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The aim of the present study was to evaluate the effects of replacing diet rice bran oil (RBO) with black soldier fly larva oil (BSFLO) on the growth performance, carcass traits, meat guality, and blood parameters of broiler chickens. At one day of age, a total of 180 male broiler chickens (Ross 308) were randomly allocated to 3 experimental groups (4 replicates and 15 birds/pen). To a basal control diet, either 50% or 75% of the RBO was replaced with BSFLO, respectively. The growth performance was monitored throughout the rearing period (divided into 3 periods: 1-10, 11-24, and 25-42 days). On days 24 and 42, Blood samples were taken from each treatment for hemato-biochemical index determination. At the termination period, 8 birds (two birds/pen) per group were slaughtered for carcass and meat quality measurement. Samples of the liver were submitted for fatty acid investigations. The results showed that the inclusion of 75% BSFLO in the broiler diet significantly increased FCR (Feed conversion ratio) in the finisher and overall periods. Interestingly, replacing 50% of RBO with BSFLO did not influence growth performance, carcass traits, and hematochemical parameters compared to 75% of BSFLO and control groups. The present study suggests that partially replacing RBO with 50% of BSFLO in broiler chicken diets has no adverse effects on growth performance, carcass-meat quality, or blood parameters.

#### KEYWORDS

insect oil, Hermetia illucens L., broiler chicken, productivity, blood profiles

# **1** Introduction

Insects are gaining traction as valuable additions to human diets in many countries (Borrelli et al., 2017). They also show potential substitutes for traditional sources of protein, such as fishmeal and soybean meal in livestock feed (Loponte et al., 2017; Bovera et al., 2018; Cutrignelli et al., 2018). The black soldier fly (*Hermetia illucens*) is a particularly attractive option. During its growth stage, this fly can efficiently convert large amounts of organic waste, which would otherwise be pollutants, into usable protein and fats for animal feed (Zheng et al., 2012; Li et al., 2016). Black soldier fly larvae (BSFL) boast a high nutrient content, containing up to 40% protein rich in essential amino acids, over 28% lipids, and essential minerals like calcium and phosphorus (Makkar et al., 2014; Wang and Shelomi, 2017).

Despite the nutritional advantages offered by insects as animal feed, concerns have emerged regarding potential hazards associated with their use. This is because insects raised on contaminated substrates may accumulate heavy metals and toxins, which could be passed on to the animals they are fed (Wang and Shelomi, 2017). However, insects have garnered attention as a promising alternative feed for monogastric animals due to their nutrient-rich composition and minimal environmental impact (Henry et al., 2015; Biasato et al., 2016; Overa et al., 2016). They boast low greenhouse gas and ammonia emissions, exhibit favorable feed conversion ratios as cold-blooded creatures, and require minimal water and soil for cultivation (van Huis, 2013; Makkar et al., 2014). Consumers appear increasingly receptive to products derived from these unconventional raw materials (Mancuso et al., 2016). Furthermore, insects can serve as bio-converters of food waste into animal feed, thus avoiding competition with humans for natural resources (Diener et al., 2011; Makkar et al., 2014).

Kierończyk et al. (2018) proposed that incorporating insect oils, such as those derived from Tenebrio molitor and Zophobas morio, into broiler chicken diets could effectively replace soybean oil (SBO) without compromising growth performance or nutrient digestibility. Additionally, Li et al. (2016) demonstrated that dietary black soldier fly larvae oil (BSFLO) led to increased deposition of omega-3 fatty acids in muscle tissues while reducing intraperitoneal fat accumulation in juvenile carp. BSFLO is particularly abundant in medium-chain fatty acids (MCFA) like lauric acid (C12:0), akin to coconut oil (CCO) (Li et al., 2016; Ushakova et al., 2016), which uniquely comprises approximately 50% lauric acid in its fatty acid composition (Dayrit, 2014). Wang et al. (2015) indicated that MCFA might contribute to abdominal fat reduction due to their preferential utilization for energy over long-chain saturated or unsaturated fatty acids. Furthermore, The MCFA, including lauric acid, exhibits antimicrobial properties against gut bacteria (Zeitz et al., 2015; Schiavone et al., 2017). Hence, it is possible to postulate that insect oils containing high levels of lauric acid may influence the growth performance and gut health of rapidly growing broiler chickens (Schiavone et al., 2018). The fatty acid composition of broiler diets has been found to influence that of meats and visceral organs, which are closely correlated with the storage and metabolism of fat (Taulescu et al., 2010; Khatun et al., 2018). Schiavone et al. (2017) observed that substituting SBO with BSFLO changed the fatty acid profile of broiler chickens. Nevertheless, a comprehensive understanding of the suitability of BSFLO as a poultry feed ingredient remains limited. Given the antimicrobial properties and capacity to modify body fatty acid composition associated with mediumchain fatty acids-rich insect oils, it is suggested that dietary BSFLO could enhance growth performance and gut health, influence the fatty acid composition of abdominal fat pads, and improve meat quality in broiler chickens. Previous studies have reported that dietary fat sources enhance broiler chickens' meat quality (Zeitz et al., 2015; Khatun et al., 2018).

In this study, we used two oil sources, including rice bran oil (RBO) and BSFLO, to see their effects on growth performance, carcass characteristics, meat quality, liver fatty acid composition, and serum parameters in broiler chickens.

## 2 Materials and methods

#### 2.1 Ethics statement

This study was conducted following the ethics of animal experimentation recommendations of the National Research Council of Thailand. The research protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University, Thailand. (Record no. IACUC-KKU-60/66)

# 2.2 Source of BSFLO, experimental design, birds, and diets

The BSFLO was purchased from BSFLY Co. Ltd. (Udonthani, Thailand), which is a reputable supplier that prioritizes quality, sustainability, and adheres to strict regulatory standards. The fatty acid content of BSFLO and rice bran oil was quantitatively determined using the gas chromatographic method 996.06 (Association of Official Analytical Chemists, 1990) and was expressed as g/100g of the total fatty acid identified (Table 1).

