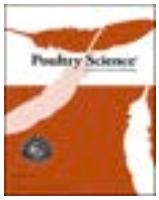
104 (2025) 104831

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj



Black soldier fly larvae oil downregulated gene expression related to fat metabolism of broilers fed low protein diet

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ARTICLE INFO

Keywords:
Broiler
Low protein
Diets
Black soldier fly
Oil

ABSTRACT

Feeding low crude protein (**LCP**) diets supplemented with crystalline amino acids improves environmental and welfare parameters of broilers. However, increased body fat contents in broilers fed LCP diets have become a concern. Black soldier fly larvae oil (**BSFLO**), rich in lauric acid, has been reported to inhibit lipogenesis and reduce body fat. A 3 × 2 factorial experiment was conducted to evaluate the effect of BSFLO on performance, blood biochemistry, carcass quality, fat metabolism gene expression, and litter quality in broilers fed protein-reduced diets. A total of 288 broilers were divided into 6 treatments: three CP levels (200, 185, or 170 g/kg; high [**HCP**], medium [**MCP**], or low [**LCP**]) and two oil sources (BSFLO and Crude Palm Oil [CPO]), with 6 replicate pens of 8 birds each. Results showed a 15 g/kg CP reduction had no effect on body weight and feed intake ($P > 0.05$) but increased FCR ($P = 0.001$). A 30 g/kg CP significantly reduced the body weight and feed intake with inferior FCR ($P < 0.05$). However, negative effect of low CP diets on FCR was mitigated by BSFLO ($P = 0.008$). Reducing CP by 30 g/kg increased fat pads ($P = 0.033$), whereas BSFLO reduced fat pads ($P = 0.049$) at all three CP levels. Protein-reduced diets increased blood cholesterol ($P = 0.002$), HDL ($P < 0.001$), and LDL ($P = 0.002$). BSFLO decreased blood triglyceride ($P = 0.026$) and cholesterol ($P < 0.001$). Reducing 30 g/kg CP increased meat cooking loss ($P = 0.035$), while BSFLO decreased cooking loss ($P < 0.001$). BSFLO increased meat protein ($P < 0.001$) and decreased cholesterol ($P = 0.003$). The inclusion of BSFLO in protein-reduced diet down-regulated the gene expression of FAS, ACC, SREBP-1, and HMGR in broilers ($P < 0.001$). Reducing CP levels decreased litter pH ($P = 0.011$), nitrogen ($P < 0.001$), ammonia ($P < 0.001$) and moisture ($P = 0.018$). The study concludes that BSFLO reduced body fat by down-regulating the lipogenesis gene expression. In addition, BSFLO enhanced feed efficiency in broilers fed protein-reduced diet.

Introduction

In recent years, there has been an increasing interest among both the industry and scientific community regarding the feeding of low crude protein (**LCP**) diets supplemented with essential amino acids to broilers. Numerous studies have shown that moderate reduction of 20–30 g/kg CP in feed did not impact broiler performance and processing yield (Chrystal et al., 2020a; Van Harn et al., 2019). Low CP diets improved nitrogen utilization thus reducing nitrogen excretion (Brink et al., 2022; Lemme et al., 2019; Selle et al., 2021; Strifler et al., 2023) and litter ammonia emission, and environmental impact indicators such as global warming potential (kg CO_2eq/kg) (Lambert et al., 2022; Ogino et al., 2021). Reduction of dietary CP level lowers the litter moisture content thereby reducing ammonia emission and the risk of infections (Woyengo et al., 2023). Moreover, low-protein diets reported to influence poultry behaviour and welfare positively (Li et al., 2018).

Despite a number of production, welfare and environmental advantages, low CP diets reported to increase the carcass (Chrystal et al.,

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https://doi.org/10.1016/j.psj.2025.104831
Received 22 September 2024; Accepted 17 January 2025
Available online 20 January 2025

2020b; Hejdysz et al., 2022; Maynard et al., 2022; Song et al., 2023; Anas et al., 2024a) and meat (Liu et al., 2017) fat contents of broilers. Low-protein diets have a higher energy-to-protein ratio (MJ kg-1) resulting in higher energy intake, retention, and fat deposition (Aletor et al., 2000; Jariyahatthakij et al., 2018). Furthermore, the reduction of dietary protein levels impacted the upregulation of lipid-related metabolic pathways. Ma et al. (2023) demonstrated that a decrease of 30 g/kg in dietary protein increased serum metabolites (triglycerides, total cholesterol, and free fatty acids) and liver enzyme activity (**ACC**, acetyl-CoA carboxylase; **FAS**, fatty acid synthase) associated with fat metabolism. Moreover, there was an upsurge of the expression of lipogenic genes (**LXRα**, liver X receptors-a; **SREBP1**, sterol regulatory element-binding protein 1; **ACC**, acetyl-CoA carboxylase; **FAS**, fatty acid synthase) while decreasing the expression of lipolytic genes (**PPARα**, peroxisome proliferator-activated receptor-a; **CPT-1**, carnitine palmitoyl-transferase1; **ACOX1**, acyl-CoA oxidase 1). Therefore, to mitigate the nagative effects on fat pad and meat fat in broilers fed low-protein diets, a better understanding about the expression of genes associated with lipid metabolism is required.

During recent years, there has been a great enthusiasm to utilize black soldier fly larvae (**BSFL**) as a protein source in poultry feed formulations (Aldis et al., 2024; Kawasaki et al., 2019; Schiavone et al., 2017; Seyedalmoosavi et al., 2022a; Vilela et al., 2021). Use of BSFL in poultry feeds reduces pressure on conventional protein sources such as soybean meal and fish meal (Chang et al., 2023; Hossain and Bhuiyan, 2023; Hosseindoust et al., 2024; Mlambo et al., 2023; Salahuddin et al., 2024). Moreover, production of BSFL has demostrated a significant potential for profitability and scalability to an industrial level (Siddiqui et al., 2024; Scala et al., 2020; Joly and Nikiema, 2019).

Processing of BSFL into meal yields oil which can be used as an energy-dense ingredient in poultry diets (Chen et al., 2022; Schiavone et al., 2018). Black soldier fly larvae oil is rich in medium-chain fatty acids (Anas et al., 2024b; Ewald et al., 2020; Kierończyk et al., 2022; Kim et al., 2020). Our previous studies shown that BSFLO in broiler diets reduced fat and cholesterol contents in meat (Aprianto et al., 2023). The lauric acid present in BSFLO significantly downregulated the expression of lipid synthesis genes (FAS and ACC). Furthermore, Xia et al. (2021) demonstrated that lauric triglyceride inhibited the expression of lipogenesis-related genes and proteins (SREBP-1c, ACC1, and FASN) while increasing the expression of lipolysis (ATGL, HSL, and LPL) and β-oxidation (PPARα, CPT-1a, and PCG-1α) related genes in obese rats. In these circumstances, it is hypothesized that negative effects of low CP diets on lipid metabolism related genes and in turn on the carcass and meat fat contents could be mitigated by using BSFLO as a feed ingredient. Therefore, this study aims to determine the effect of BSFLO addition to LCP diets on broiler productivity, fat pad, meat quality, and the expression of genes involved in fat metabolism.

Materials and methods

Animal ethics

The experimental procedures were approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta Province, Indonesia (No. 036/EC-FKH Eks./2023).

