JAST (Journal of Animal Science and Technology) TITLE PAGE

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Evaluation of black soldier fly larvae oil in broiler diets: effects on growth performance, meat quality, and fatty acid profile
Running Title (within 10 words)	Black soldier fly larvae oil in broiler diets
Author	Ha Neul Lee, Gyu Lim Yeom, Yeong Bin Kim, Ju Yeong Park, Geun Yong Park, Ji Won Shin, Jong Hyuk Kim* * Corresponding author
Affiliation	Department of Animal Science, Chungbuk National University, Cheongju 28644, Republic of Korea
ORCID (for more information, please visit https://orcid.org)	Ha Neul Lee (https://orcid.org/0009-0007-8352-4182) Gyu Lim Yeom (https://orcid.org/0009-0006-1849-053X) Yeong Bin Kim (https://orcid.org/0009-0007-9151-0135) Ju Yeong Park (https://orcid.org/0009-0008-1938-977X) Geun Yong Park (https://orcid.org/0009-0001-3453-6941) Ji Won Shin (https://orcid.org/0009-0000-7167-5425) Jong Hyuk Kim (https://orcid.org/0000-0003-0289-2949)
Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources **State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.**	This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (RS-2024-00465159). This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (RS-2023-00210634).
Acknowledgements	Not applicable.
Availability of data and material	No potential conflict of interest relevant to this article was reported.
Authors' contributions **Please specify the authors' role using this form.**	Conceptualization: Kim JH Data curation: Lee HN, Kim JH Formal analysis: Lee HN, Yeom GL, Kim YB, Park JY, Park GY, Shin JW

	Methodology: Lee HN, Kim JH Validation: Kim JH Investigation: Lee HN, Yeom GL, Kim YB, Park JY, Park GY, Shin JW Writing - original draft: Lee HN, Kim JH Writing review & editing: Lee HN, Yeom GL, Kim YB, Park JY, Park GY, Shin JW, Kim JH
Ethics approval and consent to participate	All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Chungbuk National University (CBNUA-25-0060-01).

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Jong Hyuk Kim
Email address - this is where your proofs will be sent	jonghyuk@chungbuk.ac.kr
Secondary Email address	mrdgj7@naver.com
Address	Department of Animal Science, Chungbuk National University, Cheongju 28644, Republic of Korea
Cell phone number	82 - 10 - 4935 - 8583
Office phone number	82 - 43 - 261 - 2546
Fax number	82 - 43 - 273 - 2240

Abstract

The objective of this experiment was to investigate black soldier fly larvae (BSFL) oil as a sustainable energy source in broiler diets by comparing its effects with those of soybean oil and tallow. A total of 180 1-d-old broiler chickens were randomly allotted to 1 of 3 dietary treatments with 5 replicates. Each replicate had consisted of 6 male and 6 female birds. Experimental diets were formulated to contain 3% of either soybean oil, tallow, and BSFL oil. These diets were provided on an ad libitum basis for 5 wk. Results indicated that replacing soybean oil and tallow with BSFL oil had no significant effects on growth performance, relative organ weight, stress indicator, liver antioxidant, jejunal morphology, jejunal permeability, and animal welfare assessment among treatment groups. However, broiler chickens fed diets containing BSFL oil and tallow had less ($P < 0.05$) of 24 h postmortem pH than those fed diets containing soybean oil. For meat color, redness values were greater ($P < 0.05$) in the soybean oil group than in the other groups. The BSFL oil group showed greater ($P < 0.05$) saturated fatty acid concentrations in the breast meat than the soybean oil and tallow groups. On the other hand, the soybean oil and tallow groups had greater ($P < 0.05$) unsaturated fatty acid concentrations in the breast meat than the BSFL oil group. The BSFL oil group had greater ($P < 0.05$) docosapentaenoic acid and docosahexaenoic acid in the breast meat than the other groups. Serum aspartate aminotransferase concentration was greater ($P < 0.05$) in the BSFL oil group than in the soybean oil group, while serum uric acid concentration was less ($P < 0.05$) in the BSFL oil group than in the tallow and soybean oil groups. These results suggest that BSFL oil can be used as an alternative energy source in broiler diets without affecting growth performance, but influencing meat quality and fatty acid profiles.