The present study used a completely randomized design with three treatments and four replications. A total of 180 one-day-old, male-sex, Ross 308 broiler chicks (initial body weight 47.49 ± 0.04 g) were obtained from a commercial hatchery (Charoen Pokphand Group Co. Ltd., Nakhon Ratchasima, Thailand). Chickens were randomly allocated into one of three dietary treatments (4 replication pens per treatment, with 15 chicks per pen) for a 42-day study period. Dietary treatments were as follows: the control group was basal diet based on corn-soybean meal and RBO; the BSFLO groups replaced RBO with BSFLO at 50% and 75%. The treatment diets and nutrition values were formulated to meet or exceed the nutrition specification (2022) of Ross broiler chickens in three phases - starter (1-10 days), grower (11-24 days), and finisher (25-42 days; Table 2). All broilers had free access to feed and water during the experiment. The temperature of the chicken coop was maintained at 32°C at the age of the first crucial 7 days and then reduced by 1 to 2°C each week in open housing under tropical conditions, to ensure a comfortable and healthy environment for growing chicks.

TABLE 1 The fatty acid profiles of black soldier fly larvae oil (BSFLO) and rice bran oil (RBO)

Fatty acid compositions (g/100g)	BSFLO	RBO
Saturated fatty acid	45.83	24.99
Butyric acid (C4:0)	0.71	0.29
Lauric acid (C12:0)	12.79	0.02
Myristic acid (C14:0)	2.98	0.42
Pentadecanoic acid (C15:0)	0.12	0.02
Palmitic acid (C16:0)	22.81	20.70
Heptadecanoic acid (C17:0)	0.17	0.05
Stearic acid (C18:0)	6.02	2.29
Arachidic acid (C20:0)	0.19	0.91
Behenic acid (C22:0)	0.04	0.29
Unsaturated fatty acid	47.53	74.15
Myristoleic acid (C14:1)	0.05	ND
Palmitoleic acid (C16:1n7)	0.81	0.22
cis-10-Heptadecenoic acid (C17:1n10)	0.04	0.01
cis-9-Oleic acid (C18:1n9)	21.67	38.99
cis-11-Eicosenoic acid (C20:1n11)	0.06	0.38
cis-9,12-Linoleic acid (C18:2n6)	23.23	31.77
gamma-Linolenic acid (C18:3n6)	ND	0.08
alpha-Linolenic acid (C18:3n3)	0.89	1.05
cis-11,14-Eicosadienoic acid (C20:2n6)	0.72	1.10
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.06	0.06
Arachidonic acid (C20:4n6)	ND	0.02
cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	ND	0.47
Monounsaturated fatty acid (MUFA)	22.63	39.60
Polyunsaturated fatty acid (PUFA)	24.90	34.55
PUFA/MUFA	1.10	0.87
n-3	0.89	1.52
n-6	24.01	33.03
n-6/n-3	26.98	21.73
n-3, omega 3; n-6, omega 6; n-6/n-3, ratio of omega	6 per 3.	

#### 2.3 Sample collections and measurements

The broiler chickens were weighed, and their feed intake was recorded on days 1, 10, 24, and 42. Subsequently, the body weight gain (BWG), feed conversion ratio (FCR), survivor rate (SR), and productive index (PI; Equation 1) were calculated for each study period to comprehensively assess the growth performance.

$$PI = \{ [BWG (kg) x SR (\%)] / [Age x FCR] \} x 100$$
(1)

At the end of days 24 and 42, four birds (one bird from each replicate pen) were randomly selected from each treatment, and 5 ml blood samples per bird were gently collected from the wing vein using a sterile syringe with a needle. The blood samples were then divided and transferred into the anticoagulant-free blood collecting tube 4 ml for analyzing blood serum biochemistry, i.e., alanine transaminase (ALT), aspartate transaminase (AST) alkaline phosphatase (ALP), glucose, albumin, globulin, total protein, triglyceride, cholesterol, low-density lipoprotein (LDL), and highdensity lipoprotein (HDL). For serum biochemistry analysis, the blood samples were centrifuged at 1,500 g at 4°C for 15 min to separate the serum and stored at -20°C for analysis. The remaining 1 ml blood went into another tube that contained ethylenediamine-tetra-acetic acid (EDTA) for plasma hematological analysis, i.e., complete blood count.

Upon completion of the 6-week experimental period, two birds per replication then proceeded to the euthanasia and slaughtering process. Prior to sample collection, the chicks were fasted for 12 h. Then, they were individually weighed, euthanized (by cervical dislocation), slaughtered and trimmed carcasses. The weight of each whole hot carcass was recorded. Following slaughter, the carcasses underwent immediate evisceration and subsequent sectioning. Visceral and edible meat organs were weighed and recorded to analyze carcass quality. The liver sample was immediately kept and stored at -20°C until the determination of fatty acid composition by gas chromatographic method 996.06 (25). The left side of the breast of each bird was pH-analyzed using a pH meter (CyberScan Ion 510, Eutech Instruments Pet. Ltd., Singapore), which was inserted directly into the breast muscle within 45 min and 24 h after disassembling the broiler. On the right side of each breast sample, meat color, drip loss, cooking loss, and texture profile were analyzed sequentially. About 45 min after the cut-apart, the meat was color-analyzed using the CIELAB system (Lightness:  $L^*$  value, Redness:  $a^*$  value, and Yellowness:  $b^*$ value), along with the Colorimeter (Chroma Meter CR-410 Series, Konica Minolta Sensing Inc., Tokyo, Japan), then the samples were then individually bagged in polyethylene and stored at 4°C for further evaluation of meat quality.

