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ORIGINAL PAPER

Effects of including chaya leaf meal and black soldier fly larvae in the diet of broiler chickens

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ABSTRACT. This study evaluated the effects of incorporating chaya leaf meal (CM) and black soldier fly larvae (BSFL) into broiler chicken diets. Ninety-six 7-day-old male Arbor Acre broilers were divided into six dietary treatments with four replicates, four birds each. Diets included a control basal diet, 5% CM, 5% BSFL, a combination of 5% CM and 5% BSFL, and two finisher diets containing either 5% CM or 5% BSFL from days 22 to 42 (CM-F and BSFL-F, respectively). Chickens in the control and BSFL groups had the lowest feed conversion ratio during days 7–21 ($P < 0.05$). Blood profiles and immunoglobulin Y levels were not statistically different between the groups. The BSFL group had a stronger ileal immunoglobulin A fluorescence signal and lower liver weight compared to the control, while jejunum and ileum weights were higher in the CM + BSFL group ($P < 0.05$). Lauric acid was not detected in the breast meat of the control group. In contrast, it was present in the breast meat of chickens fed 5% BSFL, constituting 0.92% and 1.43% of total fatty acids in the chickens fed 5% BSFL (BSFL and BSFL-F, respectively). These preliminary results indicate that CM and BSFL may be an alternative protein source in broiler nutrition. However, given the small sample size of this study, further large-scale research is required.

Introduction

The United Nations projects that the global population will reach 10.2 bln by 2050 (WPP, 2024), which will further intensify the global demand for food. Poultry products, especially broiler chickens, are an important food source owing to their elevated protein content and rapid production rate. Modern broilers, typically raised for only 4–5 weeks, require higher-protein and energy supply for optimal development. Soybean meal (SBM) is the primary protein source in poultry feed, and its demand is consequently rising. In Thailand, the livestock sector annually imports approximately 2.2 mln t of SBM, and the ongoing conflict between Ukraine and Russia has disrupted supply chains, leading to increased costs (Wongtangtintharn et al., 2025). Identifying alternative, nutrient-rich plant or insect protein sources could help reduce feed costs and dependence on imported SBM.

Chaya (*Cnidoscolus chayamansa* McVaugh) foliage has long been utilised for culinary and therapeutic purposes in the Mayan region. Currently, chaya leaves are widely cultivated in various areas of the world, including Thailand. The leaves possess a substantial protein content, ranging from 29.01 to 32.41% (Ramírez Rodrigues et al., 2021; Kongphapa et al., 2022; Wongnhor et al., 2023), and

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are rich in flavonoids and phenolic compounds, which exhibit antioxidant properties. In a previous study, the authors found that dietary inclusion of 15% chaya leaves did not adversely affect chicken performance (Sarmiento-Franco et al., 2002), while Wongnhor et al. (2023) recently suggested that a 20% inclusion could serve as a protein source for indigenous chickens. However, as a plant-based protein source, chaya leaves are deficient in certain essential amino acids, including lysine and methionine, and have relatively low energy content. Therefore, the identification of complementary alternative protein sources for supplementation is a practical approach to formulate balanced diets that meet animal nutritional requirements. Black soldier fly larvae (BSFL) are increasingly used for decomposition of food waste due to their rapid life cycle and high efficiency in converting organic matter into larval biomass. Moreover, BSFL have proven to be an effective feed ingredient due to their high crude protein content (36.9–43.1%) and favourable amino acid profile, with higher levels of methionine, lysine, threonine, valine, and isoleucine in comparison to SBM. The crude fat content of BSFL ranges from 21.8 to 38.36% (De Marco et al., 2015; Spranghers et al., 2017; Zulkifli et al., 2022), providing an additional energy source when used in full-fat form, with lauric acid as the predominant fatty acid. The inclusion of low BSFL levels (5%) in broiler diets has shown no negative effect on body weight gain (Bellezza Oddon et al., 2021; Attia et al., 2023) and has been associated with improved immune responses (Lee et al., 2018; Seyedalmoosavi et al., 2022). Furthermore, increasing the dietary dose of BSFL has been shown to elevate the concentration of medium-chain fatty acids, particularly lauric and myristic acids, in chicken meat (de Souza Vilela et al., 2021; Kierończyk et al., 2023; Tognoli et al., 2025). Lauric acid contributes to improved food safety by reducing *Campylobacter coli* contamination in broiler meat (Zeiger et al., 2017). Therefore, BSFL represent a valuable source of both protein and energy. This study aimed to evaluate the combined inclusion of chaya leaf meal and BSFL in broiler diets and its effects on growth performance, blood profile, immunity, visceral organ weight, and meat fatty acid composition.