Keywords: Black soldier fly larvae oil, breast meat, broiler chicken, fatty acid, growth performance

INTRODUCTION

As the global population steadily increases, meat consumption is also rising, which subsequently elevates the demand for protein and energy sources [1]. Recently, various fat sources have been utilized in poultry nutrition, including animal by-products such as tallow, lard, and poultry fat, as well as vegetable oils such as soybean oil, corn oil, and sunflower oil [2]. Soybean oil is used in poultry diets due to its high metabolizable energy and digestibility compared with other vegetable oils [3]. However, soybean oil relatively expensive and its supply is limited [4]. Beef tallow is one of the animal fats that is commonly utilized due to lower cost compared with vegetable oils, although its rendering process generates considerable greenhouse gas emissions [5, 6].

Accordingly, insects have attracted increasing attention as alternative protein and fat sources in animal feed production [4, 7]. Lipids constitute a major component of insect biomass, and substantial amounts are obtained as by-products during protein extraction [4]. Among insect species, the black soldier fly (*Hermetia illucens*) has gained considerable interest for its ability to convert livestock manure and other organic waste into larval biomass rich in protein and fat [8, 9]. Black soldier fly larvae (BSFL) contain approximately 42% protein and 35% fat [10, 11]. The fatty acid composition of BSFL is characterized by high levels of medium-chain fatty acids (MCFAs) such as lauric acid and myristic acid [12], with lauric acid is particular known for its antimicrobial effects on gut bacteria [13, 14]. Furthermore, the lipid content and fatty acid profile of insects are influenced by the chemical composition of the substrate in the rearing environment [15, 16]. In contrast, lauric acid is synthesized de novo by the larvae and its concentration in the insect biomass is not affected by the substrate [17].

Previous studies have demonstrated that replacing 50% and 100% of soybean oil with BSFL fat in diets did not adversely affect growth performance, internal organ weight, blood parameters, and gut morphology in finisher broiler chickens [4]. Similarly, replacing soybean oil with BSFL oil in broiler chicken diets did not significantly affect growth performance [18]. These findings indicate that BSFL fat may be a viable fat source in broiler feed formulations. However, few studies have investigated the effects of replacing both soybean oil and tallow with BSFL oil in broiler diets. Moreover, information on the effects of dietary BSFL oil on meat quality and fatty acid profile remains limited.

Therefore, the objective of this experiment was to evaluate BSFL oil as a sustainable energy source in broiler diets by comparing its effects with those of soybean oil and tallow.

MATERIALS AND METHODS

Animals, diets, and experimental design

All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Chungbuk National University (CBNUA-25-0060-01). A total of one hundred eighty 1-d-old Arbor Acres broiler chickens were obtained from a local commercial hatchery (Dongsan Hatchery, Cheonan, Republic of Korea) and housed in 15 cages (104 × 122 × 84 cm = width × length × height) within an environmentally controlled room. All birds (body weight; BW = 40.11 ± 0.06 g) were randomly allotted to 1 of 3 dietary treatments with 5 replicate cages in a completely randomized design. Each replicate had consisted of 6 male and 6 female birds. Experimental diets were formulated to contain 3% of either soybean oil, tallow, and BSFL oil. The BSFL oil used in the experimental diets was obtained from Entomo Co., Ltd. (Cheongju, Republic of Korea). The oil was extracted from BSFL harvested at the late larval stage. The assumed AME_n values, based on Kierończyk et al. [19] and Rostagno et al. [20] were as follows: 8,790 kcal/kg for soybean oil, 7,401 kcal/kg tallow, and 9,019 kcal/kg for BSFL oil. These estimated AME_n values were applied in the formulation of experimental diets, and differences in AME_n values among dietary treatments were compensated by adjusting the proportions of corn, corn gluten meal, and other ingredients. All diets were formulated to meet or exceed the Arbor Acres nutrients specifications for energy and nutrients in broiler chickens [21] (Table 1). During the experiment, birds were provided all diets and water ad libitum for 5 wk. The temperature was maintained at 30.4°C ± 0.3 for the first 3 d and gradually reduced to 24°C by the end of the experiment. The relative humidity was held constant at 61.7% ± 5.4. Birds were exposed to a lighting schedule of 23L:1D. The BW gain (BWG) and feed intake (FI) were recorded at the conclusion of the experiment. Feed conversion ratio (FCR) was calculated by dividing FI with BWG. Specifically, FI was corrected by accounting for both the number of dead birds and their survival duration.