Afterward, the meat was weighed, encased in multiple layers of gauze cloth, and suspended at 4°C for 24 h. Following this, the meat was carefully patted dry with a paper towel and weighed again. Drip loss (Equation 2) was calculated as a percentage based on the difference in weight before (W1) and after suspension (W2). The meat was then cooked in water at 85°C until the core temperature of the thickest section of the meat reached 80°C. Once cooked, the meat was cooled in a running tap water bath and allowed to rest at 4°C for 2 hours before being weighed once more. Cooking loss (Equation 2) was determined as the difference in weight before (W1) and after cooking (W2).

Drip loss or Cooking loss (%) =  $\{[W1 - W2]/W1\} \times 100$  (2)

The cooked meat was subsequently sliced into cubes measuring  $1 \text{ cm} \times 1 \text{ cm} \times 1 \text{ cm}$  for texture profile analysis (TPA: Hardness), and into specimens measuring 1 cm  $\times$  2 cm  $\times$  1 cm for the shear force test. During the TPA and shear force analyses, the meat

TABLE 2 Formulation and nutritional composition of experimental diets (% dry matter).

Items		1-10 days		11	-24 days		2	5-42 day	S
	Control	BSFLO	BSFLO	Control	BSFLO	BSFLO	Control	BSFLO	BSFLC
	0%	50%	75%	0%	50%	75%	0%	50%	75%
ngredients (kg)						1		1	
Corn	47.80	47.00	47.00	51.20	50.00	50.00	56.50	56.70	57.50
Soybean meal (CP 45%)	30.00	26.25	26.25	26.50	22.50	20.00	21.00	17.80	16.00
Full-fat soybean (CP 36%)	15.30	20.00	20.00	14.80	20.00	22.50	15.00	18.00	19.00
RBO	2.30	1.15	0.58	3.00	1.50	0.90	3.00	1.50	0.75
BSFLO	0.00	1.15	1.72	0.00	1.50	2.10	0.00	1.50	2.25
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Limestone	1.60	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55
Dicalcium phosphate (P 21%)	1.80	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
Premix <sup>a</sup>	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.40	0.35	0.35	0.40	0.40	0.40	0.40	0.40	0.40
DL-methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Calculated nutrient values (	(%)	1	1	1		1		1	1
Metabolizable energy (kcal/kg)	3005.00	2978.00	2945.00	3078.00	3029.00	3017.00	3128.00	3071.00	3044.0
Crude protein	22.50	22.45	22.45	21.07	21.00	20.75	19.07	18.70	18.30
Crude fat	7.28	8.11	8.11	7.97	8.88	9.33	8.14	8.16	8.26
Crude fiber	3.43	3.52	3.52	3.23	3.34	3.36	2.97	2.95	2.95
Available phosphate	0.73	0.73	0.73	0.71	0.72	0.72	0.69	0.70	0.70
Calcium	1.24	1.21	1.21	1.20	1.19	1.19	1.18	1.17	1.17
Methionine	0.67	0.67	0.67	0.65	0.65	0.65	0.63	0.62	0.62
Lysine	1.43	1.43	1.43	1.33	1.34	1.33	1.19	1.18	1.15

BSFLO, black soldier fly larvae oil; RBO, rice bran oil.

<sup>a</sup>Provided per kilogram of diet: 4.80 MIU of vitamin A, 2.00 MIU of vitamin D3, 30,000 IU of vitamin E, 1.20 g of vitamin K3, 1.20 g of vitamin B1, 3.20 g of vitamin B2, 2.00 g of vitamin B6, 0.0064 g of vitamin B1, 2.4.00 g of niacin, 0.80 g of folic acid, 0.08 g of biotin, and 6.00 g of pantothenic acid. 40.00 g of Zn, 48.00 g of Mn, 16.00 g of Fe, 6.40 g of Cu, 0.50 g of I, 0.04 g of Co, and 0.12 g of se. 0.20 g of antioxidant, 0.88 g of anticaking, and 1.00 kg of carrier.

underwent either double compression or perpendicular cutting against the alignment of muscle fibers, utilizing a texture analyzer (model TA.HDplusC, Stable Micro Systems, Godalming, UK) equipped with either a 50 mm cylindrical aluminum probe or a V-slot WB Blade, respectively. All textural parameters were automatically computed and recorded by the Exponent software (Stable Micro Systems, Godalming, UK).

#### 2.4 Statistical analysis

The data were checked for normal distribution using the Shapiro-Wilk test and consistency of variance using Levene's test. Any outliers were eliminated. Outliers were identified using the interquartile range (IQR) method and were excluded from the analysis if they significantly deviated from the overall dataset and were deemed unrelated to the experimental conditions. Statistical evaluations were carried out utilizing one-way analysis of variance (ANOVA) and conducted using SAS version 9.1 software. The significance of group differences was assessed using Tukey's studentized range test at a significance level of p < 0.05. The statistical model is as follows:

$$Y_{ijk} = \mu + \alpha_i + \Sigma_{ij}$$

where  $Y_{ijk}$  = observation,  $\mu$  = overall mean,  $\alpha_i$  = BSFLO levels (i = 0, 50, and 75%), and  $\Sigma_{ij}$  = the experimental error.

# **3** Results

The study investigated the effect of BSFLO in broiler chicken diets on growth performance across different stages, as presented in

TABLE 3	The effect of inclusion of black soldier fly larvae oil (BSFLO) in
broiler di	ets on performance.