Material and methods

Ethical statement

The present experiment was approved by the Animal Care and Use Committee of Maejo University, Chiang Mai, Thailand, for scientific and technological research (MACUC048P/2566).

Chaya leaf meal (CM) and black soldier fly larvae (BSFL) preparation

Chaya leaves (*Cnidoscolus chayamansa* McVaugh) were collected from the agricultural area of the Maejo University Phrae Campus. Fresh leaves were sun-dried for 1–2 days, then dehydrated at 60 °C and ground before being mixed with the diet. BSFL were reared at the Maejo University Phrae Campus using food and agricultural waste. BSFL were harvested at 20–30 days of age, before pupation, rinsed with tap water and stored at −40 °C. After thawing, they were desiccated in a hot-air oven at 60 °C and pulverised prior to incorporation into the diet.

Animal, experimental diet, and housing

One-day-old male Arbor Acre broiler chickens were obtained from a commercial hatchery (Charoen Pokphand Group Co., Ltd., Lamphun, Thailand) and raised in an electrically heated brooder with a commercial starter basal diet containing 21% crude protein. Due to the constraints at the experimental site and the requirement to comply with animal care and use guidelines limiting the number of animals, we relied on preliminary data to determine the inclusion levels of BSFL and chaya meal (CM) in the diet of Thai native chickens using G*power 3.1. The calculation indicated an effect size of 0.5 and an estimated sample size of 80 birds. Consequently, ninety-six 7-day-old chicks were individually weighed and assigned to six groups of similar body weight, with four replicates of four birds each. The experimental groups included: a control group receiving a basal diet (C), a group fed a basal diet with 5% CM, a group fed a basal diet with 5% BSFL, a group fed a basal diet with 5% CM and 5% BSFL (CM + BSFL), a group fed a basal diet from day 7 to 21 followed by a basal diet with 5% CM from day 22 to 42 (CM-F), and a group fed a basal diet from day 7 to 21 followed by a basal diet with 5% BSFL from day 22 to 42 (BSFL-F). All diets were formulated to be isoenergetic and isoproteinaceous, containing approximately 23% crude protein and 3200 ME kcal/kg from day 7 to 21, and 20% crude protein and 3200 kcal/kg from day 22 to 42. The components and chemical formulation of the basal diets are presented in Table 1. The chicks were housed in floor pens (85 × 75 × 60 cm) with rice hull bedding, and provided feed and water *ad libitum.* Lighting was maintained for 22 h per day. Body weight and feed intake were recorded weekly on a pen basis, and feed conversion ratio (FCR) was calculated as feed intake (FI) divided by body weight gain (BWG). All birds were reared until 42 days of age.

Table 1. Composition and nutrient content of the experimental diets

Ingredient, %	7–21 days of age						22–42 days of age					
	C	CM	BSFL	CM +BSFL	CM-F	BSFL-F	C	CM	BSFL	CM + BSFL	CM-F	BSFL-F
Maize	53.00	47.80	55.70	49.50	53.00	53.00	59.34	54.74	61.34	55.54	54.74	61.34
SBM (45% CP)	29.00	27.50	23.80	22.50	29.00	29.00	27.90	26.30	22.80	21.50	26.30	22.80
Fish meal (60% CP)	9.00	9.00	9.00	9.00	9.00	9.00	4.00	4.00	4.00	4.00	4.00	4.00
Palm oil	6.20	7.90	3.70	6.20	6.20	6.20	5.90	7.10	4.00	6.10	7.10	4.00
CM	–	5.00	–	5.00	–	–	–	5.00	–	5.00	5.00	–
BSFL	–	–	5.00	5.00	–	–	–	–	5.00	5.00	–	5.00
Dicalcium phosphate	1.20	1.20	1.20	1.20	1.20	1.20	1.40	1.40	1.40	1.40	1.40	1.40
Calcium carbonate	0.60	0.60	0.60	0.60	0.60	0.60	0.70	0.70	0.70	0.70	0.70	0.70
Sodium chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-lysine	0.05	0.05	0.05	0.05	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01
Vit. premix	0.50	0.50	0.50	0.50	0.50	0.50	0.30	0.30	0.30	0.30	0.30	0.30
Calculated composition												
ME, kcal/kg	3202	3172	3203	3197	3202	3202	3195	3150	3215	3197	3150	3215
CP, %	22.96	22.91	23.00	23.01	22.96	22.96	20.00	19.97	20.00	20.01	19.97	20.00
CF, %	2.62	3.01	2.99	3.36	2.62	2.62	2.71	3.10	3.06	3.44	3.10	3.06