Preparation of feed ingredients

Black soldier fly larvae oil was procured from PT Magalarva Sayana Indonesia (Banten, Indonesia), while crude palm oil (**CPO**) was obtained from PT. Rosa Asih (Yogyakarta, Indonesia). The BSFLO and CPO were analyzed to determine gross energy (**GE**) and fatty acid contents. The results of fatty acid analysis and GE value are given in Table 1. Gross energy (GE) was analyzed using CAL3K-S oxygen bomb calorimeter system (Digital data system, Ranburg, South Africa) following the method by Hopper et al. (2024). All feed ingredients were analyzed for dry matter (**DM**; method 930.15; AOAC, 2005), crude protein (**CP**; method 990.03; AOAC, 2005), extract ether (method 954.02; AOAC, 2005), and crude fiber (**CF**; method 973.18; AOAC, 2005). Additionally, all experimental diets (Table 2) were analyzed to determine proximate composition, GE, fatty acid, and amino acid profiles. Amino acid content analysis was carried out at the Master Lab Asia Laboratory, Trouw Nutrition Indonesia Company, using the TNI/MLA/WI-D-7.2.58 - C1, C2, and C3 (UHPLC) methodology. The analyzed nutrient composition of the experimental diets is presented in Table 3, while the fatty acid composition is shown in Table 4.

Table 1
Fatty acid composition (%) and gross energy (kcal/kg) of crude palm oil and black soldier fly larvae oil.

Items	CPO	BSFLO
Decanoic (C10:0)	1.79	0.34
Lauric (C12:0)	0.50	40.40
Myristic (C14:0)	1.15	6.90
Pentadecanoic (C15:0)	0.19	0.20
Palmitic (C16:0)	50.80	14.45
Heptadecanoid (C17:0)	0.37	0.54
Stearic (C18:0)	6.50	nd.
Heneicosanoic (C21:0)	nd.	0.88
Lignoceric (C24:0)	0.16	0.53
Palmitoleic (C16:1)	8.35	4.27
Oleic (C18:1)	8.32	0.44
Linoleic (C18:2)	20.12	30.36
Gamma-lonoleic (C18:3)	0.55	nd.
Eicosanoic (C20:1)	nd.	0.14
Linolenic (C18:3)	0.85	0.23
Eicosatrienoic (C20:3)	0.20	0.28
Nervoic (C24:1)	0.15	nd.
SFA	61.46	64.28
MUFA	16.82	4.85
PUFA	21.72	30.87
Gross energy (kcal/kg)	8,995	9,066

Abbreviations: BSFLO, Black soldier fly larvae oil; CPO, Crude palm oil; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids.

Birds and experimental treatments

Day-old male chicks of the New Lohmann Indian River (MB 202 Platinum) that had been vaccinated against Newcastle disease (**ND**) and Gumboro (infectious bursal disease) were procured from the commercial hatchery (PT. Widodo Makmur Poultry company, Yogyakarta Province, Indonesia) and brooded for 10 d. Birds were given commercial feed for up to 21 days.

The experiment followed completely randomized design in 3 × 2 factorial arrangement consisting of three dietary CP levels (200, 185, or 170 g/kg; high [**HCP**], medium [**MCP**], or low [**LCP**]) and two oil sources (BSFLO or CPO). Each treatment consisted of 6 replications with 8 birds per replication. Birds were weighed on d 21 (initial BW of 1080 ± 25 g) and randomly allocated into 36 colony cages (1 × 1 m). Experimental diets were fed ad libitum from 21 days to 35 days of age. The rations were formulated to meet or exceed the Aviagen (2022) recommendations (Table 2). Fresh litter was added to each pen at the start of the experiment as bedding for the chicks. The broiler management practices followed the recommendations of the Indian River broiler management handbook (Aviagen, 2018).

Samples collection

On day 35, blood samples were collected from one randomly selected bird per cage. Blood was drawn by cutting the jugular vein and collected into a plain vacuum tube containing EDTA. The separated blood serum was transferred to an Eppendorf tube and stored at −20 °C until analysis. Liver samples were also collected from each bird to microtubes.

Table 2
Feed ingredients and calculated nutrient composition of experimental diets.

Items	Percentage (%)					
	HCP+CPO	MCP+CPO	LCP+CPO	HCP+BSFLO	MCP+BSFLO	LCP+BSFLO
Feed ingredients						
Corn	65.00	66.76	68.52	65.00	66.76	68.52
Rice bran	6.86	9.93	13.00	6.86	9.93	13.00
Soybean meal	20.15	15.08	10.00	20.15	15.08	10.00
CPO	3.50	3.00	2.50	2.00	1.50	1.00
BSFLO	0.00	0.00	0.00	1.50	1.50	1.50
Limestone	1.00	1.05	1.10	1.00	1.05	1.10
Dicalcium phosphate	1.65	1.63	1.60	1.65	1.63	1.60
Common salt	0.45	0.43	0.40	0.45	0.43	0.40
Vitamin Mix[1]	0.05	0.05	0.05	0.05	0.05	0.05
Mineral Mix[2]	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride	0.05	0.06	0.07	0.05	0.06	0.07
Toxin binder	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine HCL	0.34	0.53	0.72	0.34	0.53	0.72
DL-Methionine	0.18	0.24	0.29	0.18	0.24	0.29
L-Threonine	0.07	0.15	0.23	0.07	0.15	0.23
L-Tryptophan	0.00	0.03	0.06	0.00	0.03	0.06
L-Isoleucine	0.05	0.17	0.28	0.05	0.17	0.28
L-Arginine	0.08	0.25	0.42	0.08	0.25	0.42
L-Valine	0.07	0.17	0.26	0.07	0.17	0.26
Calculated nutrients						
Dry matter, %	89.52	89.58	89.63	89.52	89.58	89.63
Metabolizable energy, Kcal/kg	3251	3270	3289	3253	3272	3291
Crude protein, %	19.64	18.14	16.64	19.64	18.14	16.64
Extract Ether, %	6.02	5.87	5.71	6.02	5.87	5.71
Crude fiber, %	4.13	4.28	4.43	4.13	4.28	4.43
Calcium, %	0.82	0.82	0.82	0.82	0.82	0.82
Available Phosphorus, %	0.40	0.40	0.40	0.40	0.40	0.40
Digestible Lysine, %	1.10	1.11	1.11	1.10	1.11	1.11
Digestible Methionine, %	0.46	0.49	0.51	0.46	0.49	0.51
Digestible Methionine+Cystine, %	0.73	0.73	0.73	0.73	0.73	0.73
Digestible Threonine, %	0.73	0.73	0.73	0.73	0.73	0.73
Digestible Tryptophan, %	0.20	0.20	0.19	0.20	0.20	0.19
Digestible Isoleucine, %)	0.75	0.75	0.75	0.75	0.75	0.75
Digestible Leucine, %	1.53	1.41	1.28	1.53	1.41	1.28
Digestible Arginine, %	1.18	1.18	1.18	1.18	1.18	1.18
Digestible Valine, %	0.84	0.84	0.84	0.84	0.84	0.84

Abbreviations: HCP, High crude protein; MCP, Medium crude protein; LCP, Low crude protein; BSFLO, Black soldier fly larvae oil; CPO, Crude palm oil.
(1) Supplied per kg of diet: Vitamin A, 50,000,000 IU, Vitamin D3, 10,000,000 IU; Vitamin E, 80,000 mg; Vitamin K3, 10,000 mg; Vitamin B1, 10,000 mg; Vitamin B2, 30,000 mg, Vitamin B3, 225,000 mg; Vitamin B5, 62,000 mg, Vitamin B6, 10,000 mg; Vitamin B9, 5,000 mg; Vitamin B12, 100 mg; Vitamin H, 100 mg; Vitamin C, 20,000 mg.
(2) Supplied per kg of diet: Mn, 40,000 mg; Fe, 32,000 mg; Cu, 6,050 mg; Zn, 32,000 mg; I, 404 mg; Se, 100 mg.

Microtubes were immediately frozen in liquid nitrogen and stored at −80 °C until analysis. Birds that were slaughtered for blood sample collection were subsequently defeathered, gutted and cleaned. Breast meat samples were collected for physical and chemical analysis. The litter samples collected on 35 d at six points in each pen were mixed to obtain a composite sample for each pen (Xu et al., 2017). Litter samples werestored at −20 °C immediately after collection.