D(	Control	Levels o	f BSFLO		CEM				
Performances	0%	50%	75%	<i>p</i> -value	SEM				
IBW (g/b)	47.44	47.52	47.50	0.77	0.11				
FBW (g/b)	3,029.32	2,889.01	2,894.82	0.18	78.59				
Starter period (1-10 Days)									
BW (g/b)	273.38	290.70	301.25	0.15	13.00				
BWG (g/b)	225.93	243.19	253.75	0.15	13.06				
FI (g/b)	273.46	267.92	271.85	0.56	5.19				
FCR	1.22	1.11	1.08	0.06	0.06				
SR (%)	100.00	100.00	100.00	N/A	N/A				
PI	186.52	221.75	237.65	0.13	22.87				
Grower period	(11-24 Day	/s)		1	1				
BW (g/b)	890.52	892.37	893.68	0.96	15.83				
BWG (g/b)	865.96	870.51	874.43	0.86	15.44				
FI (g/b)	1,456.93	1,469.34	1,446.40	0.22	12.21				
FCR	1.68	1.69	1.66	0.65	0.04				
SR (%)	100.00	100.00	100.00	N/A	N/A				
PI	214.48	215.10	220.48	0.73	8.27				
Finisher period	(25-42 Da	ys)							
BW (g/b)	3,039.36	2,889.01	2,894.72	0.16	80.94				
BWG (g/b)	2,138.85	1,996.65	2,000.04	0.12	70.63				
FI (g/b)	3,528.20	3,229.60	3,596.80	0.19	160.67				
FCR	1.65 <sup>b</sup>	1.62 <sup>b</sup>	1.79 <sup>a</sup>	0.04	0.06				
SR (%)	81.67	88.33	88.33	0.35	0.25				
PI	253.72	260.36	235.23	0.41	18.48				
Overall period (	1-42 Days	)							
BWG (g/b)	2,981.93	2,841.50	2,847.22	0.18	78.64				
FI (g/b)	5,182.00	4,966.90	5,288.00	0.22	174.06				
FCR	1.74 <sup>b</sup>	1.75 <sup>b</sup>	1.85 <sup>a</sup>	0.05	0.04				
SR (%)	81.67	88.33	88.33	0.35	5.05				
PI	334.84	342.65	321.75	0.68	23.68				

IBW, initial body weight; FBW, final body weight; FI, feed intake; FCR, feed conversion ratio; SR, Survivor rate; PI, productive index; NA, Not Applicable.

<sup>a,b</sup>Different superscripts within a row indicate significant differences (p < 0.05).

SEM, Standard error of means.

N/A, Not Applicable.

Table 3. Results indicated that BSFLO inclusion at 50% and 75% did not significantly affect initial body weight (IBW), final body weight (FBW), body weight gain (BWG), feed intake (FI), survival rate (SR), and production index (PI) compared to the control group during the starter, grower, and finisher periods (p > 0.05). However, a significant difference in FCR was observed in the finisher period (p < 0.05). Additionally, when considering the overall 1-42 days period, BSFLO inclusion did not significantly difference in BWG, FI, SR, and PI (p > 0.05), but differences were observed in FCR (p < 0.05), with a lower ratio in the BSFLO50% and control groups compared to 75% BSFLO.

The blood serum parameters of the broiler are shown in Table 4. The data found that replacing soybean oil with BSFLO in broiler chicken diets may affect blood cell counts. On day 21 (D21), the 50% BSFLO was significantly different in hemoglobin and white blood cells (WBCs) count (p < 0.05), higher than in other groups, and lower lymphocytes (p < 0.05), similar to those in the control group. By day 42, most blood cell parameters showed no significant differences between the groups. However, eosinophils were slightly lower with 50% BSFLO compared to the control and 75% BSFLO groups (p < 0.05).

The effects of BSFLO on blood biochemistry in broiler chicken diets are shown in Table 5. At day 21, the 50% BSFLO showed the lowest aspartate transaminase (AST) and LDL (low-density lipoprotein) cholesterol levels significantly compared to the control group (p < 0.05). By day 42, The alanine transaminase (ALP) was significantly lower in both BSFLO groups (p < 0.05). Meanwhile, triglyceride was highly significant in 50% BSFLO than the other groups (p < 0.05). On the contrary, the dietary BSFLO inclusion did not significantly (p > 0.05) influence the liver enzyme (alanine transaminase; ALT) and serum protein (albumin, globulin, and total protein).

The carcass quality parameters are shown in Table 6. The data found that replacing rice bran oil with BSFLO in broiler chicken diets may have some effects on carcass yield. The dressing percentage was slightly lower with higher BSFLO inclusion (p < 0.05). There were no significant differences (p > 0.05) in the part of giblets, including the percentage of liver, heart, pancreas, spleen, and gizzard. However, broilers in BSFLO groups had higher abdominal fat percentage than those in control group (p < 0.05). Part of external organs, the percentage of the wing tended to increase when supplementing high levels of BSFLO (p < 0.05). Meanwhile, the thigh, breast, and drumstick percentages did not differ significantly among the treatments.

The results of the breast meat quality values when replacing with BSFLO to broiler chicken diets until termination at 42 days are shown in Table 7. There were no significant changes in breast meat pH at processing (Hour-1; p > 0.05) but a slight decrease at 24 hours (Hour-24; p < 0.05) with higher BSFLO levels. Alternatively, the meat color ( $L^*$ ,  $a^*$ , and  $b^*$ ), drip loss, cooking loss, shear force, and hardness were not significantly affected (p > 0.05).