Sample collection

At the end of the experiment, 2 male broiler chickens with BW close to the mean BW per cage were selected and euthanized by CO_2 asphyxiation. One bird was used for the collection of feather, blood, internal organs, and breast meat, while the other bird was used for the assessment of intestinal permeability. Feather samples were immediately collected and stored at -20°C for corticosterone analysis. Blood samples were collected via cardiac puncture into both plasma and serum tubes. Breast meat and internal organs, including liver, spleen, bursa of Fabricius, thymus, and abdominal fat, were excised and weighed to determine their relative weights as a percentage of BW. After weighing, breast meat samples were divided according to the purpose of analysis. Samples for pH, water holding capacity (WHC), thiobarbituric acid reactive substances (TBARS) measurements were stored at 4°C, whereas those for fatty acid composition analysis were stored at -20°C. Liver samples were placed into 1.7 mL microtubes and stored at -80°C for subsequent evaluation of antioxidant capacity. Jejunal samples were fixed in 10% neutral buffered formalin for histological analysis of intestinal morphology.

Breast meat quality

The pH at 1 h and 24 h postmortem in the breast meat was analyzed using a pH meter (HI99163, Hanna Instruments, Woonsocket, RI, USA). Meat color was measured using a spectrocolorimeter (Konica Minolta, Tokyo, Japan), and values for lightness (L*), redness (a*), and yellowness (b*) were obtained. WHC was determined using the method described by Lee et al. [22]. Briefly, a 1.0 g breast meat sample was wrapped in Whatman filter paper (NO. 3, Whatman, Maidstone, UK), placed in a 50 mL tube, and centrifuged at 3,000 rpm for 15 min. TBARS was assessed using the method of Lee et al. [23]. In short, a 5.0 g breast meat sample was homogenized with 50 uL of butylated hydroxytoluene and 15 mL of distilled water for 30 sec. Then, 1 mL of the mixture was transferred to a 15 mL tube and vortexed with TBA/trichloroacetic acid. The mixture was heated in a water bath at 90°C for 15 min and subsequently cooled. After cooling, the mixture was centrifuged at 3,000 rpm for 10 min, and the supernatant was collected. The absorbance was measured at 531 nm using a microplate reader (INNO Microplate Spectrophotometer, LTEK, Seongnam, Republic of Korea).

Fatty acid composition

The fatty acid composition of oils and breast meat was analyzed according to the method described by Folch et al. [24]. A 10 g ground sample was mixed with 100 mL chloroform:methanol (2:1, v/v) solution and extracted for 24 h. The extract was concentrated under vacuum, and the residue was methylated using 14% Boron trifluoride-methanol solution (Sigma-Aldrich, St. Louis, MO, USA). The resulting fatty acid methyl esters were measured using gas chromatograph (GC, HP6890, Hewlett-Packard Ltd., CA, USA). The GC system was equipped with an HP-88 capillary column (100 m × 0.25 mm i.d., film thickness 0.20 μm; Agilent Technologies, Palo Alto, CA, USA). The oven temperature was initially held at 140°C for 5 min, then gradually increased to 240°C held for 20 min. The injector and detector temperatures were set at 260°C and 270°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min with a split ratio of 1:50. Individual fatty acids were identified by comparing their retention times with those of known standard fatty acid methyl esters. The composition of each fatty acid was calculated as the percentage of its peak area relative to the total peak area.