Growth performance, carcass characteristics, and litter quality measurement

On day 35, all birds and remaining feed in each cage were weighed to determine final BW, gain, feed intake (**FI**) and feed conversion ratio (**FCR**). On the day 35, a total of 36 birds representing each replicate, with a BW close to the mean for each group were selected, weighed and slaughtered by decapitation and cutting the jugular vein using halal methods at a commercial slaughterhouse for carcasses evaluation. After defeathering and evisceration, the neck, head, and feet were manually removed from each carcass. The abdominal fat pad was collected and weighed. The hot carcasses were then placed in ice water (at 4 °C) for approximately 4 h and transported to the laboratory. The carcass, breast, thighs, and wing were weighed. Carcass and fat pad yield percentages were calculated relative to live weight, while breast, thigh, and wing yield percentages were calculated relative to chilled carcass. Fresh litter samples were immediately analyzed to determine pH using a pH meter (PHS-3C; Shanghai Yueping Scientific Instrument Co., Ltd., Shanghai, China), while ammonia (NH_3) levels were measured following the method described (Weatherburn, 1962). The litter was then analyzed for dry matter (method 930.15; AOAC, 2005) and nitrogen content (method 990.03; AOAC, 2005).

Blood biochemical measurement

The blood profile analyzed in this study consisted of measurements of protein, albumin, cholesterol, high-density lipoprotein (**HDL**), low-density lipoproteins (**LDL**), triglycerides, and uric acid. These measurements were obtained using a UV-visual photometer (Microlab 200: Merck Vital Scientific, Darmslandt, Netherlands) equipped with commercial equipment (DiaSys Diagnostic System GmbH, Holzheim, Germany).

Meat quality measurements

The breast meat samples were analyzed to determine the moisture (method 930.15; AOAC, 2005), ash (method 942.05; AOAC, 2005), crude protein (method 990.03; AOAC, 2005), ether extract (method 954.02; AOAC, 2005), and cholesterol content using the Liebermann-Burchard method (Shafiq et al., 2022). The physical

Table 3
Analysed nutrient composition of experimental diets.

Items	Treatments					
	HCP+CPO	MCP+CPO	LCP+CPO	HCP+BSFLO	MCP+BSFLO	LCP+BSFLO
Dry matter, %	89.64	89.45	89.25	89.75	89.60	89.45
Crude protein, %	19.85	18.30	16.75	19.65	18.10	16.55
Ether extract, %	3.35	3.50	3.65	3.15	3.30	3.45
Crude fiber, %	5.64	5.49	5.35	5.45	5.51	5.56
Ash, %	7.65	7.34	7.02	7.88	7.37	6.85
Gross energy, kcal/kg	3305	3367	3430	3325	3382	3440
Amino acids, %[1]						
Indispensable						
Arginine	1.03	1.05	1.06	1.03	1.03	1.02
Histidine	0.53	0.49	0.44	0.53	0.49	0.45
Isoleucine	0.71	0.74	0.77	0.70	0.69	0.68
Serine	0.89	0.74	0.59	0.89	0.70	0.51
Leucine	1.48	1.35	1.21	1.45	1.28	1.11
Lysine	1.11	1.18	1.24	1.14	1.11	1.08
Methionine	0.49	0.46	0.43	0.41	0.42	0.43
Phenylalanine	0.95	0.82	0.68	0.90	0.81	0.72
Threonine	0.71	0.71	0.71	0.70	0.68	0.65
Dispensable						
Alanine	0.79	0.71	0.63	0.79	0.68	0.57
Aspartic Acid	1.32	1.15	0.97	1.26	1.06	0.85
Cystine	0.32	0.29	0.25	0.30	0.28	0.25
Glutamic Acid	2.68	2.41	2.14	2.69	2.29	1.89
Proline	1.20	1.06	0.91	1.20	1.07	0.94
Glycine	0.70	0.59	0.47	0.70	0.57	0.44
Valine	0.80	0.82	0.83	0.91	0.83	0.74

Abbreviations: HCP, High crude protein; MCP, Medium crude protein; LCP, Low crude protein; BSFLO, Black soldier fly larvae oil; CPO, Crude palm oil.
[1] Analyzed using the TNI/MLA/WI-D-7.2.58 - C1, C2, and C3 (UHPLC) methods, Master Lab Asia Laboratory, Trouw Nutrition Indonesia Company.

characteristics of the meat (pH, amount of moisture loss during cooking, and meat tenderness) were assessed using the methodology described by Khan et al. (2021). The meat color was assessed using a colorimeter (CR-400, Minolta Camera Co., Osaka, Japan) and measured in terms of L* (lightness), a* (redness), and b* (yellowness) 45 min (blooming time) after slaughtered directly, using a 65 light source and a 2 °C observer (Bai et al., 2022).

Quantitative real-time PCR (RT-PCR)

For the gene expression analysis, RNA was extracted from liver samples weighing up to 20 mg. This was done using a Quick-RNA miniprep kit (Zymo Research Corp., Irvine, California) and following the the recommended procedures. The RNA purity and amount were assessed using a Nanodrop Spectrophotometer (Maestrogen Inc., Hsinchu City, Taiwan). The whole RNA was utilized as a template for cDNA synthesis employing reverse transcriptase enzyme with the ReverTrace qPCR RT Master Mix (Toyobo Co., Ltd., Osaka, Japan, Cat No. FSQ-301). The QuantStudio 3 Real-Time PCR machine (Thermo Fisher Scientific, Waltham, MA) and Thunderbird SYBR qPCR Mix (Toyobo Co., Ltd., Osaka, Japan, Cat No. QPX201) were used to perform relative gene expressions, following the specified technique. In summary, a total of 2 microliters of diluted cDNA, 6 picomoles of forward primer, 6 picomoles of reverse primer, 0.04 microliters of ROX reference dye, and 10 microliters of qPCR Mix were combined in a tube. This mixture was then brought up to a total reaction volume of 20 microliters using nuclease-free water.

The primer pairs utilized for the expression analysis of the genes fatty

Table 4
Fatty acid composition of experimental diets (% relative).

Fatty acids	Treatments					
	HCP+CPO	MCP+CPO	LCP+CPO	HCP+BSFLO	MCP+BSFLO	LCP+BSFLO
Lauric (C12:0)	0.27	0.17	0.17	6.32	6.73	7.14
Myristic (C14:0)	0.80	0.66	0.66	2.56	2.62	2.68
Palmitic (C16:0)	22.49	22.76	22.76	22.31	21.09	19.88
Heptadecanoid (C17:0)	0.08	0.07	0.07	0.08	0.07	0.06
Stearic (C18:0)	4.98	4.19	4.19	3.79	3.53	3.27
Arachidate (C20:0)	0.46	0.51	0.51	0.34	0.365	0.39
Docosanoic (C22:0)	0.12	0.14	0.14	0.08	0.095	0.11
Lignoceric (C24:0)	0.16	0.18	0.18	0.21	0.215	0.22
Palmitoleic (C16:1)	0.23	0.15	0.15	0.71	0.74	0.77
Oleic (C18:1)	42.94	43.31	43.31	37.86	38.07	38.28
Linoleic (C18:2)	26.20	26.89	26.89	24.66	25.3	25.94
Gamma-linolenic (C18:3)	1.04	0.74	0.74	0.87	0.84	0.81
Eicosanoic (C20:1)	0.17	0.19	0.19	0.12	0.13	0.14
Linolenic (C18:3)	1.04	0.74	0.74	0.87	0.84	0.81
Nervoic (C24:1)	0.16	0.18	0.18	0.22	0.22	0.22
SFA	29.36	28.68	28.68	35.69	34.67	33.65
MUFA	43.05	43.83	43.83	38.91	39.16	39.41
PUFA	28.28	28.37	28.37	23.52	25.54	27.56

Abbreviations: HCP, High crude protein; MCP, Medium crude protein; LCP, Low crude protein; BSFLO, Black soldier fly larvae oil; CPO, Crude palm oil; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids.

acid synthase (**FAS**), acetyl-CoA carboxylase (**ACC**), carnitine palmitoyltransferase 1 (**CPT-1**), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), sterol regulatory element-binding transcription factor 1 (**SREBP-1**), and peroxisome proliferator-activated receptor alpha (**PPAR-α**) may be located in Table 5. The amplification schedule consisted of a hold stage at 95 °C for 2 min, followed by a PCR stage with 40 cycles of 1 s at 95 °C and 30 s at 60 °C. At the conclusion of the run, the melt curve was examined to ascertain the amplification of the given product. There was a total of 6 samples in each group, and each sample was conducted twice. The mRNA levels were normalized using the $2^{-\Delta\Delta C(t)}$ method, with β-actin as the reference gene, and expressed as relative values compared to the control group (Livak and Schmittgen, 2001).