The fatty acid profiles of the liver are presented in Table 8. Broilers fed diets with high levels of BSFLO (75%) had significantly higher (p < 0.05) levels of saturated fatty acids compared to the control or 50% BSFLO groups, particularly butyric acid (C4:0), palmitic acid (C16:0), and stearic acid (C18:0). Conversely, 50% BSFLO inclusion significantly decreased (p < 0.05) unsaturated fatty acids compared to the control and 75% BSFLO groups, leading to a notable decrease in palmitoleic acid (C16:1n7) and cis-9-oleic acid (C18:1n9c) levels. Moreover, polyunsaturated fatty acid (PUFA) levels were also lowered (p < 0.05) in both BSFLO groups, reducing linoleic acid (C18:2n6) and alpha-linolenic acid (C18:3n3) content. The total levels of PUFA, n-3, n-6, and n-9 were significantly higher (p < 0.05) in the control group compared to the BSFLO treatments.

TABLE 4 The effect of inclusion of black soldier fly larvae oil (BSFLO) in broiler diets on hematology.

Blood serum	Control		vels SFLO	<i>p-</i> value	SEM	
parameters	0%	50%	75%	value		
Days 21						
RBCs (x10 <sup>6</sup> cells/mm <sup>3</sup> )	2.14	2.34	2.09	0.28	0.09	
Hemoglobin (g/dl.)	17.90 <sup>b</sup>	19.97 <sup>a</sup>	19.20 <sup>ab</sup>	0.04	0.40	
Hematocrit (%)	26.67	30.00	27.00	0.06	0.62	
WBCs (cells/µl)	4,066.67 <sup>b</sup>	9,400.00 <sup>a</sup>	5,500.00 <sup>b</sup>	0.01	546.71	
Heterophils (%)	27.00	31.50	25.00	0.28	2.17	
Basophil (%)	8.00	9.00	5.50	0.20	1.05	
Eosinophils (%)	0.00	0.00	0.00	N/A	N/A	
Lymphocytes (%)	64.00 <sup>ab</sup>	58.50 <sup>b</sup>	68.67 <sup>a</sup>	0.02	1.33	
Monocytes (%)	1.00	1.00	1.00	N/A	N/A	
H/L ratio	0.42	0.54	0.37	0.08	0.03	
Days 42						
RBCs (x10 <sup>6</sup> cells/mm <sup>3</sup> )	2.35	2.30	2.35	0.76	0.06	
Hemoglobin (g/dl.)	10.55	10.13	9.25	0.30	0.43	
Hematocrit (%)	29.50	28.00	26.00	0.12	0.74	
WBCs (cells/µl)	9,863.33	6,086.67	6,297.50	0.12	1,262.00	
Heterophils (%)	32.00	19.33	28.00	0.26	4.95	
Basophil (%)	3.00	2.67	2.67	0.88	0.54	
Eosinophils (%)	2.50 <sup>a</sup>	1.00 <sup>b</sup>	2.00 <sup>ab</sup>	0.05	0.24	
Lymphocytes (%)	61.67	76.00	66.33	0.17	4.72	
Monocytes (%)	1.00	1.00	1.00	N/A	N/A	
H/L ratio	0.52	0.28	0.42	0.21	0.8	

RBCs, red blood cells; WBCs, white blood cells; NA, Not Applicable,

<sup>a,b</sup>Different superscripts within a row indicate significant differences (p < 0.05).

SEM, Standard error of means.

N/A, Not Applicable.

In contrast, broilers receiving the 75% BSFLO diet had a higher concentration of monounsaturated fatty acid (MUFA) and a lower PUFA/MUFA ratio (p < 0.05). Additionally, the n-6/n-3 ratio was significantly lower (p < 0.05) in both the 50% BSFLO and control groups.

# 4 Discussion

The main constituents of BSFL are proteins and lipids, with their composition varying depending on the substrate used for rearing. Typically, the BSFL contains around 40% protein and 30% lipid on a dry matter basis. While BSFL are primarily valued as a TABLE 5 The effect of inclusion of black soldier fly larvae oil (BSFLO) in broiler diets on blood plasma biochemistry.

Blood plasma	Control	Lev of BS	vels SFLO	p- value	SEM	
biochemistry	0%	50%	75%	value		
Days 21						
ALT (U/L)	5.33	6.00	4.00	0.30	0.84	
AST (U/L)	213.50 <sup>a</sup>	198.33 <sup>b</sup>	223.00 <sup>a</sup>	0.01	3.11	
ALP (U/L)	18,682.50	22,398.67	18,733.50	0.38	1754.04	
Glucose (mg/dl)	227.00	215.33	229.33	0.15	4.41	
Albumin (g/dl)	1.17	1.15	1.15	0.98	0.07	
Globulin (g/dl)	1.87	1.65	1.45	0.15	0.11	
Total protein	3.06	2.80	2.60	0.13	0.10	
Triglyceride (mg/dl)	23.33	14.33	16.00	0.10	2.55	
Cholesterol (mg/dl)	126.67	126.00	145.33	0.22	7.86	
LDL (mg/dl)	26.50 <sup>a</sup>	21.33 <sup>b</sup>	25.33 <sup>a</sup>	0.01	0.62	
HDL (mg/dl)	52.67	58.50	61.00	0.34	3.31	
Days 42		1				
ALT (U/L)	6.00	5.50	5.00	0.90	0.20	
AST (U/L)	436.67	345.00	341.67	0.07	25.91	
ALP (U/L)	7,190.00 <sup>a</sup>	3,090.00 <sup>b</sup>	3,850.00 <sup>b</sup>	0.02	393.19	
Glucose (mg/dl)	214.00	239.00	236.67	0.07	6.18	
Albumin (g/dl)	1.35	1.20	1.33	0.09	0.03	
Globulin (g/dl)	2.00	1.95	1.87	0.85	0.15	
Total protein	3.35	3.15	3.20	0.78	0.17	
Triglyceride (mg/dl)	33.00 <sup>b</sup>	49.00 <sup>a</sup>	34.00 <sup>b</sup>	<0.01	1.03	
Cholesterol (mg/dl)	126.67	137.00	133.33	0.38	4.87	
LDL (mg/dl)	28.67	33.00	24.33	0.14	2.87	
HDL (mg/dl)	44.67	45.50	45.00	0.92	1.24	

ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

<sup>a,b</sup>Different superscripts within a row indicate significant differences (p < 0.05).