C. – basal diet, CM – 5% Chaya leaf meal in basal diet, BSFL – 5% black soldier fly larvae in basal diet, CM + BSFL – 5% Chaya leaf meal and 5% black soldier fly larvae in basal diet, CM-Finisher (CM-F) – 5% Chaya leaf meal in basal diet (22–42 days), BSFL-Finisher (BSFL-F) – 5% black soldier fly larvae in basal diet (22–42 days); SBM – soybean meal, CM – chaya leaf meal, ME – metabolisable energy, CP – crude protein, CF – crude fibre; vit. premix provided per kg of diet (7–21 days of age): IU: vit. A 10 000, vit. D_3 2 000, vit. E 11; mg: vit. K_3 2; vit. B_1 2.5, vit. B_2 5, vit. B_6 3, vit. B_{12} 0.03, pantothenic acid 10, nicothenic acid 25, biotin 0.12, folic acid 1.5, vit. C 7.5; vit. premix provided per kg of diet (22–42 days of age): IU: vit. A 6 000, vit. D_3 1200, vit. E 6.6; mg: vit. K_3 1.2, vit. B_1 1.5, vit. B_2 3, vit. B_6 1.8, vit. B_{12} 0.02, pantothenic acid 6, nicothenic acid 15, biotin 0.07, folic acid 0.9, vit. C 4.5

Blood collection, haematology, serum biochemistry, and immunoglobulin Y analysis

At 42 days, blood samples were collected from the wing vein of eight birds per group. Each sample was divided into two tubes. The first tube contained an anticoagulant (K2 EDTA, BD Vacutainer®, Franklin Lakes, NJ, USA) and was stored at 4 °C for haematological analysis, including packed cell volume (PCV), red blood cells (RBC), and white blood cell (WBC) counts. The second tube contained a clot activator (BD Vacutainer®) and was used for serum collection. After centrifugation at 4 200 *g* for 5 min, the separated serum was stored at −40 °C for subsequent biochemical and immunological assays. Aspartate transaminase (AST) and alkaline phosphatase (ALP) activities were determined using an automated blood chemistry analyser (SPOTCHEMTMEZ™ EZ SP-4430, Arkray, Japan). Serum immunoglobulin Y (IgY) concentrations were quantified with an ELISA kit (AB157693; Abcam, Cambridge, UK) following the manufacturer's protocol. Serum samples were diluted 1:2000 with dilution buffer, and 100 µl of the diluted serum or standard solution was added to IgY-coated 96-well plates. Plates were incubated in the dark for 30 min and then rinsed four times with washing solution. Next, 100 µl of enzyme-conjugated antibody was added, and plates were incubated in the dark at ambient temperature for 30 min. After washing, 100 µl of tetramethylbenzidine substrate was added to each well, followed by incubation in the dark for 10 min. The reaction was stopped by adding stop solution, and absorbance was measured at 450 nm using a microplate reader (BioTek Synergy H1M; Agilent Technologies, Santa Clara, CA, USA).

Tissue sampling and immunofluorescence analysis

At 42 days, four birds with a body weight closest to the replicate mean were selected for intestinal sampling. The birds were anesthetised with Zoletil® (Virbac, Carros, France) prior to decapitation. The small intestines were then excised, rinsed with phosphate-buffered saline (PBS, pH 7.4), and fixed in 10% paraformaldehyde for 48 h, and subsequently embedded in paraffin. Sections (5 µm) were prepared with a microtome (RM2235, Leica, Germany). For immunohistochemical analysis, the tissue sections were deparaffinised and subjected to antigen retrieval by microwave heating in sodium citrate buffer for 10 min. Non-specific binding was blocked by incubating the sections with 2% bovine serum albumin (Sigma-Aldrich, Saint Louise, MO, USA) in a humidified chamber at room temperature for 1 h. The sections were then incubated with a primary antibody against chicken IgA (AB112814; Abcam) at a 1:500 dilution for one hour. After two washes with PBS, the sections were incubated with a fluorescent secondary antibody (AB150129; Abcam) at a 1:500 dilution for 1 h. Following three final washes with PBS, the slides were mounted (AB104139; Abcam) and examined under a digital fluorescence microscope (BX53; Olympus, Tokio, Japan). Four photographs were taken per section, and the fluorescence intensity was quantified using ImageJ/Fiji software, as described by Shihan et al. (2021).