Statistical analyses

All experimental data were analyzed as a completely randomize design experiment in 3 × 2 factorial arrangement using IBM SPSS version 26.0. Significant main effects were compared using DMRT procedure. Effects were considered statistically significant at $P < 0.05$.

Results

Growth performances

The effect of reduced protein diets and BSFLO on broiler performance from d 21 to 35 are presented in Table 6. LCP diets significantly decreased final BW, gain, and FI ($P < 0.05$) compared to those fed MCP or HCP diets. Broilers feed HCP diets reported significantly ($P < 0.001$) lower FCR than those fed MCP or LCP diets. BSFLO in diets improved the FCR significantly ($P < 0.001$). A significant interactive effect between dietary protein level and oil source was found for FCR ($P < 0.01$). BSFLO improved FCR significantly ($P < 0.01$) with LCP and HCP, but not with MCP diets. No interactive effects were observed for the final BW, gain, and FI ($P > 0.05$).

Nutrient intake

The effect of low protein diets containing either BSFLO or CPO on nutrient intake of broilers is shown in Table 7. The MCP diets reported significantly higher ME intake than HCP and LCP diets. The reducing CP in the diets linearly decreased CP intake ($P < 0.001$) resulting in higher ME:CP ratios ($P < 0.001$). The inclusion of BSFLO in the diets reduced nutrient intake. A significant interaction between crude protein level and the type of oil used was observed for ME:CP ratio ($P < 0.001$). A significantly higher ME:CP ratio was reported by CPO at LCP level but not at HCP or MCP.

Table 5
Gene-specific primers used for analysis of mRNA levels of lipid metabolism using quantitative real-time RT-PCR (Xie et al., 2019).

Gen	Primer sequence (50′=>3′)	Orientation	Base pairs
β-actin	GTGTGATGGTTGGTATGGGC	Forward	225
	CTCTGTTGGCTTTGGGGTTC	Reverse	
FAS	TGGTTGACTGCCACCAATTG	Forward	213
	ACCCCACTTTCCATCACGAT	Reverse	
HMGR	TCCCTGAACCCTCATCTTTG	Forward	250
	TCTGCAAGAATACGGCTCCT	Reverse	
ACC	GCTGGGTTGAGCGACTAATG	Forward	173
	GGGAAACTGGCAAAGGACTG	Reverse	
CPT-1	GAAGACGGACACTGCAAAGG	Forward	223
	GGGCAAGTTGAATGAAGGCA	Reverse	
SREBF1	TCACCGCTTCTTCGTGGAC	Forward	220
	CTGAAGGTACTCCAACGCATC	Reverse	
PPAR-α	AATCATAAAGGAGTTTAAGTGACCG	Forward	264
	GCTGGTGAAAGGGTGTCTGT	Reverse	

Abbreviations: *ACC,* Acetyl-Coa carboxylase; *CPT-1,* Carnitine palmitoyltransferase; *FAS*, Fatty acid synthase; *HMGR*, 3-hydroxy-3-methylglutaryl coenzyme A reductase; *SREBP1*, Sterol regulatory element-binding transcription factor 1; *PPAR-α*, Peroxisome proliferator-activated receptor alpha.

Table 6
Effect of low protein diets with either black soldier larvae oil or crude palm oil on broiler performance from d 21 to 35.

Protein Level	Oil	Final BW (g)	Gain (g)	FI (g)	FCR
HCP	CPO	2505.54	1420.95	2692.51	1.90[b]
	BSFLO	2534.75	1449.75	2636.56	1.82[c]
MCP	CPO	2471.56	1416.38	2706.69	1.91[b]
	BSFLO	2484.38	1399.27	2647.91	1.89[b]
LCP	CPO	2382.40	1318.25	2644.87	2.01[a]
	BSFLO	2417.14	1342.45	2504.79	1.87[bc]
SEM		23.22	17.50	33.10	0.018
Main effect					
Protein level					
HCP		2520.14[a]	1435.35[a]	2664.54[a]	1.86[b]
MCP		2477.97[a]	1407.82[a]	2677.30[a]	1.90[a]
LCP		2399.77[b]	1330.35[b]	2574.83[b]	1.94[a]
SEM		16.42	12.59	23.41	0.01
Oil					
CPO		2453.16	1385.19	2681.36[a]	1.94[a]
BSFLO		2478.76	1397.16	2596.42[b]	1.86[b]
SEM		13.41	10.28	19.12	0.01
P-value					
Protein level		<0.001	<0.001	0.008	0.001
Oil		0.187	0.417	0.004	<0.001
Protein level x Oil		0.887	0.377	0.365	0.008

[a,b,c] Means within a column with different superscripts are different ($P < 0.05$).

Abbreviations: HCP, High crude protein; MCP, Medium crude protein; LCP, Low crude protein; BSFLO, Black soldier fly larvae oil; CPO, Crude palm oil; BW, Body weight; FI, Feed intake; FCR, Feed conversion ratio; SEM, Standard error of the mean.

Table 7
Effect of low protein diets with either black soldier larvae oil or crude palm oil on crude protein (CP), metabolizable energy (ME), and ME:CP intake in broiler.

Protein Level	Oil	ME (Kcal)	CP (g)	ME:CP ratio (Kcal/g)
HCP	CPO	8898.85	470.02	18.90[c]
	BSFLO	8767.36	462.85	18.90[c]
MCP	CPO	9339.10	446.62	20.90[b]
	BSFLO	9202.68	436.45	21.00[b]
LCP	CPO	9074.20	367.92	24.60[a]
	BSFLO	8616.59	356.61	24.10[a]
SEM		112.00	5.00	0.00
Main effect				
Protein level				
HCP		8833.10[b]	466.43[a]	18.90[c]
MCP		9270.89[a]	441.53[b]	21.40[b]
LCP		8845.90[b]	362.21[c]	24.40[a]
SEM		79.2	4.00	0.00
Oil				
CPO		8862.05[a]	428.18[a]	21.50[a]
BSFLO		8495.54[b]	418.60[b]	21.40[b]
SEM		64.5	3.00	0.00
P-value				
Protein level		0.039	<0.001	<0.001
Oil		0.040	0.037	<0.001
Protein level x Oil		0.356	0.911	<0.001

[a,b,c] Means within a column with different superscripts are different ($P < 0.05$).

Abbreviations: HCP, High crude protein; MCP, Medium crude protein; LCP, Low crude protein; BSFLO, Black soldier fly larvae oil; CPO, Crude palm oil; ME, Metabolizable energy; CP, Crude protein; SEM, standard error of the mean.

Carcass yield

The effect of dietary CP levels and source of oil on carcass characteristic are shown in Table 8. Dietary protein levels had no effect on

Table 8
Effect of dietary CP level and source of oil on broiler carcass characteristics (n = 6 per treatment).