Serum parameters

Serum parameters including high-density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (TC), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and uric acid were analyzed. Levels of HDL, LDL, TC, TG, AST, and ALT were measured using an enzymatic assay with the Cobas C702 analyzer (Roche Diagnostics, Mannheim, Germany). Uric acid was measured using an enzymatic assay with the Labospect 008AS analyzer (Hitachi, Tokyo, Japan), and creatinine was analyzed using a kinetic colorimetric assay (Cobas C702 analyzer, Roche Diagnostics, Mannheim, Germany).

Liver antioxidant capacity

Liver antioxidant capacity indicators such as total antioxidant capacity (TAC) and malondialdehyde (MDA) were measured using the EZ-total antioxidant capacity (TAC) assay kit (DG-TAC200, DoGenBio, Seoul, Republic of Korea) and EZ-Lipid peroxidation (TBARS) assay kit (DG-TBA200, DoGenBio, Seoul, Republic of Korea), respectively, according to the manufacturer’s instructions. Protein concentration was determined using the Pierce™ BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL, USA). The concentrations of TAC and MDA were normalized to protein concentration and expressed accordingly.

Liver characteristics

Liver samples were used to evaluate liver color, liver hemorrhage, fatty liver score, and liver fat concentrations. The liver color (L*, a*, and b* values) was measured using a color reader (Konica Minolta Optics Inc., Tokyo, Japan). The liver hemorrhage was scored on a scale from

0 to 5, where 0 indicates no hemorrhages and 5 indicate severe hemorrhaging [25]. The fatty liver score was determined on a scale from 1 to 5, with 1 indicating a normal liver and 5 indicating a pale yellow liver [26]. Crude fat concentrations in the liver were measured using Soxhlet method [27] after freeze-drying for 72 h and grinding of the sample.

Stress indicator

The heterophil to lymphocyte (H:L) ratio was determined using the method described by Samour and Howlett [28]. A 10 μL blood sample was smeared onto a glass slide and air-dried at room temperature. After drying, smeared sample sequentially stained with Wright's and Giemsa's stain solutions. The stained slides were examined under a microscope (OS-370DVM, Osun Hitech, Goyang, Republic of Korea). The heterophils and lymphocytes were counted independently by two independent evaluators and the H:L ratio was calculated by dividing the number of heterophils by the number of lymphocytes. Feather corticosterone (CORT) concentration was determined following the described by Bortolotti et al. [29]. In short, feathers were cut into fragments less than 5 mm in length. Approximately 80 to 100 mg of feather samples were placed in a 50 mL microtube, and 10 mL of methanol was added. The samples were subjected to extraction using ultrasonic water bath (MaXturdy™45, DAIHAN Scientific, Wonju, Republic of Korea) for 30 min, followed by incubation in a water bath at 50°C for 24 h. After extraction, the feather extracts were filtered to remove feathers and dried extract samples in water bath at 50°C for 24 h. The dried samples were analyzed using CORT ELISA kit (Enzo Life Sciences Inc., Farmingdale, NY, USA).

Jejunal morphology

Jejunal samples were fixed in 10% neutral buffered formalin and sliced into sections approximately 2 to 3 mm in thickness. The tissue samples were then embedded in paraffin and sectioned at a thickness of 4 μm. Following sectioning, the samples were stained with hematoxylin and eosin. The stained samples were examined using a microscope (OS-370DVM, Osun Hitech, Goyang, Republic of Korea). Jejunal morphology was measured to villus height (VH), crypt depth (CD), villus width (VW), VH:CD ratio, and villus surface area (VSA). The VSA was calculated from VH and VW, using the method described by Sabry and El-Ghany [30]: VH × VW × π.

