value
	Control	BSFL	4% DL	8% DL	
Ingredients, %					
Corn[2]	59.23	61.48	61.90	62.68	
DL[3]	0.00	0	4.00	8.00	
BSFL[4]	0.00	4.00	0.00	0.00	
Soybean meal[5]	31.50	27.01	25.99	24.01	
Corn gluten meal[6]	1.36	1.59	2.01	0.59	
Soybean oil	3.45	1.77	1.90	0.62	
Dicalcium phosphate	1.40	1.20	1.22	1.20	
Limestone	1.36	1.31	1.33	1.24	
Salt	0.30	0.30	0.30	0.30	
$_{L}$-Lysine HCl	0.21	0.18	0.19	0.19	
$_{DL}$-Methionine	0.18	0.16	0.17	0.17	
Premix[7]	1.00	1.00	1.00	1.00	
Calculated nutrient levels, %					
Metabolizable energy, MJ/kg	12.46	—	—	—	
Gross energy, MJ/kg	19.11	19.11	19.11	19.11	
Crude protein	20.08	20.08	20.08	20.08	
Calcium	1.01	0.98	0.99	1.00	
Total phosphorus	0.70	0.68	0.68	0.70	
Lysine	1.22	1.18	1.17	1.21	
Methionine	0.50	0.51	0.52	0.54	
Methionine + cysteine	0.85	0.84	0.85	0.85	
Analytical nutrient levels[8]					
Gross energy, MJ/Kg	19.35 ± 0.12	19.44 ± 0.31	19.59 ± 0.12	19.58 ± 0.40	0.650
Crude protein, %	20.08 ± 0.02	20.07 ± 0.02	20.09 ± 0.01	20.10 ± 0.01	0.097

[1]BSFL, 4% DL, and 8% DL represent the treatment diets containing 4% black soldier fly larvae meal, 4% and 8% *Drosophila* larvae meal (days 1–21), respectively.
[2]The crude protein content was 10.47%.
[3]DL, *Drosophila* larvae.
[4]BSFL, black soldier fly larvae.
[5]The crude protein content was 41.42%.
[6]The crude protein content was 61.38%.
[7]Premix provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 2500 IU; vitamin E, 20 IU; menadione sodium bisulfate, 1.3 mg; thiamin, 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; calcium pantothenate, 10 mg; pyridoxine HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B12(cobalamin), 0.013 mg; choline chloride, 400 mg; Fe (from ferrous sulfate), 80 mg; Cu (from copper sulfate), 8 mg; Mn (from manganese sulfate), 110 mg; Zn (from zinc sulfate), 60 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg.
[8]Nutrient levels were analyzed and confirmed on day 21 of the animal experiment. Data are expressed as mean ± SD (n = 3).

Meat quality

Meat pH, color, and shear force were determined using breast muscles and leg muscles from the slaughtered broiler chick. Muscle pH was determined by an electronic pH meter (HI9125, HANNA, Italy) 24 h after postmortem. Meat color was determined by a Chroma Meter and described as lightness (L*), redness (a*), and yellowness (b*) according to the CIE-Lab trichromatic system (CR-10 Plus, Konica Minolta, Japan). The shear force of each sample was measured 96 h after postmortem using a Digital Meat Tenderness Meter (C-LM3B, Northeast Agricultural University, Ha'erbin, China).

Serum biochemical parameters

Blood was collected from the wing veins of live broilers marked for slaughter. The serum was separated by centrifugation at 3,000 × g for 15 min and stored at −20°C. The levels of glucose (GLU), triglyceride (TG), total cholesterol (TCHO), uric acid (UA), total protein (TP), albumin (ALB), globulin (GLB), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), calcium (Ca), phosphorus (P) and the activities of alanine transaminase (ALT) and aspartate aminotransferase (AST) in serum were measured by an automatic biochemical analyzer (URIT-8000, Nanjing Agricultural University Animal Hospital, Nanjing, China).

Statistical analysis

Data were generated with methods of randomized complete block design and subjected to 1-way ANOVA with a Tukey's honestly significant difference to determine whether each comparison was statistically significant. ANOVA was carried out using SPSS statistical software 19.0 (IBM, Armonk, NY). Detailed information about ANOVA analysis is listed in Supplemental Table S1. All results were presented as mean ±SD. Differences among means were considered to be significant

at $P < 0.05$. For animal trials, each pen was considered as the experimental unit for the growth performance, while the individual bird was used as the experimental unit to analyze the slaughtering performance, meat traits, and blood parameters.