Weight of visceral organs and fatty acid composition of breast meat

The relative weights of visceral organs were assessed in six randomly selected birds per group. The proventriculus, gizzard, liver, and small intestine segments (duodenum, jejunum, and ileum) were weighed and values expressed as a percentage of body weight. To determine the fatty acid composition, left breast meat samples were collected from birds in the control, BSFL, and BSFL-F groups and stored at −40 °C. The procedure was carried out by an independent laboratory following AOAC methods 996.06 and 969.33 (AOAC International, 2023). Briefly, 5 g of meat were subjected to fat extraction using a Soxtec 8000 extraction unit (FOSS, Hillerød, Denmark) with petroleum ether. The extracted fat was then methylated by adding 5 ml of 0.5 N potassium hydroxide in methanol and heating at 100 °C in a water bath for 5 min. After cooling, 2 ml of 14% boron trifluoride in methanol was added, and the mixture was heated again at 100 °C for 15 min. The solution was then mixed with 10 ml of saturated sodium chloride, and fatty acid methyl esters were extracted three times with 5 ml of petroleum ether. The upper layer was evaporated to dryness using a rotary evaporator and the residue was dissolved in 2 ml of n-heptane. The solution was filtered through a 0.45-µm nylon syringe filter and analysed using an Agilent 7890B gas chromatograph (Agilent Technologies).

Statistical analysis

Data on growth performance, blood parameters, integrated density of IgA signal, relative visceral organ weight, and fatty acid composition were analysed using one-way ANOVA. Differences between groups were determined using Duncan's multiple range test in IBM® SPSS® software version 27 (IBM Co., Armonk, NY, USA).

Results

Growth performance

Values for BWG, FI and FCR are shown in Figure 1. Between days 7 and 21, BWG and FI did not differ significantly between the groups.

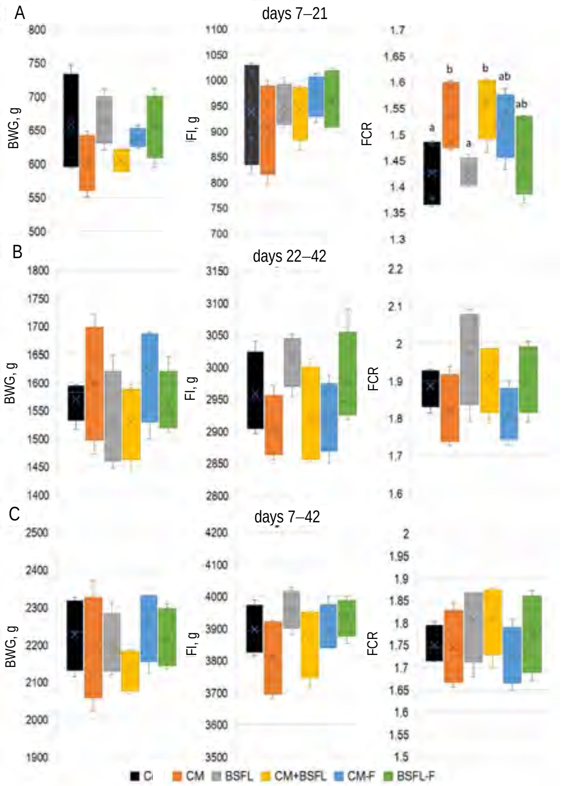

Figure 1. The growth performance of broiler chicken fed diet including Chaya leaf meal and black soldier fly larvae during 7–12 days (A), 22–42 days (B), and 7–42 days (C)

C – basal diet, CM – 5% chaya leaf meal in basal diet, BSFL – 5% black soldier fly larvae in basal diet, CM + BSFL – 5% chaya leaf meal and 5% black soldier fly larvae in basal diet, CM-F – 5% chaya leaf meal in basal diet (22–42 days), BSFL-F – 5% black soldier fly larvae in basal diet (22–42 days); BWG – body weight gain; ^{ab} – $P < 0.05$

However, FCR was higher in the CM + BSFL group compared to the control. From day 22 to 42, no significant differences were observed in BWG, FI, or FCR among the groups. Similarly, over the entire period (days 7–42), BWG, FI, and FCR did not differ significantly between the groups.

Haematology and serum biochemistry

Figure 2 presents the blood profiles of chickens fed the experimental diets. Packed cell volume (PCV), red blood cell (RBC) count, and white blood cell (WBC) count did not differ significantly between the groups ($P > 0.05$). Plasma protein levels were also similar among the experimental groups ($P > 0.05$). The activities of the enzymes aspartate transaminase (AST) and alkaline phosphatase (ALP), which are indicators of liver and tissue health, also showed no significant differences ($P > 0.05$).