Jejunal permeability

Intestinal jejunal permeability was assessed by measuring transepithelial electrical resistance (TEER) using a two-channel Ussing chamber system (P2300, Physiologic Instruments Inc., Reno, NV, USA). Approximately 3.0-5.0 cm segments of jejunum were collected from the region between the distal end of the duodenal loop and Meckel's diverticulum in broiler chickens. Immediately after excision, the samples were immersed in chilled Krebs-Henseleit buffer (pH 7.4; K3753-10L, Sigma-Aldrich, St. Louis, MO, USA) to maintain tissue viability. Adhering fat and mesenteric tissues were carefully removed, the jejunal samples were then mounted vertically between the two halves of the Ussing chamber according to the method described by Goo et al. [31] and Kim et al. [32]. The Ussing chamber was filled with Krebs-Henseleit buffer and maintained at 40°C in a water bath and continuously aerated with a gas mixture of 95% O_2 and 5% CO_2. After a 5 min stabilization period, short-circuit currents (Isc), potential difference (PD), and electrical resistance (R) were recorded at approximately 10 s intervals for 5 min. The TEER values were calculated from the recorded values of PD, Isc, and R using Ohm's law [33]. The detailed protocol for using the two-channel Ussing chamber system was described by Goo et al. [34] and Kim et al. [35].

Animal welfare assessment

At the end of study, six birds (3 males and 3 females) were randomly selected from each cage to evaluate animal welfare. Welfare evaluation was conducted in accordance with the Welfare

Quality Assessment protocol for poultry [36]. Four welfare indicators, including gait score, footpad dermatitis, hock burn, and plumage cleanliness, were examined by five trained evaluators. Gait score was assessed from 0 to 5, with 0 indicating normal gait and 5 indicating severe lameness or inability to stand or walk. Footpad dermatitis was scored from 0 to 4, with 0 representing no visible lesions and 4 indicating severe footpad dermatitis. Hock burn was scored from 0 to 4, with 0 indicating no evidence of lesions and 4 indicating severe hock burn. Plumage cleanliness was scored from 0 to 3, with 0 indicating clean and white feathers and 3 indicating dirty brown feathers.

Statistical analysis

All data were analyzed by analysis of variance as a completely randomized design using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA). The cage was considered the experimental unit for growth performance parameters (BW, FI, and FCR) and welfare assessments, as these were analyzed based on cage-level averages. For all other measurements, including fatty acid composition of breast meat, meat quality traits, serum parameters, liver antioxidant capacity, liver characteristics, stress indicators, jejunal morphology, and jejunal permeability, individual birds were considered the experimental unit. Outlier data were checked using the PROC UNIVARIATE procedure of SAS [37]. The LSMEANS procedure was used to calculate mean values and the PDIFF option was used to separate the means if the difference was significant. Significance for statistical test was set at $P < 0.05$.

RESULTS

Fatty acid concentrations in fat sources

The fatty acid concentrations in fat sources are summarized in Table 2. In this analysis, BSFL oil contained a greater proportion of saturated fatty acid (SFA; 66.4%) than soybean oil (15.9%) and tallow (39.2%). In contrast, unsaturated fatty acid (UFA) accounted for a greater proportion in soybean oil (84.0%) and tallow (60.3%) than in BSFL oil (33.3%). Each fat source exhibited a distinct fatty acid profile. The BSFL oil was characterized by a high concentration of lauric acid (C12:0; 41.8%), a MCFA. Soybean oil contained a large proportion of polyunsaturated fatty acids (PUFA), especially linoleic acid (C18:2 n-6; 53.0%). Tallow was rich in monounsaturated fatty acids (MUFA), particularly oleic acid (C18:1 n-9; 45.9%).

Growth performance

No significant differences in BWG, FI, and FCR were observed among dietary treatments (Table 3).

Breast meat quality

The pH at 24 h postmortem was less ($p < 0.05$) in the BSFL oil group than in the soybean oil group (Table 4). However, the BSFL oil and tallow groups showed similar pH at 24 h postmortem. The tallow group showed a greater ($p < 0.05$) L* values for meat color compared with the BSFL oil group. The a* values for meat color was less ($p < 0.05$) in the BSFL oil and tallow groups than in the soybean oil group. However, dietary fat sources did not affect breast yield, pH at 1 h postmortem, b* values for meat color, WHC, and TBARS.