Results

Proximate composition, gross energy, and density

The results indicated that there were significant differences between *Drosophila* and BSF in terms of the CP ($P < 0.001$) and CF ($P < 0.001$), the main sources of energy, which led to a significant difference in the GE ($P < 0.01$) for the two insects (Table 2). Overall, the BSF was lower in CP and GE, but higher in CF when compared with samples from *Drosophila*. Nevertheless, it was perceived that the ash content of BSFL was close to DA male ($P = 0.249$), and the BSFL had a density almost equal to DL ($P = 0.974$) and DA male ($P = 0.507$), although significant differences were detected between BSFL and other *Drosophila* samples regarding ash and density ($P < 0.05$).

During the life cycle of *Drosophila*, the CP relative content was 40.11% in larvae and 40.98% in pupa when calculated by their dry matters (Table 2). Then a significant upsurge ($P < 0.001$) was noticed after metamorphosis and emerged into adults (53.61% for DA females and 53.72% for DA males, respectively). On the other hand, the CF in *Drosophila* larval stage was about 27.03%, followed by a climb and stabilization in later phases of life cycle since no significant difference ($P \geq 0.184$) was detected among DP (31.50%), DA female (29.56%), and male (30.09%). As shown in Table 2, the ash content of *Drosophila* was relatively low from larva (8.07%) to pupa (7.18%), whereas it moderately but significantly ($P < 0.05$) increased to 9.94% in adult females and 10.79% in males. In contrast with the fluctuation of CP, CF, and ash content, there was limited change in GE at distinct life phases of *Drosophila* ($P \geq 0.199$). Table 2 also illustrates the variation of *Drosophila* density during development as it dropped ($P < 0.001$) from 1.20 g/mm^3 in larval stage to 0.67 g/mm^3 in pupal stage, and then returned ($P < 0.001$) to 0.99 g/mm^3 in female and 1.10 g/mm^3 in male.

Mineral contents

Table 3 summarizes the mineral contents in *Drosophila* and BSF. Both insects contained numerous minerals iron, potassium, sodium, zinc, calcium, and phosphorus. The BSFL was extremely rich in iron (58.94 mg/100g), sodium (187.14 mg/100g), and calcium (2634.95 mg/100 g) when compared with *Drosophila* samples ($P < 0.001$). As a result, the proportion of calcium to phosphorus in BSFL was much higher than that in *Drosophila* ($P < 0.001$). However, the two insects were found to have comparable levels of potassium and zinc when they were at the larval stage ($P \geq 0.095$).

Similar to its density shown in Table 2, the fruit fly also witnessed a significant decline in sodium and phosphorus from larva to pupa ($P < 0.001$), followed by a rebound ($P < 0.001$) from pupa to adult (Table 3). Additionally, the content of calcium in *Drosophila* reached up to 1,485.93 mg/100 g in DL and 1,407.10 mg/100 g in DP, and then decreased ($P < 0.01$) to 859.43 mg/100 g in DA females and 962.29 in mg/100 g males (Table 3). A similar pattern was observed as well for the proportion of calcium to phosphorus, which yet was opposite to the change of iron and zinc, high in adulthood and low in larva and pupa, except the iron level did not differ significantly between adult females and flies in earlier phases ($P \geq 0.257$).

AA and FA profiles in potential commercial stages of insects

For the aim of commercial potential, insect larvae and pupa are usually more favorable as a dietary protein source than their adults, partially due to the short cycle (Liu et al., 2017a). Therefore, only samples from larval and pupal stages were subjected to AA and FA determination in this study.

A total of 17 AA including 9 essential amino acids (EAA) for chicken were monitored in our assays as the contents of the other 3 non-essential amino acids (NEAA) were too low to be detected (Table 4). It was noteworthy that lysine, aspartate, and glutamic acid were relatively abundant in all the three samples examined, among which significant differences by ANOVA test merely displayed in the contents of lysine, methionine, alanine, and cysteine ($P < 0.05$). In general, the AA profile of BSFL, including EAA/TAA (total amino acid) and EAA/NEAA, was very similar to its *Drosophila* counterpart except that the DL was higher in lysine and alanine ($P < 0.01$), but lower in cysteine ($P < 0.01$).

The variation in FA composition of DL, DP, and BSFL is exhibited in Table 5. The proportion of saturated fatty acids (SFA) in *Drosophila* accounted for approximately half of the

Table 2. Proximate composition of samples from the two insects

Items[3]	*Drosophila*[1]				BSFL[2]	*P*-value
	DL	DP	DA-female	DA-male		
CP, %	40.11 ± 0.48^{b}	40.98 ± 0.80^{b}	53.61 ± 0.25^{a}	53.73 ± 0.79^{a}	36.90 ± 0.21^{c}	<0.001
CF, %	27.03 ± 1.87^{c}	31.50 ± 0.46^{b}	29.56 ± 0.42^{bc}	30.10 ± 0.62^{b}	39.14 ± 1.58^{a}	<0.001
Ash, %	8.07 ± 0.44^{c}	7.17 ± 0.34^{c}	9.94 ± 0.68^{b}	10.80 ± 0.92^{ab}	11.84 ± 0.85^{a}	<0.001
GE, MJ/kg	26.18 ± 0.04^{a}	26.56 ± 0.14^{a}	26.32 ± 0.63^{a}	25.95 ± 0.19^{a}	24.54 ± 0.50^{b}	<0.001
Density, g/mm^3	1.20 ± 0.02^{a}	0.67 ± 0.02^{c}	1.01 ± 0.10^{b}	1.11 ± 0.06^{ab}	1.17 ± 0.04^{a}	<0.001