Protein Level	Oil	Carcass (%)	Breast weight (%)	Thigh weight (%)	Wing weight (%)	Fat-pad (%)
HCP	CPO	73.01	48.01	41.50	10.90	1.52[b]
	BSFLO	72.53	46.94	42.10	10.79	1.14[c]
MCP	CPO	72.38	47.53	41.82	10.64	1.94[a]
	BSFLO	71.88	48.30	42.23	10.95	1.19[bc]
LCP	CPO	71.42	46.99	42.61	10.95	2.14[a]
	BSFLO	72.13	47.35	41.92	11.41	1.15[c]
SEM		0.48	0.60	0.46	0.30	0.12
Main effect						
Protein level						
HCP		72.77	47.47	41.80	10.85	1.33[b]
MCP		72.13	47.91	42.02	10.79	1.56[ab]
LCP		71.78	47.17	42.26	11.18	1.65[a]
SEM		0.34	0.42	0.33	00.22	0.08
Oil						
CPO		72.27	47.51	41.98	10.83	1.87[a]
BSFLO		72.18	47.53	42.08	11.05	1.16[b]
SEM		0.28	0.35	0.27	00.18	0.07
P-value						
Protein level		0.134	0.467	0.611	0.403	0.03
Oil		0.817	0.961	0.778	0.397	<0.001
Protein level x Oil		0.369	0.287	0.341	0.641	0.049

[a,b,c] Means within a column with different superscripts are different (P < 0.05).

Abbreviations: HCP, High crude protein; MCP, Medium crude protein; LCP, Low crude protein; BSFLO, Black soldier fly larvae oil; CPO, Crude palm oil; SEM, Standard error of the mean.

carcass, breast, thigh, and wing percentages (P > 0.05). However, broilers fed LCP had higher fat pad compared to HCP (P < 0.05). There was an interaction between dietary protein level and oil source (P < 0.05). The oil source had no effect on percentage fat pad at HCP level whereas both with LCP and MCP diets BSFLO reported significantly lower fat pad than CPO.

Blood biochemical parameters

Table 9 shows the effect of dietary CP levels and oil sources on blood biochemical profile in broilers. Compared to HCP, LCP reported significantly higher HDL, LDL and total cholesterol levels. Protein level was low in broilers fed MCP diets, compared to those fed either HCP or LCP. The addition of BSFLO decreased blood cholesterol (P < 0.05) and triglyceride (P < 0.01), but had no effect on HDL, LDL, protein, albumin, and uric acid (P > 0.05).

Meat physical quality

The effect of protein level in the diets and the type of oil on physical properties of broiler meat are presented in Table 10. The results show that reduced protein in the diets had no effect on pH and tenderness (P > 0.05). However, LCP diets had higher cooking loss compared to MCP (P < 0.05). The b* value was higher in broiler fed with lower protein diets (P < 0.05), while there was no significant difference in L* and a* values (P > 0.05). Dietary inclusion of BSFLO did not have a significant impact on pH, tenderness, and color (P > 0.05), but reduced the cooking loss (P < 0.01). An interaction between protein level and oil source was observed on the pH value at 45 min (P < 0.05). pH value of meat at 45 min was lower in the LCP than in the HCP when BSFLO was added.

Meat chemical quality

Chemical quality of broiler meat with dietary treatments of protein level in the diets and the type of oil are presented in Table 11. The protein level in the diets did not affect the moisture, ash, fat, protein, and cholesterol of the meat (P > 0.05). The addition of BSFLO to the feed increased the protein (P < 0.01), while reducing the fat and cholesterol (P < 0.01). There was no interaction between the feed protein level and oil source on the chemical composition of meat (P > 0.05).

Gene expression of lipid metabolism

Fig. 1 shows the effect of protein level in diets and BSFLO supplementation on genes expression of lipid metabolism. Lowering protein level in diets linearly increased ACC, FAS, HMGR, and SREBP-1 (P < 0.01). The addition of BSFLO decreased gene expression of ACC, FAS,

Table 9
Effect of dietary CP level and oil source on broiler blood biochemical profile (n = 6 per treatment).

Protein Level	Oil	HDL (mg/dl)	LD (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Protein (mg/dl)	Albumin (mg/dl)	Uric acid (mg/dl)
HCP	CPO	27.55	41.54	102.80	53.23	2.60	1.19	3.19[a]
	BSFLO	25.16	42.00	97.42	30.06	2.58	1.00	2.47[b]
MCP	CPO	31.10	44.04	106.64	52.70	2.35	1.03	1.92[c]
	BSFLO	32.20	40.12	101.00	37.55	2.40	1.05	2.10[bc]
LCP	CPO	33.60	46.66	123.12	48.58	2.66	1.25	1.68[c]
	BSFLO	33.78	49.48	109.73	40.28	2.45	1.19	1.73[c]
SEM		1.60	1.88	4.20	3.50	0.04	0.12	0.10
Main effect								
Protein level								
HCP		26.36[b]	41.77[b]	100.11[b]	41.64	2.59[a]	1.09	2.83[a]
MCP		31.65[a]	42.08[b]	103.82[b]	45.13	2.38[b]	1.04	2.01[b]
LCP		33.69[a]	48.07[a]	116.42[a]	44.43	2.56[a]	1.22	1.71[c]
SEM		1.14	1.30	3.01	2.48	0.06	0.09	0.10
Oil								
CPO		30.75[b]	44.08	110.85[a]	51.50[a]	2.54[b]	1.15	2.26
BSFLO		30.38[b]	43.87	102.72[b]	35.96[b]	2.48[b]	1.08	2.10
SEM		0.93	1.06	2.46	2.03	0.05[b]	0.07	0.08
P-value								
Protein level		<0.001	0.002	0.002	0.583	0.040	0.338	<0.001
Oil		0.780	0.888	0.026	<0.001	0.380	0.447	0.177
Protein level x Oil		0.540	0.197	0.571	0.124	0.311	0.701	0.008

[a,b,c] Means within a column with different superscripts are different (P < 0.05).

Abbreviations: HCP, High crude protein; MCP, Medium crude protein; LCP, Low crude protein; BSFLO, Black soldier fly larvae oil; CPO, Crude palm oil; LDL, Low density lipoprotein; HDL, High density; SEM, Standard error of the mean.

Table 10
Effect of dietary CP level and source of oil on physical quality of broiler meat ($n = 6$ per treatment).

Parameter	Oil	pH		Cooking loss (%)	Tenderness (Kg/cm^2)	Color		
		45 min	24 h			L*	a*	b*
HCP	CPO	5.80[ab]	6.07	30.65	1.81	52.15	2.27[ab]	10.10
	BSFLO	6.00[a]	6.05	27.88	1.78	51.87	1.45[c]	9.31
MCP	CPO	5.76[b]	5.97	30.43	1.74	51.12	1.76[bc]	13.72
	BSFLO	5.89[ab]	6.11	27.31	1.84	52.17	2.84[a]	13.08
LCP	CPO	5.89[ab]	5.93	31.37	1.52	52.35	1.78[bc]	10.64
	BSFLO	5.74[b]	5.98	28.86	1.72	53.13	2.02[bc]	12.86
SEM		0.07	0.07	00.45	0.14	0.98	0.23	0.80
Main effect								
Protein level								
HCP		5.90	6.06	29.27[ab]	1.79	52.01	1.86	9.71[c]
MCP		5.83	6.04	28.87[b]	1.79	51.65	2.30	13.40[a]
LCP		5.82	5.95	30.12[a]	1.62	52.74	1.90	11.75[b]
SEM		0.05	0.05	0.33	0.10	0.69	0.17	0.57
Oil								
CPO		5.82	5.99	30.82[a]	1.69	51.87	1.94	11.49
BSFLO		5.88	6.05	28.02[b]	1.78	52.39	2.10	11.75
SEM		0.40	0.04	0.27	0.08	0.56	0.13	0.46
P-value								
Protein level		0.447	0.299	0.035	0.396	0.528	0.137	<0.001
Oil		0.299	0.308	<0.001	0.446	0.521	0.392	0.691
Protein level x Oil		0.047	0.539	0.802	0.725	0.772	0.001	0.128

[a,b,c] Means within a column with different superscripts are different ($P < 0.05$).

Abbreviations: HCP, High crude protein; MCP, Medium crude protein; LCP, Low crude protein; BSFLO, Black soldier fly larvae oil; CPO, Crude palm oil; L*, Lightness; a*, Redness; b*, Yellowness; SEM, Standard error of the mean.