Relative visceral organ weight and intestinal length

The relative weights of visceral organs are shown in Figure 4. The proventriculus weight of chickens fed 5% BSFL was significantly higher than that of chickens receiving 5% CM between days 21 and 42 (CM-F). In the 5% BSFL group, liver weights were significantly lower compared to the control, CM, and CM-F groups, ($P < 0.05$). No significant differences were observed between the experimental groups in the relative weights of the gizzard, pancreas, spleen, or duodenum ($P > 0.05$). The jejunum and ileum weights in the CM + BSFL group were significantly higher than in the control and BSFL-F group, with a statistically significant difference ($P < 0.05$). Small intestine length did not differ significantly between the experimental treatments (data not shown).

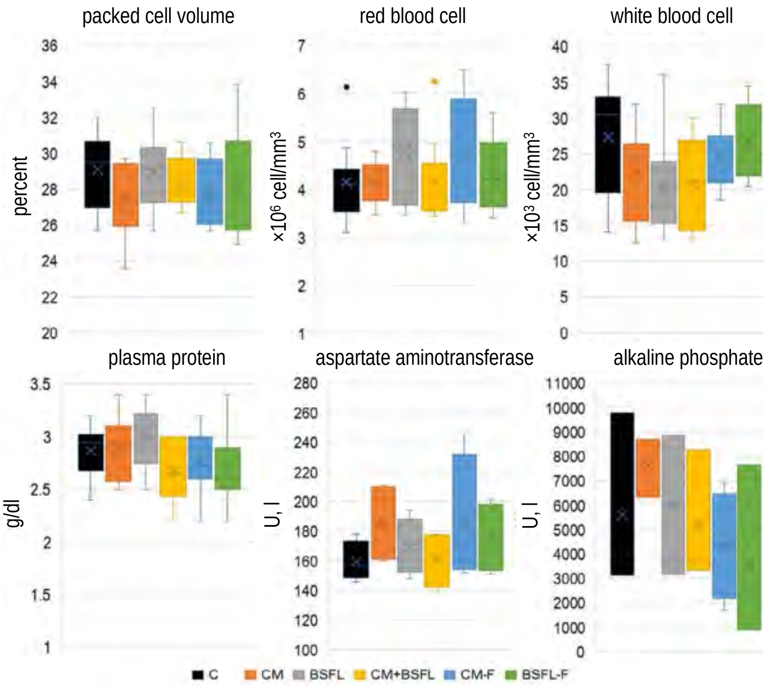

Figure 2. Blood profiles of broiler chicken fed experimental diet

C – basal diet, CM – 5% chaya leaf meal in basal diet, BSFL – 5% black soldier fly larvae in basal diet, CM + BSFL – 5% chaya leaf meal and 5% black soldier fly larvae in basal diet, CM-F – 5% chaya leaf meal in basal diet (22–42 days), BSFL-F – 5% black soldier fly larvae in basal diet (22–42 days)

Serum IgY and IgA staining

There were no significant differences recorded between the groups in relation to serum IgY levels (Figure 3A). IgA concentration in the ileum was measured using immunofluorescence, (Figure 3C; green colour). Chickens fed 5% BSFL showed significantly higher ileal IgA fluorescence intensity than the control, while no significant differences were observed compared to the other groups (Figure 3B; C).

Fatty acid content in breast meat

The fatty acid composition of breast meat from the control, BSFL, and BSFL-F groups is presented in Table 2. Lauric acid was not detected in the control group, whereas chicken fed 5% BSFL (BSFL and BSFL-F) contained 0.92% and 1.43% of total fatty acids, respectively. No significant differences were observed among the groups for other types of fatty acids ($P > 0.05$).