[1]DL, *Drosophila* larva; DP, *Drosophila* pupa. DA, *Drosophila* adult.
[2]BSFL, black soldier fly larvae.
[3]CP, crude protein; CF, crude fat; GE, gross energy.
$^{a–c}$ Means that rows not sharing the same superscript letter are statistically different ($P < 0.05$). All results were based on dry matter. Data are expressed as mean ± SD ($n = 4$).

Table 3. Mineral contents of samples from the two insects (mg/100 g in dry matter)

Minerals	*Drosophila*[1]				BSFL[2]	*P*-value
	DL	DP	DA-female	DA-male		
Fe	11.87 ± 1.13^{c}	12.24 ± 0.28^{c}	16.67 ± 2.21^{c}	33.06 ± 3.61^{b}	58.94 ± 4.08^{a}	<0.001
K	1,907.06 ± 181.31^{a}	1,321.23 ± 19.85^{b}	1,603.39 ± 151.15^{ab}	1,555.78 ± 30.76^{b}	1,624.59 ± 123.20^{ab}	<0.01
Na	96.67 ± 2.71^{b}	46.04 ± 3.42^{d}	75.92 ± 5.58^{c}	71.10 ± 1.06^{c}	187.14 ± 2.36^{a}	<0.001
Zn	17.89 ± 1.03^{b}	17.95 ± 0.26^{b}	26.76 ± 2.81^{a}	27.94 ± 0.98^{a}	17.59 ± 4.07^{b}	<0.001
Ca	1,485.93 ± 198.14^{b}	1,407.10 ± 107.79^{b}	859.44 ± 59.19^{c}	962.29 ± 62.94^{c}	2,634.95 ± 39.26^{a}	<0.001
P	900.45 ± 17.72^{b}	717.73 ± 22.54^{c}	1,147.90 ± 41.39^{a}	1,167.43 ± 78.46^{a}	734.39 ± 50.36^{c}	<0.001
Ca/P	1.50 ± 0.19^{b}	1.96 ± 0.16^{b}	0.75 ± 0.04^{c}	0.83 ± 0.11^{c}	3.60 ± 0.28^{a}	<0.001

[1] DL, *Drosophila* larvae; DP, *Drosophila* pupa; DA, *Drosophila* adult.
[2] BSFL, black soldier fly larvae.
[3] Fe, iron; K, potassium; Na, sodium; Zn, zinc; Ca, calcium; P, phosphorus.
[a-c] Means that rows not sharing the same superscript letter are statistically different (P<0.05). All results were based on dry matter. Data are expressed as mean ± SD (n = 3).

Table 4. Amino acid profiles of samples from the two insects (g/kg in dry matter).

Amino acids[3]	*Drosophila*[1]		BSFL[2]	*P*-value
	DL	DP		
EAA				
Arginine	20.78 ± 1.60	21.49 ± 2.76	17.67 ± 0.32	0.080
Histidine	9.91 ± 0.78	10.11 ± 0.96	9.77 ± 0.47	0.853
Isoleucine	15.06 ± 1.12	15.74 ± 1.76	14.18 ± 0.80	0.360
Leucine	24.92 ± 1.84	26.01 ± 2.96	23.13 ± 0.64	0.273
Lysine	26.93 ± 2.04^{a}	22.75 ± 2.84^{ab}	20.57 ± 0.15^{b}	<0.05
Methionine	12.55 ± 3.84^{b}	22.57 ± 2.17^{a}	14.40 ± 4.56^{b}	<0.01
Phenylalanine	15.54 ± 1.06	16.23 ± 1.52	14.17 ± 1.85	0.238
Threonine	16.10 ± 1.11	17.59 ± 3.80	12.80 ± 1.14	0.093
Valine	19.71 ± 1.54	20.12 ± 2.22	18.57 ± 0.35	0.492
NEAA				
Alanine	31.82 ± 2.73^{a}	18.26 ± 3.56^{b}	19.19 ± 0.77^{b}	<0.001
Aspartate	26.30 ± 1.65	27.63 ± 3.93	29.17 ± 0.75	0.403
Cysteine	1.38 ± 0.08^{c}	2.32 ± 0.07^{a}	1.96 ± 0.26^{b}	<0.001
Glycine	16.45 ± 1.32	16.53 ± 1.47	16.32 ± 1.11	0.979
Glutamic acid	47.31 ± 5.16	50.10 ± 10.00	37.22 ± 1.26	0.097
Proline	13.38 ± 2.17	10.65 ± 5.14	14.56 ± 0.42	0.338
Serine	14.57 ± 1.43	14.82 ± 1.89	12.63 ± 0.05	0.170
Tyrosine	18.79 ± 1.42	16.45 ± 1.87	17.77 ± 2.32	0.259
TAA	331.48 ± 23.95	326.70 ± 30.63	294.11 ± 5.49	0.156
EAA	130.82 ± 9.82	141.01 ± 16.89	117.82 ± 8.36	0.115
EAA/TAA, %	39.46 ± 0.62^{b}	43.11 ± 2.04^{a}	40.04 ± 2.26^{ab}	<0.05
EAA/NEAA, %	65.19 ± 1.71^{b}	75.94 ± 6.36^{a}	66.94 ± 6.32^{ab}	<0.05