Table 11
Effect of dietary CP level and source of oil on broiler meat chemical quality ($n = 6$ per treatment).

Parameter	Oil	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Cholesterol, (mg/100 g)
HCP	CPO	73.28	5.41	10.25	23.95	803.33
	BSFLO	72.83	5.27	07.65	27.44	746.40
MCP	CPO	73.67	5.42	11.06	24.23	901.84
	BSFLO	72.95	5.26	07.58	27.00	780.09
LCP	CPO	72.98	4.65	11.37	24.38	875.06
	BSFLO	72.80	4.85	08.00	26.58	801.02
SEM		0.35	0.39	0.56	0.82	31.70
Main effect						
Protein level						
HCP		73.06	5.34	08.95	25.70	774.86
MCP		73.31	5.34	09.32	25.61	840.96
LCP		72.89	4.75	09.69	25.48	838.04
SEM		0.25	0.28	0.40	0.58	22.41
Oil						
CPO		73.31	5.16	10.89[a]	24.19[b]	860.08[a]
BSFLO		72.86	5.12	7.74[b]	27.01[a]	775.83[b]
SEM		0.21	0.23	0.33	0.48	18.30
P-value						
Protein level		0.498	0.247	0.435	0.965	0.078
Oil		0.129	0.904	<0.001	0.000	0.003
Protein level x Oil		0.751	0.880	0.700	0.738	0.576

[a,b,c] Means within a column with different superscripts are different ($P < 0.05$).

Abbreviations: HCP, High crude protein; MCP, Medium crude protein; LCP, Low crude protein; BSFLO, Black soldier fly larvae oil; CPO, Crude palm oil; SEM, Standard error of the mean.

HMGR, and SREBP-1 ($P < 0.01$), while increasing the expression of CPT-1 and PPARα ($P < 0.01$). There was an interaction between protein level and BSFLO supplementation on gene expression of lipid metabolism ($P < 0.01$). The gene expression of ACC and FAS was the lowest in broilers fed HCP and MCP with the addition BSFLO and highest in LCP with or without the addition of BSFLO. The gene expression of CPT-1 increased as the feed protein level decreased with the addition of BSFLO ($P < 0.01$). The highest value of CPT-1 expression was observed in broilers fed LCP with the addition of BSFLO. The gene expression of HMGR increased in chickens fed a reduced protein diets (LCP and MCP) with BSFLO ($P < 0.01$). The addition of BSFLO increased the SREBP-1 gene expression in chicken fed a low protein diets ($P < 0.01$).

Litter quality

The effect of protein level in diets and BSFLO on litter quality is presented in Table 12. Reducing the protein level in the diets (MCP and LCP) increased dry matter and decreased pH of litter ($P < 0.05$). Decreasing dietary protein by 30 g/kg (LCP) decreased ammonia and nitrogen of litter ($P < 0.01$). The addition of BSFLO had no significant effect on litter quality parameters ($P > 0.05$).

Discussion

Although the reduction of 30 g/kg in dietary protein resulted in lower body weight, those birds exceeded the Indian River Performance

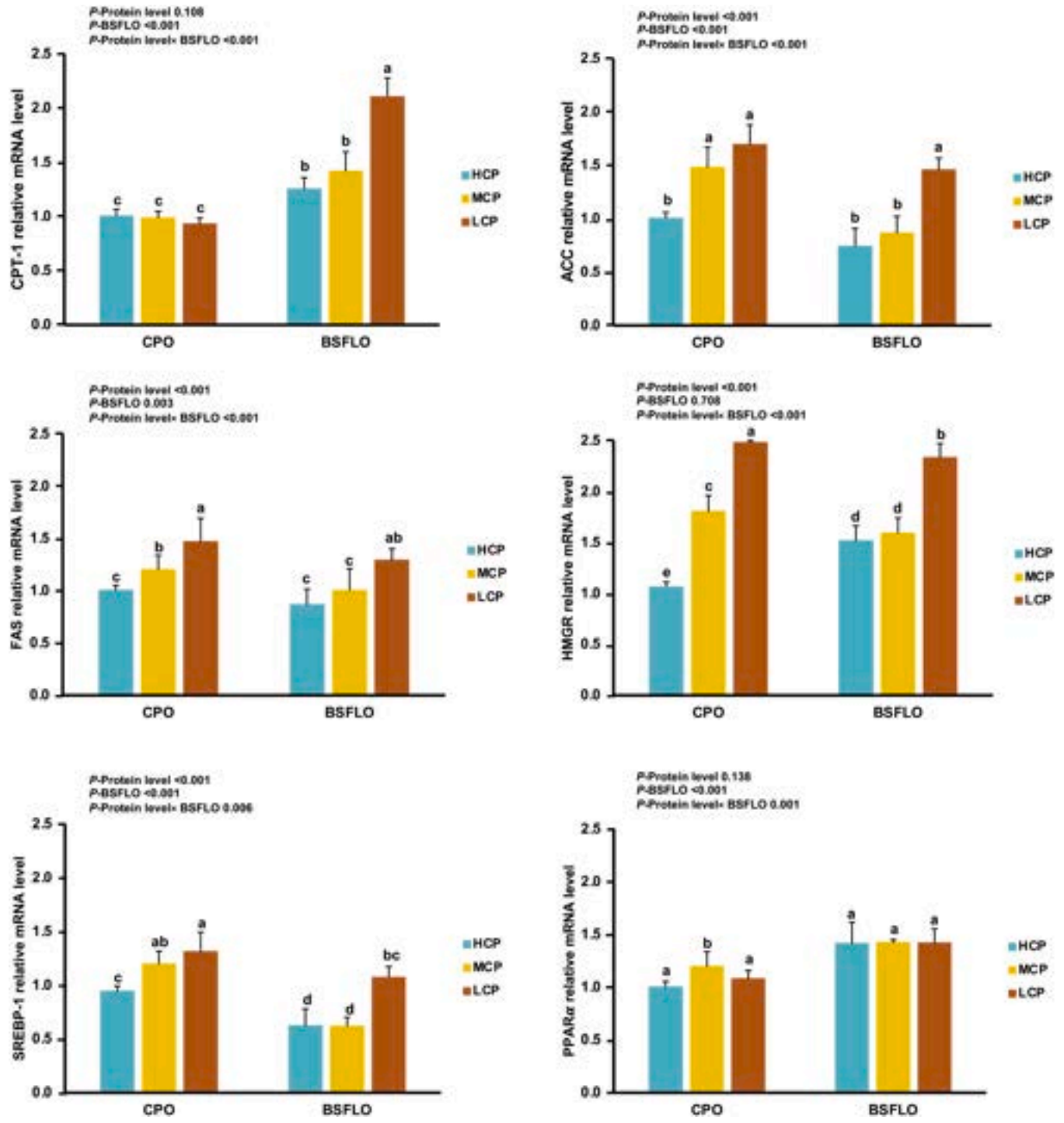

Fig. 1. Effect of black soldier fly larvae oil calcium salt (BSFLO) supplementation and protein level in diets on mRNA expression of lipid metabolism in liver. Data represented by means ± SEM ($n = 6$ per treatment).[a–d]Bars with different letter are different under different treatment group when interaction effect occurs ($P < 0.05$). P-values of BSFLO supplementation, protein level, and their interaction are indicated in the figure.

Objective (Aviagen, 2022) for live weight (2295 vs 2399 g). The inferior growth performance reported in birds fed LCP diets compared to those fed HCP and MCP diets is consistent with findings from others studies (Wang et al., 2020; Macelline et al., 2023). Chrystal et al. (2020a) revealed that 30 g/kg of dietary CP reduction had no significant impact on broiler performance. In line with Chrystal et al. (2020a) and Wang et al. (2020), a moderate level of reduction in dietary CP to 180 g/kg from 200 g/kg had negligible effects on body weight and weight gain, though increased the FCR significantly. Interestingly, though a reduction in CP level to 170 g/kg resulted in inferior growth performance parameters, as discussed later, FCR was significantly overcome when BSFLO replaced CPO.