Fatty acid concentrations in the breast meat

The BSFL oil group had greater ($p < 0.05$) SFA concentrations in the breast meat than the soybean oil and tallow groups (Table 5). In particular, the BSFL oil significantly increased ($p <$ 0.05) SFAs such as lauric acid, myristic acid, stearic acid concentrations in the breast meat compared with the soybean oil and tallow groups. On the other hand, the soybean oil and tallow groups had greater ($p < 0.05$) UFA concentrations in the breast meat than the BSFL oil group. The soybean oil group showed greater ($p < 0.05$) PUFAs such as linoleic acid, γ-linolenic acid, and α-linolenic acid concentrations in the breast meat than the BSFL oil and tallow groups. The tallow group had greater ($p < 0.05$) MUFA, particularly oleic acid and palmitoleic acid concentrations in the breast meat than the BSFL oil and soybean oil groups. However, the BSFL oil group had greater ($p < 0.05$) docosapentaenoic acid and docosahexaenoic acid (DHA) in the breast meat than the soybean oil and tallow groups. In addition, the BSFL oil group had greater ($p < 0.05$) PUFA/MUFA and n-3 fatty acid in the breast meat than the tallow group.

Relative organ weight

Liver, spleen, bursa of Fabricius, thymus, and abdominal fat did not differ significantly among dietary fat sources (Table 6).

Serum parameters

Serum TG concentration was greater ($p < 0.05$) in the tallow group than in the soybean oil group. The BSFL oil group had greater ($p < 0.05$) serum AST concentration compared with the soybean oil group (Table 7). Serum uric acid concentration was less ($p < 0.05$) in the BSFL oil group than in the tallow and soybean oil groups. However, dietary fat sources did not affect serum HDL, LDL, TC, ALT, and creatinine concentrations.

Liver antioxidant capacity and characteristics

MDA and TAC levels in the liver were not significantly different among dietary fat sources (Table 8). The a* values for liver color were greater ($p < 0.05$) in the tallow group than in the soybean oil group (Table 9). However, dietary fat sources did not significantly affect liver characteristics, such as L* and b* values for liver color, liver hemorrhagic score, fatty liver score, and liver fat concentrations.

Stress indicator

Dietary fat sources did not significantly affect CORT and H:L ratio (Table 10).

Jejunal morphology and permeability

The VH, VW, CD, VH:CD ratio, and VSA did not differ significantly among dietary fat sources (Table 11). In addition, PD, Isc, and TEER values were not significant among dietary fat sources (Table 12).

Animal welfare assessment

Dietary fat sources had no significant effect on gait score, footpad dermatitis, hock burn, and plumage cleanliness (Table 13).

DISCUSSION

Soybean oil contains high levels of PUFA, particularly linoleic acid, which is an essential fatty acid for animals and humans [38, 39, 40]. In its role as a component of membrane phospholipids, linoleic acid contributes to maintaining membrane fluidity of the transdermal water barrier [41]. In addition, arachidonic acid is a major metabolite derived from linoleic acid [42]. It serves as the principal for eicosanoid synthesis, and eicosanoids play key roles in inflammation and immune regulation [43]. Animal fats such as tallow are rich in oleic acid [44]. An important function of oleic acid is the regulation of lipids in the membrane structure [45]. By modulating membrane lipids, oleic acid influences signal-dependent guanine nucleotide-binding protein activity and is associated with reduced blood pressure [46, 47]. In contrast to animal fats such as tallow rich in oleic acid, prepupa BSFL oil contains a high amount of SFA, with lauric acid as the predominant MCFA [48, 49]. Lauric acid is a primary MCFA with 12 carbon atoms and exerts modulatory roles in metabolism, immune functions, and antimicrobial activity [50, 51]. Similarly, our results showed the predominant fatty acids of soybean oil, tallow, and BSFL oil were linoleic acid, oleic acid, and lauric acid, respectively, which is in agreement with previous observations [52, 53, 54]. These results suggest that the distinct fatty acid profiles of each fat source may underlie their differential physiological effects.