[1]DL, *Drosophila* larvae; DP, *Drosophila* pupa.
[2]BSFL, black soldier fly larvae.
[3]EAA, essential amino acid; NEAA, nonessential amino acid; TAA, total amino acid.
[a-c] Means that rows not sharing the same superscript letter are statistically different (P < 0.05). All results were based on dry matter. Data are expressed as mean ± SD (n = 4).

total FA assayed, namely, 46.27% in larva and 50.82% in the pupa, whereas BSFL had a higher proportion (57.86%, P < 0.001) of unsaturated fatty acids. As per Table 5, insect samples studied here were mostly composed of FA with 14, 16, and 18 carbon atoms, such as palmitic (C16:0), palmitoleic (C16:1), and oleic (C18:1) acids. Of these FA mentioned, only palmitoleic acid showed a significant difference between *Drosophila* and BSF (P < 0.001). Furthermore, the level of linoleic acid (C18:2, ω-6) in the two insects was much higher than their linolenic acid (C18:3, ω-3), with the BSF containing more of both (P < 0.01).

Growth performance

To maximize the effect of insect proteins on growth, a low-protein diet containing ~20% crude protein (Table 1),

Table 5. Fatty acid profiles of samples from the two insects (g/kg DM in dry matter).

Fatty acids [3]	*Drosophila*[1]		BSFL[2]	*P*-value
	DL	DP		
Butyric acid C4:0	0.01 ± 0.00	—	0.02 ± 0.01	
Caproic acid C6:0	0.01 ± 0.00	0.01 ± 0.00	—	
Caprylic acid C8:0	0.05 ± 0.01^{a}	0.01 ± 0.01^{b}	0.01 ± 0.00^{b}	<0.001
Capric acid C10:0	0.02 ± 0.00^{b}	0.01 ± 0.00^{b}	0.13 ± 0.03^{a}	<0.001
Lauric acid C12:0	0.69 ± 08^{b}	0.58 ± 0.17^{b}	1.79 ± 0.47^{a}	<0.001
Myristic acid C14:0	5.30 ± 0.65^{a}	5.58 ± 1.64^{a}	0.43 ± 0.11^{b}	<0.001
Tetradecenoic acid C14:1	0.16 ± 0.02^{a}	0.18 ± 0.05^{a}	0.01 ± 0.01^{b}	<0.001
Palmitic acid C16:0	7.20 ± 0.85	7.66 ± 2.25	4.72 ± 1.40	0.061
Palmitoleic acid C16:1	6.92 ± 0.88^{a}	5.70 ± 1.67^{a}	0.35 ± 0.10^{b}	<0.001
Stearic acid C18:0	0.58 ± 0.05^{b}	0.46 ± 0.13^{b}	1.56 ± 0.46^{a}	<0.01
Oleic acid C18:1n9	6.92 ± 0.88	5.85 ± 1.72	6.71 ± 1.94	0.616
Linoleic acid C18:2n6	2.20 ± 0.28^{b}	1.75 ± 0.50^{b}	4.49 ± 1.27^{a}	<0.01
Linolenic acid C18:3n3	0.12 ± 0.02^{b}	0.13 ± 0.04^{b}	0.45 ± 0.13^{a}	<0.001
Arachidic acid C20:0	0.17 ± 0.02	0.09 ± 0.09	0.16 ± 0.03	0.115
Eicosenoic acid C20:1	0.01 ± 0.01	—	0.04 ± 0.01	
Eicosadienoic acid C20:2	0.01 ± 0.01	0.18 ± 0.21	—	
Eicosatrienoic acid C20:3n6	—	0.02 ± 0.04	—	
Arachidonic acid C20:4n6	—	—	0.17 ± 0.06^{a}	
Eicosapentaenoic acid C20:5n3	0.04 ± 0.03	0.04 ± 0.07	0.05 ± 0.04	0.986
Docosanoic acid C22:0	0.07 ± 0.05^{ab}	0.02 ± 0.03^{b}	0.11 ± 0.05^{a}	0.056
Docosadienoic acid C22:2	—	0.03 ± 0.04	—	
Docosahexaenoic acid C22:6n3	0.04 ± 0.05	0.01 ± 0.17	0.01 ± 0.01	0.301
Lignoceric acid C24:0	—	0.01 ± 0.02	0.01 ± 0.01	
Osenic acid C24:1	—	—	0.01 ± 0.02	
SFA	14.10 ± 1.65	14.43 ± 4.16	8.92 ± 2.42	0.062
MUFA	14.01 ± 1.78^{a}	11.74 ± 3.44^{ab}	7.11 ± 2.06^{b}	<0.05
PUFA	2.39 ± 0.31^{b}	2.23 ± 0.64^{b}	5.17 ± 1.44^{a}	<0.01
SFA/TFA, %	46.27 ± 0.53^{b}	50.82 ± 0.41^{a}	42.14 ± 0.45^{c}	<0.001