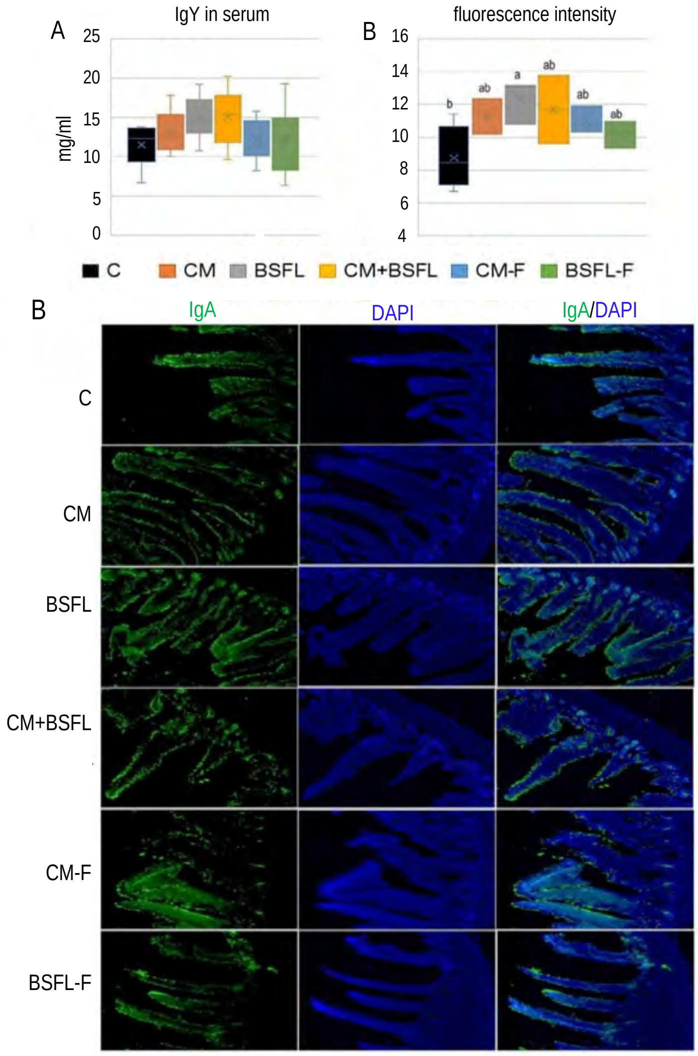

Figure 3. The IgY in serum of broiler chicken fed experimental diet (A). Fluorescence intensity of IgA staining in the ileum segment of chicken fed experimental diet (B). The representatives of immunofluoresce staining of ileal IgA (green), and DAPI (blue) staining of chicken fed experimental groups (C).

C – basal diet, CM – 5% chaya leaf meal in basal diet, BSFL – 5% black soldier fly larvae in basal diet, CM + BSFL – 5% chaya leaf meal and 5% black soldier fly larvae in basal diet, CM-F – 5% chaya leaf meal in basal diet (22–42 days), BSFL-F – 5% black soldier fly larvae in basal diet (22–42 days); ^{ab} – $P < 0.05$

Table 2. Fatty acid composition (% of total fatty acids) in the breast meat of broiler chickens fed experimental diets

Item	Diets				
	C	BSFL	BSFL-F	SEM	*P*-value
Lauric (C:12)	ND	0.92	1.43	–	–
Myristic (C:14)	0.35	0.92	0.52	0.19	0.57
Palmitic (C16:0)	30.52	32.25	29.91	0.97	0.70
Palmitoleic acid (C16:1)	3.32	3.23	1.95	0.37	0.29
Stearic acid (C18:0)	10.80	12.44	12.48	0.73	0.68
Oleic acid (C18:1n-9)	43.32	39.17	37.71	1.25	0.14
Linoleic acid (C18:2n-6)	10.73	10.61	14.83	1.46	0.51
Arachidonic acid (C20:4n-6)	0.97	0.46	2.08	0.63	0.68
SFA	41.66	46.53	44.34	1.71	0.62
USFA	58.34	53.47	56.57	1.55	0.54
MUFA	46.64	42.40	39.66	1.49	0.13
PUFA	11.70	11.07	16.91	2.07	0.56

C. – basal diet, BSFL – 5% black soldier fly larvae in basal diet, BSFL-Finisher (BSFL-F) – 5% black soldier fly larvae in basal diet (22–42 days); ND – not detected, SFA – saturated fatty acid, USFA – unsaturated fatty acid, MUFA – monounsaturated fatty acid, PUFA – polyunsaturated fatty acid, SEM – standard error of mean