SEM, Standard error of means.

feed source due to their high protein content, their lipid content is often overlooked and considered a by-product in animal feed production. As a result, lipids or fatty acids derived from BSFL are frequently underutilized despite research suggesting their potential for alternative fat sources. The present experiment tests the BSFLO as a partial replacement in half and three-quarters of the RBO in the diet for growing chickens.

In the present study, the replacement of RBO with BSFLO for the broilers' diet did not significantly affect their growth performance, except FCR, which was identified as increasing in the finisher and overall phase when levels of BSFLO inclusion

TABLE 6	The effect of inclusion of black soldier fly larvae oil (BSFLO) in
broiler di	ets on carcass yield.

Carcass	Control		Levels of BSFLO		SEM
compositions	0%	50% 75%		value	
Dressing percentage (%)	75.26 <sup>a</sup>	74.29 <sup>b</sup>	74.03 <sup>b</sup>	0.03	0.23
Internal organ (%)					
Liver	1.78	1.78	1.76	0.97	0.04
Heart	0.37	0.39	0.41	0.10	0.01
Pancreas	0.18	0.19	0.19	0.75	< 0.01
Spleen	0.16	0.17	0.20	0.46	0.02
Gizzard	1.14	1.15	1.10	0.33	0.02
Abdominal fat	1.03 <sup>b</sup>	1.32 <sup>a</sup>	1.16 <sup>ab</sup>	0.02	0.05
External organ (%)					
Breast fillet	29.22	28.99	28.43	0.27	0.28
Breast inner fillet	5.50	5.59	5.63	0.59	0.07
Wing	10.49 <sup>ab</sup>	10.32 <sup>b</sup>	10.74 <sup>a</sup>	0.03	0.09
Thigh	17.99	17.73	18.11	0.76	0.31
Drumstick	13.70	14.15	13.55	0.06	0.15

<sup>a,b</sup>Different superscripts within a row indicate significant differences (p < 0.05).

SEM, Standard error of means.

increased (75% BSFLO). The current study was not consistent with previous research, which also reported that the partial (50%) or total (100%) inclusion of BSFLO in a broiler diet had no significant impact on performance in all periods, as well as nutrient digestibility, intestinal morphological features, and even carcass and meat quality were not adversely affected by supplementation or addition of BSFLO to the diet (Schiavone et al., 2017; Kim et al., 2020; Dabbou et al., 2021). The disparity among all the studies could be attributed to variations in factors such as age, breed, trial conditions, management practices, dietary composition, and the wide variation in fatty acid compositions of BSFLO (Danieli et al., 2019; Gao et al., 2019; Kawasaki et al., 2019). Comparing the effect of three oil sources, i.e., BSFLO, coconut oil (CCO), and corn oil (CO) in broiler chickens, Kim et al. (2020) did not observe any impact on BW, BWG, or FI in chicks at 15 and 30 days old, they did report a noteworthy decrease in FCR for chicks fed CCO and BSFLO compared to the CO group. The authors suggest that this improvement in FCR is likely due to the MCFA abundant in insect oils, which can promote better nutrient digestion and absorption. According to our results, the inclusion of 75% BSFLO likely did not negatively affect the growth performance, especially the BW and FCR of starter and grower periods, compared to a middle level of BSFLO or control groups (Table 3).

The blood serum parameters of broiler chickens play a crucial role in assessing their health and physiological status. The findings presented in Table 4 indicate that replacing RBO with BSFLO in broiler diets exerts effects on some blood cell counts. Specifically, chickens fed with a diet containing 50% BSFLO exhibited higher TABLE 7 The effect of inclusion of black soldier fly larvae oil (BSFLO) in broiler diets on meat quality.

Meat profiles	Control	Levels of BSFLO		p- value	SEM	
	0%	50%	75%	value		
pН						
Hour-1	6.07	6.02	6.03	0.73	0.05	
Hour-24	6.03 <sup>a</sup>	5.93 <sup>ab</sup>	5.85 <sup>b</sup>	0.05	0.25	
Color (CIELAB S	ystem)					
L*	54.82	50.93	51.08	0.25	1.56	
a*	1.26	1.00	1.31	0.55	0.21	
b*	6.51	6.03	6.46	0.90	0.81	
Drip loss (%)	3.94	4.28	2.62	0.34	0.81	
Cooking loss (%)	19.29	15.76	17.49	0.17	1.25	
Shear force (g/cm <sup>2</sup> )	3,219.72	3,421.47	3,526.66	0.25	105.88	
Hardness (g)	633.37	639.74	480.91	0.31	70.36	

<sup>a,b</sup>Different superscripts within a row indicate significant differences (p < 0.05). SEM. Standard error of means.

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levels of hemoglobin and WBCs counts on day 21, although increasing WBCs were identified for 50% BSFLO. This finding appears difficult to explain since none of the broilers showed any signs of physical distress. Meanwhile, at day 42, most blood cell parameters showed no significant differences among the groups, indicating potential adaptation or normalization of blood cell counts over time. Notably, eosinophil levels were slightly lower in chickens fed with 50% BSFLO compared to the control and 75% BSFLO groups. Nevertheless, all the hemato-chemical parameters analyzed for the broilers in the present study fell within the physiological reference intervals reported for chicken (Mat et al., 2022), thus suggesting that the BSFLO utilization did not negatively influence the health status or immune system of the broiler chickens. Moreover, The H/L ratio usually has been used for the measurement of distress conditions in chickens in which an increased H/L ratio may indicate that the animals suffered from infections, inflammation, or stress (Salamano et al., 2010; DeMarco et al., 2013; Pozzo et al., 2013). In the present study, the H/L ratio of chickens fed the experimental diets did not present any significant differences compared with the control group.