[1]DL, *Drosophila* larvae; DP, *Drosophila* pupa.
[2]BSFL, black soldier fly larvae.
[3]SFA, saturated fatty acids: C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0, C22:0; MUFA, monounsaturated fatty acids: 14:1, C16:1, C17:1, C18:1, C20:1, C24:1; PUFA, polyunsaturated fatty acids: C18:2n6, C18:3n3, C20:2, C20:3n6, C20:4n6, C20:5n3, C22:2, C22:6n3; TFA, total fatty acids; UFA, unsaturated fatty acids.
$^{a-c}$ Means that rows not sharing the same superscript letter are statistically different (P<0.05). All results were based on dry matter. Data are expressed as mean ± SD (n=4).
"—" Means the levels of fatty acids were too low to be detected.

compared to the 23% crude protein recommended by (NRC., 1994), was adopted as a basal diet to feed 1-d-old Arbor Acres broiler chicks for 21 d. Parameters in Table 6 reveal that the growth performance of the broiler was not affected by dietary treatment of 4% BSFL in comparison to the control group ($P \geq 0.112$). Differently, the ADFI, ADG, and LW on day 21 in the two DL groups were significantly higher than in the other groups ($P < 0.01$). In addition, birds supplemented with 8% DL performed even better than 4% DL in ADFI and ADG ($P < 0.001$), and eventually a better LW on day 21 ($P < 0.001$) although no significant difference in FCR was identified among all treatments.

Slaughtering performance, meat quality, and serum parameters

As represented in Table 7, the same trend as LW on day 21 was also found for the carcass weight, which led to a comparable percentage of dressing among the four experimental groups by their final LW. Generally, all organ indexes reported in Table 7 were not significantly influenced by the dietary treatments, apart from the intestine relative weight being higher in the birds administered with 4% DL when compared to 8% DL ($P < 0.05$), but neither of them differed from the control significantly based on the final LW ($P \geq 0.103$).

Interestingly, dietary DL supplementation was also noted to elevate the pH ($P < 0.001$) of muscles from both breast and thigh as compared with the control (Table 8). This increase in pH was positively correlated with the inclusion level of DL ($P < 0.001$). Otherwise, the insect diets analyzed in this study overall had no significant impact on the meat quality and serum biochemical parameters as presented in Tables 8 and 9 respectively.

Discussion

Insects, especially the BSF, have been considered as a potential sustainable nutritious dietary source alternative to the

Table 6. Effects of the dietary treatments on the growth performance of broilers from 1 to 21 d

Items[2]	Dietary treatments[1]				*P*-value
	Control	BSFL	4% DL	8% DL	
LW, g					
1 d	45.67 ± 0.59	46.00 ± 0.59	45.44 ± 0.33	45.87 ± 0.31	0.311
21 d	765.88 ± 22.88^{c}	733.62 ± 5.69^{c}	852.80 ± 28.84^{b}	982.30 ± 19.49^{a}	<0.001
ADFI, g					
1-21 d	45.99 ± 1.82^{c}	45.46 ± 1.82^{c}	51.29 ± 0.45^{b}	61.64 ± 0.63^{a}	<0.001
ADG, g					
1–21 d	35.56 ± 2.20^{c}	34.93 ± 0.27^{c}	39.94 ± 2.21^{b}	46.77 ± 0.93^{a}	<0.001
FCR, g/g					
1—21 d	1.34 ± 0.04	1.38 ± 0.03	1.34 ± 0.04	1.38 ± 0.03	0.072

[1]BSFL, 4% DL, and 8% DL represent the treatment diets containing 4% black soldier fly larvae meal, 4% and 8% *Drosophila* larvae meal (days 1–21), respectively.
[2]LW, live weight; ADFI, average daily feed intake; ADG, average daily growth; FCR, feed conversion ratio.
[a–c] Means that rows not sharing the same superscript letter are statistically different ($P < 0.05$). Data are expressed as mean ± SD ($n = 5$).