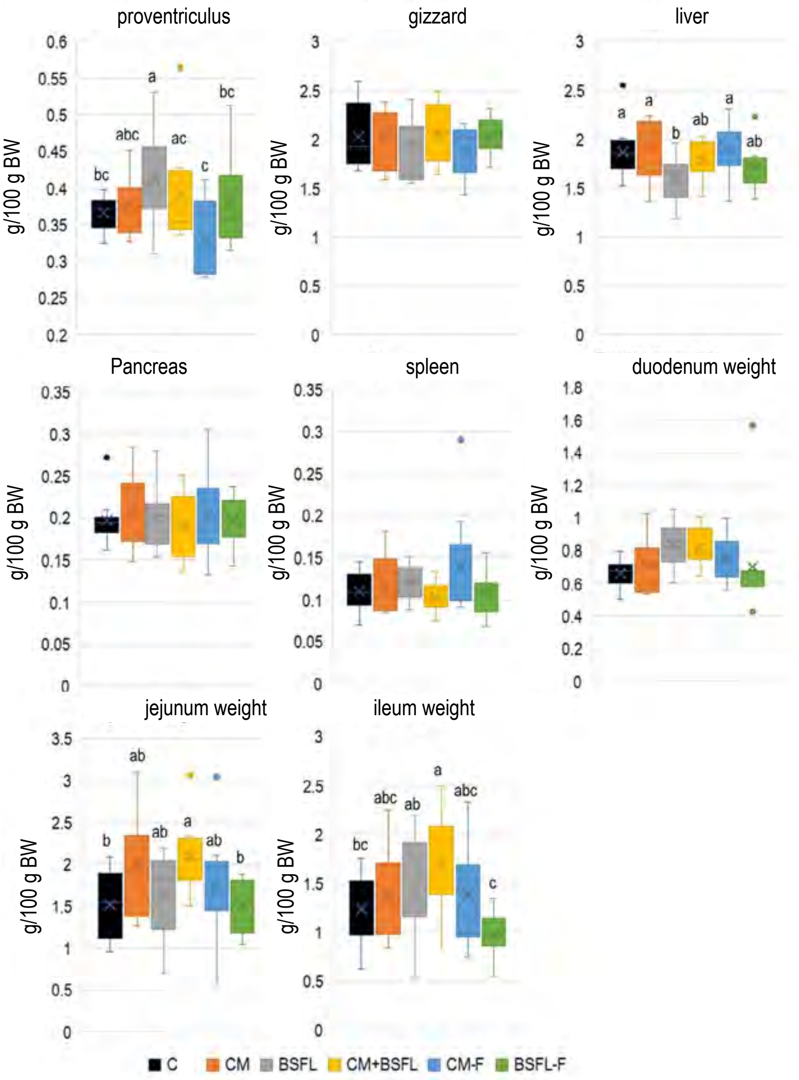

Figure 4. Relative visceral organ weight of broiler chicken fed experimental diet.

C – basal diet, CM – 5% chaya leaf meal in basal diet, BSFL – 5% black soldier fly larvae in basal diet, CM + BSFL – 5% chaya leaf meal and 5% black soldier fly larvae in basal diet, CM-F – 5% chaya leaf meal in basal diet (22–42 days), BSFL-F – 5% black soldier fly larvae in basal diet (22–42 days); BW – body weight; ^{abc} – $P < 0.05$

Discussion

The aim of the current study was to evaluate alternative protein sources, specifically CM and BSFL, as substitutes for soybean meal in broiler diets. Chaya leaves are rich in protein, ranging from 29.01 to 32.41% (Ramírez Rodrigues et al., 2021; Kongphapa et al., 2022; Wongnhor et al., 2023), but are a low energy source; therefore, combining them with BSFL may better meet the nutritional requirements of broilers. Growth performance results showed that diets containing 5% CM, as well as the combination of 5% CM + BSFL, reduced feed efficiency during the starter period (days 7–21) compared to the control. However, no differences were observed during the finisher period (days 22–42) or over the entire 7–42 day trial. Previous studies have reported that diets with 10–30% chaya leaf reduced body weight gain as the inclusion rate increased in Thai indigenous chickens (Wongnhor et al., 2023). This study investigated lower inclusion levels of CM combined with BSFL as alternative protein sources to meet the nutritional requirements of broilers. The findings are consistent with Sarmiento-Franco et al. (2002), who demonstrated that a 15% addition of chaya leaf meal to an isoenergetic and isoproteinaceous diet for Ross chickens aged 7–21 days did not affect performance relative to the control group. Conversely, Donkoh et al. (1999) found that 5–7.5% CM reduced body weight gain and feed efficiency. Moula et al. (2018) observed that 2% BSFL supported growth comparable to a commercial diet, while Lee et al. (2025) reported improved feed efficiency at 1% BSFL. However, higher inclusion levels of BSFL (13.6–21.1%) have been shown to decrease body weight gain during the first 49 days of rearing (Facey et al., 2023).