In the present study, inclusion of different fat sources did not significantly affect growth performance in broiler chickens. These findings are in line with previous studies, which collectively suggest that partial or complete replacement of soybean oil with insect-derived fats has no adverse effect on growth performance in poultry. Schiavone et al. [4] demonstrated that the replacement of 50% and 100% of soybean oil with *Hermetia illucens* (HI) larvae fat did not significantly affect ADG, ADFI, and FCR in finisher broiler chickens. Similarly, Kierończyk et al. [18] reported that dietary inclusion of soybean oil and HI larvae fat had no significant effect on BWG, FI, and FCR in broiler chickens. In addition, Sypniewski et al. [54] observed that the replacement of 50% and 100% of soybean oil with BSFL fat in turkey diets did not significantly affect growth performance. Although soybean oil and BSFL oil differ markedly in fatty acid compositions, with soybean oil rich in PUFAs and BSFL oil containing high concentrations of MCFAs, both fat sources provide comparable energy availability for broiler chickens [18]. This similarity in energy utilization may be attributed to the metabolic properties of the MCFAs abundant in BSFL oil. Notably, BSFL oil and coconut oil exhibit similar fatty acid profiles, both characterized by high concentrations of SFAs and MCFAs, especially lauric acid [4, 55]. MCFAs are rapidly absorbed in the small intestine and transported via the portal vein to the liver, where the absorbed MCFAs undergo efficient β-oxidation to provide readily available energy [56, 57, 58]. This metabolic characteristic may contribute to the observation that BSFL oil, similar to coconut oil, has no detrimental impact on growth performance in broiler chickens. Wang et al. [59] demonstrated that the replacement of 25%, 50%, 75%, and 100% of soybean oil with coconut oil had no significant effect on BWG, FI, and FCR in broiler chickens. In addition, dietary inclusion of coconut oil and BSFL oil showed no significant differences in BWG, FI, and FCR in broiler chickens [53]. Therefore, the similarity in growth performance between broiler chickens fed BSFL oil and those fed soybean oil may be attributed to the efficient energy utilization of MCFAs, as also demonstrated in diets containing coconut oil. On the other hand, BSFL oil and soybean oil have generally shown no adverse effects on growth performance in broiler chickens, results have been less consistent with other fat sources. Aslam et al. [60] reported that dietary supplementation of tallow resulted in higher FCR than BSFL oil and soybean oil. This discrepancy may be explained by the levels of fat inclusion in diets, as the impact of dietary fat on growth performance depends not only on the amount but also on the fatty acid composition [61]. Okur [6] demonstrated that broiler chickens fed diet containing 2.0-2.5% soybean oil, poultry fat, and tallow showed no significant differences in growth performance. Moreover, Poorghasemi et al. [61] reported that dietary inclusion of 4% tallow, canola oil, and sunflower oil had no significant effect on growth performance of broiler chickens. However, Azman et al. [62] demonstrated that dietary inclusion of 6% tallow increased ADG, ADFI, and FCR compared to soybean oil and poultry fat. Aslam et al. [60] reported that broiler chickens fed diets containing 3.4%-7.9% palm oil, poultry fat, and tallow increased FCR than those fed diets containing BSFL oil. In the present study, dietary supplementation of 3.0% fat sources did not significantly affect growth performance in broiler chickens. Considering that higher inclusion levels of dietary fat have been associated with impaired growth performance in previous studies, these results suggest that BSFL oil may provide comparable energy availability to soybean oil and tallow at moderate inclusion levels, thereby supporting its use as an effective alternative fat source in broiler diets.