Table 7. Effects of the dietary treatments on the slaughtering performance of broilers on day 21

Items[2]	Dietary treatments[1]				*P*-value
	Control	BSFL	4% DL	8% DL	
Carcass weight, g	695.62 ± 27.87^{c}	671.54 ± 21.13^{c}	788.40 ± 26.55^{b}	919.98 ± 17.35^{a}	<0.001
Dressing, %	90.81 ± 1.33	91.54 ± 2.73	92.46 ± 1.16	93.66 ± 0.52	0.073
Breast, %	19.73 ± 1.67	21.10 ± 2.18	21.41 ± 1.37	21.98 ± 1.75	0.261
Thigh, %	16.89 ± 1.81	18.52 ± 1.83	18.06 ± 1.49	18.51 ± 0.88	0.335
Liver, %	2.46 ± 0.41	2.89 ± 0.50	3.11 ± 0.85	2.57 ± 0.18	0.257
Heart, %	0.86 ± 0.18	0.86 ± 0.12	0.93 ± 0.11	0.86 ± 0.03	0.707
Kidney, %	0.25 ± 0.04	0.26 ± 0.09	0.36 ± 0.04	0.30 ± 0.08	0.089
Intestine, %	3.92 ± 0.54^{ab}	3.86 ± 0.41^{ab}	4.16 ± 0.80^{a}	3.04 ± 0.35^{b}	<0.05

[1]BSFL, 4% DL, and 8% DL represent the treatment diets containing 4% black soldier fly larvae meal, 4% and 8% *Drosophila* larvae meal (days 1–21), respectively.
[2]Intestine, sum of duodenum, jejunum, ileum. and cecum.
[a–c]Means that rows not sharing the same superscript letter are statistically different ($P < 0.05$). Data are expressed as mean ± SD ($n = 5$).

conventional plant-derived meals for poultry, livestock, and aquaculture (Makkar et al., 2014; Henry et al., 2015; Jozefiak et al., 2016). Previous studies thereby intensively investigated the nutritional ingredients and feed application of BSF by mainly focusing their attention on the larval and pupal stage (Liu et al., 2017a; Heuel et al., 2021; Patterson et al., 2021). In the present report, we provided insight into the potential of *Drosophila* as an edible protein source for broilers in comparison with a commercial BSFL product from the market. The informational evidence described here is valuable to the mass-breeding poultry industry in order to meet the future challenge of supplying food security for the global community (van Huis, 2013).

Drosophila, unlike the BSF, has never been utilized as a potential animal feed although *Drosophila* has been prevalent in scientific fields all over the world for more than 100 yr. Here we attempted to address this issue and systematically assess the nutritional composition of *Drosophila* as a dietary source by concentrating on the variation in different developmental phases, in particular the larval and pupal phases. This is distinct from the majority of research topics on BSF as a "standard" diet formulation with a high breeding rate has been well-established in laboratories for *Drosophila*, whereas the conversion efficiency of diverse feed sources by BSFL is not fully explored (Hafen, 1997; Diener et al., 2011; Sprangers et al., 2017).

Chemical composition from this study indicated that *Drosophila* CP accounted for about 40.11% at the larval phase and 40.98% at the pupal stage, and then rose up to 53.61% in female and 53.73% in male adults. In contrast, CP in BSFL on a dry matter basis (about 39.9%) was significantly lower than any *Drosophila* sample evaluated under the same experimental setting. The current findings are consistent with those of previous research on insect meals, which reported that CP approximately ranged from 23% to 76%, although CP in BSFL was also claimed to reach up to 46.3% when BSF was fed on unknown feed during larval development (St-Hilaire et al., 2007; Diener et al., 2009; Oonincx et al., 2015). It is known that fat content in insects also varies substantially with diet types. Our study found that the CF content accounted for 39.14% in BSFL, much higher than the 27.03% in DL, 31.50% in DP, and 30.10% in DA, all of which, however, are in accordance with the 10–50% range of insect fat by earlier literature (Barker et al., 1998; Hawkey et al., 2021). What is notable is the amount of minerals enriched in *Drosophila* and BSF, such as iron, potassium, sodium, zinc, calcium, and

phosphorus. Elements listed in this study implicated that both insects contain sufficient levels of minerals to meet the dietary requirements of most animals (Barker et al., 1998).