PCV, RBC, and WBC are commonly used to assess the physiological status of animals. Changes in blood chemistry, such as elevated AST and ALT activities, indicate potential liver damage in broiler chickens (Han et al., 2019; Sang et al., 2023). In the present study, blood parameters were evaluated to confirm that CM and BSFL inclusion did not affect the health status of animals. The PCV and WBC values in all groups were within normal ranges (Wakenell, 2010), except RBC, which was higher in all animals. Similarly, Wongnhor et al. (2023) reported no significant changes in PCV, RBC, or WBC in chickens fed CM. Few studies have examined CM effects on chicken blood parameters, though in rats, chaya leaf extract increased PCV in diabetic and toxin-induced models (Obichi et al., 2015; Iwu et al., 2020). Diets containing BSFL have also been reported not to impair haematologic parameters in chickens (Bongiorno et al., 2022; Bejaei and Cheng, 2023). In contrast, broilers fed defatted BSFL had lower RBC than the control group (Mat et al., 2022), while another study observed an increase in WBC value (Bongiorno et al., 2022). In the current experiment, AST, ALT, and plasma protein levels were not significantly different between the groups. On the other hand, Seyedalmoosavi et al. (2022) found that feeding chickens a high level of BSFL (10–30% of voluntary intake) increased ALP activity. Therefore, incorporating 5% CM or 5% BSFL into the diet did not adversely affect chicken health.

This study evaluated the immune response of chickens by measuring IgY and IgA levels. The results showed that dietary BSFL and CM did not affect serum IgY levels, whereas IgA fluorescence in the ileum was higher in the BSFL group compared to the control. Immunoglobulin A is the main mucosal antibody and plays a key role in pathogen defence. Intestinal IgA production is associated with balanced gut microbiota (Wang et al., 2023), whereas IgA deficiency may lead to dysbiosis and ileal inflammation (Nagaishi et al., 2022). Seyedalmoosavi et al. (2022) also reported that BSFL feeding did not alter serum IgY but tended to increase IgA levels, while broilers fed 1–3% BSFL showed immune response activation through increased production of CD4+T cells (Lee et al., 2018). The immunomodulatory effects of BSFL may result from its lauric acid and antimicrobial peptide content, which exert antimicrobial activity (Koutsos et al., 2022). In addition, chitin, an insoluble fibre present in BSFL, may act as a prebiotic and support microbial balance. Therefore, the current study suggests that dietary BSFL may stimulate ileal IgA activity through the combined action of chitin and lauric acid. However, further work is necessary to confirm this hypothesis.

The inclusion of BSFL in broiler diets has been reported to increase the relative weight of the gizzard, small intestine, pancreas, and liver (Facey et al., 2023). The present study showed that BSFL-fed chickens had a heavier proventriculus than the control group, while their relative liver weight decreased. Lee et al. (2025) found no effect of BSFL inclusion on liver weight. Chickens fed high-fibre diets typically display increased proventriculus weight (González-Alvarado et al., 2008; Jiménez-Moreno et al., 2009). The reduction in liver weight in the BSFL group, which received a fat source from the larvae, compared to the control group that received palm oil, may be attributed to the distinct fatty acid profile of BSFL. Specifically, its high lauric acid content, a medium-chain fatty acid, is readily absorbed and undergoes rapid beta-oxidation in the mitochondria (Roopashree et al., 2021), leading to reduced hepatic fat deposition. Furthermore, the relative weights of the jejunum and ileum were higher in chickens fed a combined CM and BSFL diet, likely due to the higher fibre content compared with other groups. Sadeghi et al. (2015) and Tejeda and Kim (2020) similarly reported that increased dietary fibre elevated the relative weights of the jejunum and ileum in broilers.

This study analysed the fatty acid composition of breast meat in chickens fed BSFL and the control group. BSFL-fed chickens showed higher lauric acid levels, which were undetected in the control group. This corresponds with previous reports showing increased lauric acid in the breast meat of chickens fed BSFL (Kierończyk et al., 2023; Tognoli et al., 2025). Whole BSFL, used in this study, contained both protein and fat (43.15 crude protein and 36.31% ether extract), including lauric acid (Spranghers et al., 2017), which accumulated in the breast tissue. Dietary lauric acid has been shown to reduce *C. coli* counts in meat, thereby improving its microbiological safety (Zeiger et al., 2017).

Conclusions

Our findings demonstrate that including 5% chaya leaf meal (CM) or 5% black soldier fly larvae (BSFL) in broiler diets does not influence growth performance. However, the combination of CM and BSFL affected the digestive system due to its elevated fibre content, which tended to reduce broiler growth performance. Diets containing BSFL improved immunoglobulin A levels and increased lauric acid accumulation in breast meat.

Therefore, both CM and BSFL represent readily available and inexpensive alternative protein ingredients for broiler chicken diets, offering a potential solution for managing high feed costs. It is important to note that this study involved a limited sample size, and thus further large-scale research is recommended.

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Conflicts of interest

The Authors declare that there is no conflict of interest.

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