Kidd et al. (2005) demonstrated that the use of synthetic amino acids to augment amino acid density in feed resulted in improved growth performance and FCR. Additionally, the incorporation of unbound amino acids derived from synthetic sources exhibited higher absorption rates compared to amino acids from feed ingredients (bound amino acids), potentially compromising the growth performance of broilers fed

Table 12
Effect of dietary CP level and source of oil on broiler litter quality ($n = 6$ per treatment).

Parameter	Oil	Dry matter (%)	pH	NH_3 (mg/100g)	Nitrogen (%)
HCP	CPO	56.97	7.40	166.28	1.06
	BSFLO	59.49	7.18	191.78	1.07
MCP	CPO	63.39	7.04	164.66	0.83
	BSFLO	65.06	7.08	173.58	0.84
LCP	CPO	65.58	6.98	120.27	0.83
	BSFLO	66.25	7.00	118.39	0.77
SEM		2.60	0.09	13.80	0.04
Main effect					
Protein level					
HCP		58.23[b]	7.29[a]	179.03[a]	1.07[a]
MCP		64.22[a]	7.06[b]	169.12[a]	0.83[b]
LCP		65.92[a]	6.99[b]	119.33[b]	0.80[b]
SEM		1.88	0.07	9.80	0.03
Oil					
CPO		61.98	7.14	150.40	0.91
BSFLO		63.60	7.09	161.25	0.89
SEM		1.54	0.06	8.00	0.02
P-value					
Protein level		0.018	0.011	<0.001	<0.001
Oil		0.463	0.524	0.345	0.687
Protein level x Oil		0.941	0.331	0.614	0.667

[a,b,c] Means within a column with different superscripts are different ($P < 0.05$).

Abbreviations: HCP, High crude protein; MCP, Medium crude protein; LCP, Low crude protein; BSFLO, Black soldier fly larvae oil; CPO, Crude palm oil; NH_3, ammonia; SEM, Standard error of the mean.

low-protein diets (Liu et al., 2021; Macelline et al., 2023). Results of this study support that dietary CP level can be reduced up to 15 g/kg with minimum effects on live weight and gain

Chrystal et al. (2020a) revealed that reducing dietary protein by 30 g/kg from 210 to 180 g/kg had no significant impact on broiler performance when provided with sufficient essential amino acid. However, the provision of non-essential amino acids is reported to be crucial. Furthermore, decreasing dietary protein levels may increase the ileal starch-protein disappearance rate ratio, negatively affecting feed efficiency (Chrystal et al., 2020a; 2020b; Liu et al., 2021; Strifler et al., 2023). These findings highlight that a delicate balance of amino acids is needed in formulating low-protein diets to ensure optimal broiler performance.

The results of this research reported that medium-chain fatty acids (MCFAs) in BSFLO, especially lauric acid (C:12) was much higher compared to palm oil (4.04 vs 0.05 g/kg). MCFAs are known to be absorbed more efficiently in the intestine than long-chain fatty acids (LCFAs). Moreover, the oxidation of MCFAs as an energy source occurs more rapidly than that of LCFAs. Energetic catabolism of LCFAs requires carnitine-mediated transport for entry into mitochondria whereas MCFAs are believed to undergo oxidation independently of carnitine (Baltić et al., 2019; Houten et al., 2016; Montgomery et al., 2013; Pereyra et al., 2023; Schönfeld and Wojtczak, 2016). Decreased feed intake without affecting body weight and, better feed efficiency (1.94 vs 1.86) of BSFLO fed birds could be attributed to above mechanisms.

The inclusion of BSFLO in standard CP diets enhanced feed efficiency by 4.21 % (1.90 vs 1.82). Meanwhile, in diets with a 30 g/kg CP reduction (LCP), BSFLO had a more prominent impact in improving FCR where FCR was improved by 6.96 % (2.01 vs 1.87). Baltić et al. (2019) showed that MCFAs increased body weight and feed efficiency in broilers. These findings highlighted the potential of MCFAs, particularly from BSFLO, to improve feed efficiency in broiler. In the present study, adverse effects of low CP on FCR were more prominent when CPO was the dietary oil source. However, the above effect was corrected to a level comparable with the FCR of HCP-BSFLO diets when BSFLO was used with LCP diet. These findings suggest the potential of using BSFLO as a mean of mitigating adverse effects of LCP diets on FCR of broilers.

The present study showed that dietary CP reduction and BSFLO inclusion had no effect on carcass, breast, thigh, and wing percentage. This was consistent with the previous studies (Wang et al., 2020; Belloir et al., 2017). On the other hand, in the present study, the fat pad percentage was increased in broilers fed a low protein diet. Contrary to a number of previous research (Ma et al., 2023; Sharma et al., 2022) a moderate level of dietary CP reduction (200-185 g/kg), had no significant effects on fat pad. Dietary supplementation of BSFLO markedly decreased fat pad of broilers (Aprianto et al., 2023). The increase in fat pad percentage in the present study could be explained by the increase of dietary ME:CP ratio as CP was reduced. This was consistent with previous studies (Belloir et al., 2017).

Increase in ME:CP ratio with the reduction of dietary CP level, resulted in higher ACC and FAS gene expression while lowering the protein levels. The level of lipid accumulation is determined by key enzymes such as ACC and FAS that involve in lipid synthesis and CPT-1 which involve in β-oxidation (Ma et al., 2023). Moreover, the use of BSFLO in place of CPO with increasing of ME:CP ratio decreased ACC and FAS gene expression, while increasing CPT-1 gene expression (Fig. 1). The BSFLO inclusion reduced lipid deposition by reducing the key enzymes for lipid synthesis and increasing the key enzymes for β-oxidation. Kang et al. (2022) also reported that downregulated ACC and FAS and upregulated CPT-1 gene expression resulted in lower lipid deposition.

Dietary protein can influence the metabolism of cholesterol and lipoproteins. Certain amino acids in proteins can affect the synthesis, oxidation, and excretion of cholesterol, thereby influencing the levels of cholesterol, HDL, and LDL (Cao et al., 2019). When protein intake is reduced, it could potentially lead to higher levels of cholesterol, HDL, and LDL (Caponio et al., 2021). This is in line with this study where a 30 g/kg reduction in dietary protein increased HDL and LDL cholesterol ($P < 0.01$). However, the addition of BSFLO reduced blood cholesterol ($P < 0.05$) and triglycerides compared to CPO addition ($P < 0.01$).

The BSFLO is rich in medium-chain fatty acids. which are known to reduce the speed of cholesterol synthesis by reducing HMG-CoA Reductase enzyme activity. This enzyme plays a crucial role in cholesterol synthesis in the body. It catalyses the conversion of HMG-CoA to mevalonate, an important step in the biosynthesis of cholesterol. MCFAs, particularly lauric acid, are known to reduce the activity of the HMG-CoA reductase enzyme. By inhibiting this enzyme, MCFAs can slow down the rate of cholesterol synthesis in the body (Seyedalmoosavi et al., 2022b). Therefore, it is hypothesized that BSFL being rich in MCFAs reduce cholesterol synthesis when chickens are fed diets supplemented with BSFLO.

A 30 g/kg decrease in feed protein linearly reduced uric acid ($P < 0.01$). Apart from that, there was a significant interaction between protein levels and source of oil on uric acid ($P < 0.01$). Uric acid is a by-product of protein metabolism. When broilers consume protein, it's broken down into amino acids. Some of these amino acids are further metabolized into uric acid. When the protein intake is reduced, there's less protein available for metabolism. Consequently, the production of uric acid decreases (Kidd et al., 2021b). Lower protein diets improve nitrogen utilization efficiency, thereby reducing nitrogen content in manure. This reduction in nitrogen excretion positively affects uric acid levels (Rauglaudre et al., 2023). This is in accordance with the present research where reduction of dietary protein by 15 g/kg decreased nitrogen content in the litter.