Our data further showed the AA and FA spectra of *Drosophila* and BSF. Only results from insect larvae and pupa were uncovered here because the two stages are the most economic effectiveness commercial stages (Liu et al., 2017a). In general, the levels of EAA from the two insects seemed to be sufficient to comply with the requirements for poultry (Spranghers et al., 2017). According to the AA parameters, for example, the EAA/TAA and EAA/NEAA ratio, the AA composition of DL was more similar to BSFL when compared to DP. In total, 4 out of 17 AA displayed significant differences among DL, DP, and BSFL. One of the four AA was lysine, which also was the most prevalent AA in the DL biomass. Given lysine often acts as a limiting AA critical for chicken growth, its higher level in DL than in BSFL perhaps suggests a better potential of DL as a dietary source (Eits et al., 2003). However, this may not be true when it comes to the satisfactory high level of essential FA in BSFL, including linoleic (ω-6) and linolenic (ω-3) acids, in comparison to DL and DP. These essential FA are necessary to maintain cell membrane, hemoglobin synthesis, and cell division under normal conditions (Marineli et al., 2012). Besides, the FA measured here were very similar to reported FA profiles of insect species, with palmitic acid (C16:0) as the dominant SFA and oleic acid (C18:1n9) as the main monounsaturated fatty acid (MUFA) (Hawkey et al., 2021). In addition, meals from both insects contained insufficient long-chain polyunsaturated fatty acids (PUFA), such as eicosapentaenoic (C20:5n3) and docosahexaenoic (C22:6n3) acids, which are high-quality FA associated with oily fish (Hawkey et al., 2021).

Taken together, our results indicated that DL would be a promising dietary protein source because DL possessed (1) a higher CP content and a lower content of CF than the BSFL available in the market; and (2) a balanced AA profile similar to the BSFL in a comparative analysis. This persuaded us to further investigate the potential effect of DL on the performance of broiler chicken. It is well documented that diet recipes recommended by NRC (1994) have been optimized to meet the requirement for maximizing the productive efficiency in the poultry industry,

Table 8. Effects of the dietary treatments on meat quality of broilers on 21 d of age

Items[2]	Dietary treatments[1]				*P*-value
	Control	BSFL	4% DL	8% DL	
pH, 24 h					
Breast	5.78 ± 0.01^{c}	5.78 ± 0.01^{c}	5.88 ± 0.03^{b}	5.98 ± 0.01^{a}	<0.001
Thigh	5.81 ± 0.01^{c}	5.83 ± 0.01^{c}	5.98 ± 0.02^{b}	6.08 ± 0.01^{a}	<0.001
a*					
Breast	5.93 ± 1.77	7.78 ± 1.38	7.09 ± 1.62	6.43 ± 0.83	0.433
Thigh	7.52 ± 2.07	8.99 ± 1.21	8.12 ± 1.11	8.96 ± 1.87	0.731
b*					
Breast	16.44 ± 2.58	17.22 ± 1.40	14.71 ± 1.70	15.26 ± 1.47	0.174
Thigh	18.11 ± 2.23	17.37 ± 1.58	16.97 ± 1.51	17.74 ± 1.16	0.731
L*					
Breast	51.37 ± 2.49	52.26 ± 2.28	48.93 ± 1.52	48.54 ± 2.53	0.058
Thigh	50.35 ± 1.63	52.70 ± 1.73	50.13 ± 0.46	49.94 ± 3.20	0.145
Shear force, *N*					
Breast	30.89 ± 1.66	29.13 ± 1.77	28.93 ± 3.02	28.53 ± 1.68	0.332
Thigh	25.52 ± 1.65	26.67 ± 2.29	26.10 ± 1.57	25.70 ± 0.71	0.704

[1]BSFL, 4% DL, and 8% DL represent the three treatment diets containing 4% black soldier fly larvae meal, 4% and 8% *Drosophila* larvae (days 1–21), respectively.
[a–c]Means that rows not sharing the same superscript letter are statistically different ($P < 0.05$). Data are expressed as mean ± SD ($n = 5$).

Table 9. Effects of the dietary treatments on serum biochemical indices of broilers on 21 d of age.