The activities of plasma ALT, AST, and ALP are generally related to liver damage, acting as biomarkers of liver health when they are damaged (Hyder et al., 2013). The blood biochemistry traits (Table 5) were significantly affected on AST and ALP by the dietary treatments, which showed lower value than the control group, but the value present trial fell and is still within the physiological normal ranges (Schiavone et al., 2018), suggesting that BSFLO may not cause negative effects on the hepatopancreas or liver health. The results of the present research agree with those of Schiavone et al. (2017) and Li et al. (2016), who showed that the use of BSFLO in substitution for soybean oil in broilers and juvenile Jian carp diets, TABLE 8 The effect of inclusion of black soldier fly larvae oil (BSFLO) in broiler diets on fatty acid profile of liver.

	Control	Levels c	of BSFLO			
Fatty acid composition	0%	50%	75%	<i>p</i> -value	SEM	
Saturated Fatty Acid	2.27 <sup>ab</sup>	1.90 <sup>b</sup>	2.93 <sup>a</sup>	0.023	0.37	
Buryric acid (C4:0)	0.03 <sup>b</sup>	0.01 <sup>b</sup>	0.24 <sup>a</sup>	0.006	0.09	
Myristic acid (C14:0)	0.01	0.01	0.03	0.131	0.01	
Palmitic acid (C16:0)	0.95 <sup>ab</sup>	0.75 <sup>b</sup>	1.29 <sup>a</sup>	0.012	0.19	
Heptadecanoic acid (C17:0)	0.01	0.01	0.01	0.000	0.00	
Stearic acid (C18:0)	0.91 <sup>b</sup>	0.88 <sup>b</sup>	1.09 <sup>a</sup>	0.005	0.08	
Arachidic acid (C20:0)	0.01	0.01	0.01	0.064	0.00	
Behenic acid (C22:0)	0.02	0.02	0.02	0.100	0.00	
Tricosanoic acid (C23:0)	0.33 <sup>a</sup>	0.21 <sup>b</sup>	0.24 <sup>b</sup>	0.003	0.04	
Insaturated Fatty Acid	2.41 <sup>a</sup>	1.51 <sup>b</sup>	2.18 <sup>a</sup>	0.021	0.33	
Palmitoleic acid (C16:1n7)	0.05 <sup>a</sup>	0.02 <sup>b</sup>	0.06 <sup>a</sup>	0.001	0.01	
cis-9-Oleic acid (C18:1n9c)	0.97 <sup>a</sup>	0.57 <sup>b</sup>	1.08 <sup>a</sup>	0.012	0.19	
cis-11-Eicosenoic acid (C20:1n11)	0.02	0.01	0.02	0.065	0.00	
Nervonic acid (C24:1n9)	0.01	0.01	0.01	0.100	0.00	
cis-9,12-Linoleic acid (C18:2n6)	1.17 <sup>a</sup>	0.79 <sup>b</sup>	0.87 <sup>b</sup>	0.009	0.14	
gamma-Linolenic acid (C18:3n6)	0.02	ND	0.01	0.113	0.01	
alpha-Linolenic acid (C18:3n3)	0.05 <sup>a</sup>	0.02 <sup>b</sup>	0.03 <sup>b</sup>	0.001	0.01	
cis-11,14-Eicosadienoic acid (C20:2n6)	0.02	0.02	0.02	0.100	0.00	
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.04	0.02	0.04	0.131	0.01	
Arachidonic acid (C20:4n6)	0.01	0.01	0.01	0.100	0.00	
cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	0.02	0.02	0.01	0.065	0.00	
cis-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3)	0.03	0.02	0.02	0.065	0.00	
Ionounsaturated fatty acid (MUFA)	1.05 <sup>a</sup>	0.61 <sup>b</sup>	1.17 <sup>a</sup>	0.013	0.21	
olyunsaturated fatty acid (PUFA)	1.36 <sup>a</sup>	0.90 <sup>b</sup>	1.01 <sup>b</sup>	0.011	0.17	
UFA/MUFA	1.30 <sup>ab</sup>	1.48 <sup>a</sup>	0.86 <sup>b</sup>	0.014	0.22	
-3	0.09 <sup>a</sup>	0.06 <sup>b</sup>	0.06 <sup>b</sup>	0.001	0.01	
6	1.24 <sup>a</sup>	0.83 <sup>b</sup>	0.93 <sup>b</sup>	0.009	0.15	
.9	1.24 <sup>a</sup>	0.59 <sup>b</sup>	1.09 <sup>a</sup>	0.015	0.24	
-6/n-3	13.8 <sup>b</sup>	13.8 <sup>b</sup>	15.5 <sup>a</sup>	0.043	0.69	

<sup>a,b</sup>Different superscripts within a row indicate significant differences (p < 0.05).

SEM, Standard error of means.

ND, Not detected.

respectively, had no negative effects on the blood traits of the animals and confirmed the nutritional adequacy of these diets.