Dong et al. (2023) indicated that a reduction of 30 g/kg crude protein in the diets did not affect meat quality parameters such as meat color, pH, cooking loss, and tenderness. In contrast, in the current study, MCP and HCP increased the meat b* colour and cooking loss. Similarly, Duque-Ramírez et al. (2023) reported that lowering the level of crude protein in the diets increased b* of broiler meat. Baéza et al. (2022) mentioned that chickens fed a corn-based diets tend to have meat with higher yellowness. Therefore, higher b* may be linked to the diet

composition where low CP diets had higher corn level. Moreover, Benahmed et al. (2023) reported that broiler fed by low protein diets increased cooking loss of meat. Higher fat content can result in increased cooking loss because fat tends to melt during the cooking process, leading to a greater reduction in meat weight and moisture content. This statement is applicable to this study, where an increase in fat content, particularly in the fat pad, was observed with a decrease in dietary protein (Table 8). Schumacher et al. (2022) reviewed that fatty acids with longer chains affect in higher melting points, while fatty acids with more unsaturated bonds have lower melting points. Composite fatty acids typically melt within the range of 25 °C–50 °C, saturated fats melt at higher temperatures and polyunsaturated fats (PUFAs) melt at lower temperatures.

BSFLO supplementation in the present study reduced cooking loss, which was consistent with a previous study where cooking loss was reduced when BSFLO was added to the diets (Aprianto et al., 2023). Meat pH value was reduced at 45 min after slaughter in the LCP group under BSFLO supplementation. According to Mir et al. (2017) the pH level in meat depends on the quantity of glycogen present in the muscle before slaughter and the speed at which glycogen is converted into lactic acid after slaughter. Rosenvold et al. (2001) reported that high muscle glycogen store was caused by high protein content in the diet. The lower cooking loss may be influenced by the protein content of the meat. In the current study, broilers fed diets with BSFLO had higher meat protein content. The presence of protein is associated with water holding capacity (WHC), where an increase in WHC leads to lower cooking loss (Van Laack, 1999; Bowker and Zhuang, 2015). However, further investigation is needed to fully understand the impact of BSFLO supplementation on broiler meat protein and WHC. The observed reduction in cooking loss may indicate the potential for BSFLO addition to improve broiler meat quality.

A number of studies (Sharma et al., 2022; Benahmed et al., 2023) have indicated that lowering crude protein level in the diets had no influence on meat chemical quality when amino acid requirements are adequately fulfilled. The present study also demonstrated that reduced CP diets had no effect on meat chemical quality. In the present study, the addition of BSFLO to LCP had a positive effect by reducing the carcass fat pads. This finding is in line with the result from previous studies that showed BSFLO supplementation decreased meat fat and cholesterol (Aprianto et al., 2023; Dabbou et al., 2021). The reduction in meat fat was associated with the downregulation of genes responsible for lipid (FAS and ACC) and cholesterol (HMGR) synthesis in the liver of broilers fed BSFLO. Moreover, the addition of BSFLO upregulated the expression of genes involved in β-oxidation (CPT-1). The inclusion of BSFLO in the diets increased meat protein content. Aprianto et al. (2023) also reported that dietary supplementation with BSFLO significantly increased meat protein content. According to Shokrollahi et al. (2014), MCFA promotes glucose oxidation that leads to protein synthesis in the body.

In the present study, the expression of genes ACC, FAS, HMGR, and SREBP-1 was increased in broilers fed the low protein diet. Similarly, Ma et al. (2023), observed that low protein diets upregulated the gene expression of ACC, FAS, and SREBP-1 in the liver of broilers. To our knowledge, this is the first study presenting the direct effect of protein reduction on HMGR expression. The increased expression of ACC, FAS, HMGR in the present study could be associated with the upregulation of SREBP-1 as a factor transcription, according to the results from Wan et al. (2021). Overall, the supplementation of BSFLO downregulated the gene expression of ACC, FAS, HMGR dan SREBP-1, particularly in the MCP and HCP diets. In the LCP diet, BSFLO downregulated the expression of HMGR, FAS, and SREBP-1 compared to CPO. Aprianto et al. (2023), Anas et al. (2024c) showed that the administration of BSFLO reduce the gene expression of ACC and FAS. The inclusion of MCFA reduced the activity of HMGR in line with the findings from previous research (Sung et al., 2018). The BSFLO contain high levels of MCFA, especially lauric acid. MCFA is a natural ligand of SREBP-1 that can reduce its expression. The decreased expression of SREBP-1 effects on downregulation of FAS and ACC in lipid synthesis. Additionally, SREBP-1 is also transcription factor that regulate ACC in cholesterol synthesis (Eberlé et al., 2004). In the present study, the gene expression of CPT-1 was similar across different protein levels in the CPO diet. Meanwhile, the inclusion of BSFLO upregulated the expression of CPT-1 in the LCP diets and PPARα. The low protein diets supplemented with MCFA had no effect on the relative expression of CPT-1 (Tang et al., 2023). The upregulation of the CPT-1 gene expression was associated with the increased of PPARα gene expression with supplementation of BSFLO. Arunima and Rajamohan (2014), suggested that dietary coconut oil as a source of MCFA increased the expression of CPT-1 gene, which was correlated with the upregulation of PPARα.

Broilers excrete nitrogen in various forms (Braun, 2015), with the majority being uric acid (70–80 %). Litter microbes convert these compounds into ammonia (NH_3) (Ferguson et al., 1998). The present study demonstrated that reduction of dietary CP from 200 to 170 g/kg lowered the N, pH, and NH_3 content and the moisture content ($P < 0.05$). Van Harn et al. (2019), showed also that reducing broiler dietary protein by 15 g/kg lowered nitrogen content and increased litter dry matter, without affecting NH_3 and litter pH.

Broilers fed low-protein diets (MCP and LCP) exhibited higher litter dry matter levels compared to the control (HCP). Broilers fed low-protein diets consume less water (Van Harn et al., 2019). The elevated nitrogen contents in diets lead to increased litter pH values. High pH and moisture contents in litter enhance the conversion of litter N into ammonia (Toppel et al., 2019). Less N excretion, lower pH and moisture levels observed in the litter of broilers fed low CP diets may be the reasons for reduced ammonia emission.

Conclusions

A moderate level (15 g/kg) of dietary CP reduction to 185 g/kg with crystaline amino acids could maintain feed intake, live weight and weight gain comparable with those fed 200 g/kg of dietary CP. In LCP diet, BSFLO improved the FCR significantly compared to CPO. As expected, the LCP diets increased the carcass fat pads of broilers. BSFLO reduced fat pads of broilers at all three dietary CP levels. Dietary BSFLO down-regulated the expression of genes associated with lipogenesis resulting in a significant reduction in body fat. Moreover, BSFLO increased the muscle protein content while reducing cholesterol levels. LCP diets reduced the litter N, moisture and pH levels thereby lowering the emission of ammonia. It is concluded that the inclusion of BSFLO in low crude protein diets has beneficial effects on feed conversion efficiency, lipid metabolism related gene expression, carcass characteristics, meat quality, and nitrogen excretion in broiler chickens.

Declaration of competing interest

All authors certify that they have no affiliation with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership; employment; consultancies; stock ownership; or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in subject matter or materials discussed in this manuscript.

Acknowledgments

The authors highly appreciate the assistance and facilities provided by the Laboratory of Nutritional Biochemistry and Tropical Animal Research Center, Faculty of Animal Science, Universitas Gadjah Mada. We would also like to extend our gratitude to Nur'alim Hidayaturrohman, Dimas Fakhruddin, Lanti Yulianti Rebecca Sitohang, and Patrick Erhard Latue from the ABM Research Group for their technical support during this study.

Disclosures

The authors declare that there is no conflict of interest.

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