Items[2]	Dietary treatments[1]				*P*-value
	Control	BSFL	4% DL	8% DL	
GLU, mmol/L	13.45 ± 2.72	12.16 ± 1.97	13.33 ± 0.57	12.69 ± 0.41	0.693
TG, mmol/L	0.54 ± 0.05	0.57 ± 0.11	0.55 ± 0.53	0.54 ± 0.06	0.902
TCHO, mmol/L	4.66 ± 0.41	4.42 ± 0.64	4.77 ± 0.29	4.07 ± 0.55	0.242
UA, μmol/L	181.58 ± 17.96	175.63 ± 46.58	165.00 ± 46.21	172.90 ± 14.84	0.924
TP, g/L	22.25 ± 1.80	23.85 ± 3.89	23.15 ± 1.01	22.78 ± 3.53	0.875
ALB, g/L	10.35 ± 0.70	11.20 ± 1.94	11.08 ± 0.82	10.00 ± 1.42	0.537
GLB, g/L	11.90 ± 1.24	12.65 ± 2.38	12.08 ± 0.66	12.78 ± 2.13	0.866
ALB/GLB	0.87 ± 0.07	0.89 ± 0.13	0.92 ± 0.87	0.79 ± 0.33	0.210
AST, U/L	151.28 ± 25.30	149.78 ± 58.42	193.03 ± 29.09	153.25 ± 31.30	0.357
ALT, U/L	15.15 ± 1.90	14.68 ± 3.10	16.50 ± 2.87	15.63 ± 2.63	0.796
AST/ALT	10.10 ± 1.91	9.95 ± 0.1.78	11.80 ± 1.32	9.81 ± 1.08	0.284
HDL-C, mmol/L	1.65 ± 0.24	1.50 ± 0.28	1.49 ± 0.23	1.31 ± 0.15	0.279
LDL-C, mmol/L	1.63 ± 0.09	1.58 ± 0.18	1.67 ± 0.32	1.63 ± 0.17	0.951
Ca, mmol/L	1.92 ± 0.09	1.79 ± 0.08	1.91 ± 0.24	1.71 ± 0.05	0.130
P, mmol/L	4.44 ± 0.82	3.69 ± 0.95	4.78 ± 1.12	4.90 ± 0.97	0.332
Ca/P, %	0.45 ± 0.11	0.50 ± 0.09	0.42 ± 0.13	0.36 ± 0.08	0.320

[1]BSFL, 4% DL, and 8% DL represent the treatment diets containing 4% black soldier fly larvae meal, 4% and 8% Drosophila larvae meal (days 1–21), respectively.
[2]GLU, glucose; TG, triglyceride; TCHO, total cholesterol; UA, uric acid; TP, total protein; ALB, albumin; GLB: globulin; AST, aspartate amino transferase; ALT, alanine aminotransferase; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Ca, calcium; P, phosphorus.
[a–c]Means that rows not sharing the same superscript letter are statistically different ($P < 0.05$). Data are expressed as mean ± SD ($n = 5$).

with limited room left for any remarkable improvement. Therefore, a low-protein diet containing merely ~20% CP was carried out in this study as a control diet, based on which soybean was partially replaced by DL or BSFL with all diets isonitrogenous and isocaloric for GE. As so, a 4% BSFL inclusion was set up in parallel for comparison since diets with 3–5% BSF were broadly used for diverse experiments in former reports (Onsongo et al., 2018; He et al., 2021; Mat et al., 2022). It is not surprising that the BSFL in this context had a neutral effect on the growth rate and feed consumption of broilers receiving the low-protein diet, consistent with the conclusions from many existing research although more supported a beneficial role of BSF in both broilers and laying hens (Schiavone et al., 2017; Kawasaki et al., 2019; Bellezza Oddon et al., 2021; Tahamtani et al., 2021). What was unexpected was that supplementation of 4% DL improved broiler chicken growth performance and product quality, which was significantly enhanced by elevating the DL inclusion level to 8%, but with no improvement in FCR. These findings implied that the great weight gain in the DL-fed groups was most likely due to the high CP digestibility presumably reflected by the feed intake response elicited by the DL additives. In poultry, increased dietary protein digestibility aids weight gain in chicks, and weight gain is seen as a result of protein accumulation, which asks for adequate AA nutrition and is vital for a successful feeding program (Hwangbo et al., 2009).

In addition, it is also important to observe that muscle pH for all feeding groups fell in the range of normal meat, as for values lower than 5.7 and higher than 6.2, chicken meat can be classified as PSE (pale, soft, and exudative) or DFD (dark, firm, and dry), respectively (Bovera et al., 2016). The higher pH values such as recorded in the DL groups quite often are ascribed to a lower amount of glycogen in the muscle, which is a key contributing factor to the meat quality (Cullere et al., 2016). Last but not the least, it is reasonable to speculate that the insect larvae administration had no negative effects on bird metabolic status in light that the hematological analyses led to similar results among the experimental treatments, and all blood values were within the physiological ranges.

As discussed above, we conclude that *Drosophila* is comparable or superior to BSFL meal as a nutrient source for broilers. However, concerns are raised by the standard laboratory procedures used here to culture and acquire DL, as it would be far too expensive and laborious for industrial-level production. This is different from the BSF which benefits from its cheap rearing substrates by feeding on organic wastes, yet spoiling the quality control of BSF products. When further compared with the soybean and fish meals, no doubt there has to be a trade-off between nutritional values and ecological/economic costs. Given *Drosophila* stocks must be manually maintained through the frequent transfer of breeding adults to fresh food, recycling unneeded flies from large stock centers perhaps would lower the cost if appropriate collaborations could be established. Meanwhile, collecting adults with a vacuum cleaner would be less tedious if further exploration proves DA to be a better choice for broilers as hinted by the higher CP in DA. From that aspect, our study certainly represents a stepping stone toward the translation of insect-derived feeds into industrial production.

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Conflict of Interest Statement

The authors declare no conflicts of interest exist.

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