The replacement of RBO with BSFLO in broiler chickens did not negatively affect liver function indicators, as measured by AST and ALP activity, between birds fed diets containing different fat sources, consistent with a previous report (Taulescu et al., 2010). This suggests that BSFLO supplementation did not induce liver toxicity or impair liver function. However, the effects on plasma cholesterol were inconclusive. Meanwhile, medium-chain fatty acids are generally known to decrease triglycerides, total cholesterol, and LDL cholesterol, as well as increase HDL cholesterol in chickens (Wang et al., 2015; Khatibjoo et al., 2018). Our results did not show a consistent pattern; we found that triglyceride was higher by, on average, 18.8% in 75% BSFLO-fed chickens than in 50% BSFLO-fed ones (Table 5). However, the BSFLO did not significantly impact total and HDL cholesterol levels compared to chickens fed RBO. It is not

clear how BSFLO affected cholesterol metabolism in chickens, necessitating further investigation. In line with our study, dietary BSFLO did not affect blood parameters in chickens, which aligns with previous studies where dietary BSFLO supplementation did not affect blood parameters in chickens (Schiavone et al., 2017), juvenile carp (Li et al., 2016), and rabbits (Gasco et al., 2019). Thus, BSFLO's influence on cholesterol metabolism appears to differ from RBO, although BSFLO is rich in MCFA. It should be kept in mind that RBO has a higher amount of linolenic acid than BSFLO, which is typically known to have a hypocholesterolemic effect (Lorenzo et al., 2014). It is likely that BSFLO would disturb or curb the MCFAinduced increase in plasma cholesterol. Thus, it may be likely that MCFA of BSFLO would disturb or curb-the induced increase in plasma cholesterol, similar to observations with CCO (Wang et al., 2015). Lekshmi Sheela et al. (2016) report that the coconut phytocompounds, especially lauric acid, were found effective against HMG-CoA reductase enzyme which plays a central role in the synthesis of cholesterol.

None of the viscera was significantly impacted by dietary fat sources. The results of the present study agree with the findings of Schiavone et al. (2017), which reported that the partial or total replacement SBO with BSFLO in growing broiler diets did not affect the growth performance, the feed choice or the carcass traits. In the same context, Cullere et al. (2019) showed that defatted BSFL meal could be introduced into the diet for growing broiler quails at 10% and 15% inclusion levels, partially replacing conventional soybean meal and SBO, with no negative effects on productive performance, mortality or carcass traits. However, RBO partial replacement with both BSFLO groups increased abdominal fat percentages by 32% and 16%, respectively (Table 6). BSF oil is rich in saturated fatty acids (SFA) 1.8 times compared to SBO (Table 1), particularly lauric and myristic acids. In order that, the high SFA intake may promote lipogenesis (fat synthesis) and adipocyte hypertrophy (fat cell enlargement) in abdominal adipose tissue. As research has shown, the fatty acid profile of a broiler's diet directly influences the fatty acid composition of its meat and internal organs. This influence is closely linked to fat storage and metabolism within the animal (Taulescu et al., 2010; Khatun et al., 2018).

Different fats did not affect thigh and breast meat but affected wing yield traits. Meanwhile, the pH of the breast meat was decreased in chickens fed a diet containing BSFLO compared with those fed on SBO. Nonetheless, the negative correlation between the lightness and pH of fresh meat (Qiao et al., 2001) was not observed in this study (Table 7). Of interest is the BSFLO trend in medium-chain fatty acids vs. SBO, which has increased  $b^*$  values. Increased breast meat yellowness has been postulated with an increase in carotenoid contents (da Silva et al., 2017) or lipid contents (Zhao et al., 2018) in broiler chickens. Nonetheless, all observed values regarding pH, meat colors, and cooking loss of breast meat still fall within the acceptable range according to meat quality characteristics (Cullere et al., 2019). Further analysis of the nutrient profile, fatty acid composition, and antioxidant content of breast and leg meat could provide insights into the differences in meat quality observed between the various fat sources used in the diets.

The liver plays a central role in maintaining healthy fat balance (lipid homeostasis) through a complex network of precisely regulated biochemical pathways, signaling mechanisms, and cellular processes. Hepatocytes, the main functional cells of the liver, are responsible for these biochemical and metabolic functions, including lipid metabolism. In a healthy state, the liver processes large amounts of fatty acids daily while storing only a small portion as triglycerides. Omega-3 fatty acids, particularly docosahexaenoic acid (DHA) and its precursor docosapentaenoic acid (DPA), are critical factors reflecting changes in liver health (Schiavone et al., 2017). Notably, omega-3 fatty acids and their derivatives possess potent antiinflammatory properties that help mitigate the metabolic effects of oxidative stress in the liver and other tissues (Dabbou et al., 2021; Schiavone et al., 2017). Additionally, maintaining a proper balance between different fatty acid types is crucial. This includes the n-6/n-3 and PUFA/MUFA ratios, which should not be excessively high. Our findings revealed that partially replacing refined RBO with BSFLO at 50% and 75% inclusion levels resulted in favorable n-6/n-3 (0.86) and PUFA/MUFA (1.38) ratios, respectively (Table 8).

In conclusion, the important differences in the dietary contents of lauric and myristic FAs with increasing BSFLO inclusion had no beneficial effect on amount of fat accumulated in the abdominal fat and also FCR in the final period. Despite this, the findings of this study suggest that BSFLO could partially replace RBO in chickens' diet at 50% without any adverse effects on growth performance, hematological parameters, serum biochemical indices, or carcass and meat quality. These findings suggest that BSFLO can be a suitable ingredient for poultry diets. Further research efforts are necessary to investigate the impact of BSFLO on the meat quality traits and fatty acid profile of broiler chickens in depth.

### Data availability statement

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author.

#### **Ethics statement**

The animal study was approved by the Institutional Animal Care and Use Committee of Khon Kaen University, Thailand. (Record no. IACUC-KKU-60/66). The study was conducted in accordance with the local legislation and institutional requirements.

### Author contributions

NS: Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. PP: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. WP: Conceptualization, Investigation, Methodology, Validation, Writing – original draft. NP: Investigation, Methodology, Validation, Writing – original draft. TS: Investigation, Validation, Writing – original draft. BT: Funding acquisition, Writing – review & editing. AC: Writing – review & editing. SW: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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# Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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