Meat quality has become increasingly important as consumers emphasize safety and health [63]. The pH value is one of the physical characteristics of meat quality and typically decreases postmortem due to the accumulation of lactic acid in muscle tissue [64]. Aberle et al. [65] reported that the pH of normal muscle decreased from approximately 7.0 to 5.6-5.7 within

6 to 8 h after slaughter, ultimately reaching a normal range of 5.3 to 5.7. In the present study, the pH at 24 h postmortem did not differ significantly among dietary fat sources. The values ranged from 5.6 to 5.7, which are within the normal postmortem pH range for breast meat. Therefore, dietary inclusion of BSFL oil had no adverse effect on pH value in broiler chickens. Meat color, shape, size, and texture are important factors influencing consumers' purchasing decisions regarding meat products [66]. The L* value of meat color is affected by the extent of protein denaturation, which is influenced by pH values [67]. A lower pH value increases protein denaturation, leading to greater light scattering and a lighter meat appearance [68]. The present study showed a higher L* value in the tallow group compared with the soybean oil and BSFL oil groups. In contrast, previous studies reported that replacing 50% and 100% of soybean oil with HI fat in broiler diets showed no significant L* value of meat [14, 69]. This discrepancy may be related to differences in the lipid characteristics of dietary fat sources. Supporting this, Bianchi et al. [70] demonstrated that dietary animal fats increased L* value in breast meat compared with vegetable oils. Owing to BSFL oil has been described as having lipid properties intermediate between vegetable oils and animal fats, the effects of BSFL oil on meat color may thus be more comparable to those of vegetable oils than those of animal fats [71]. Thus, the difference in L* value of meat color may be related to variations in fatty acid composition among dietary fat sources. Lipid oxidation is a major factors limiting the quality and acceptability of meat products [72]. According to a previous study, the replacement of 50% and 100% of soybean oil with BSFL oil significantly increased TBARS values at 0 d in chicken leg meat, whereas no significant differences were observed at 3 d and 6 d [73]. This result may be attributed to differences in PUFA content among the fat sources to the polymerization of MDA during storage [74]. Meat with a high PUFA content shows increased TBARS due to the greater susceptibility of PUFAs to free radical attack than SFAs [75]. Soybean oil contains high levels of PUFA, particularly linoleic acid [38], which may explain the increase in TBARS at 0 d. However, MDA has been reported to react with a wide range of compounds or to form dimers

and trimers during storage [76, 77]. These reactions decrease the amount of MDA available to react with TBA, thereby lowering TBARS [78]. In the present study, TBARS values of breast meat at 5 d did not differ among dietary fat sources, suggesting that BSFL oil had no adverse effect on lipid oxidation.

In the present study, dietary inclusion of different fat sources significantly affected the fatty acid profile in breast meat, with soybean oil, tallow, and BSFL oil increasing PUFA, MUFA, and SFA concentrations, respectively. The PUFAs are known to have beneficial effects, such as the prevention of cardiovascular diseases [79]. However, among PUFAs, long-chain PUFAs increase the susceptibility of meat to oxidation [75, 80]. In addition, our results showed that dietary inclusion of soybean oil increased PUFAs such as linoleic acid in breast meat. Linoleic acid is an essential fatty acid and has been reported to decrease blood pressure [39, 81]. Nevertheless, linoleic acid is desaturated to n-6 PUFAs, particularly arachidonic acid [41]. Arachidonic acid is a precursor of eicosanoids, including prostaglandins and leukotrienes [83]. Although eicosanoids play normal physiological roles, excessive production has been linked to inflammation and cancer [83]. Dietary inclusion of tallow increased MUFA concentrations in breast meat, in particular oleic acid. The MUFAs have been reported to reduce LDL cholesterol and LDL oxidation, thereby contributing to cardiovascular health [84, 85]. In addition, oleic acid improves the quality of refrigerated meat through its strong antioxidant activity [86]. However, dietary inclusion of tallow also increased the n-6/n-3 ratio in breast meat. An elevated n-6/n-3 ratio has been associated with metabolic changes, increased production of pro-inflammatory mediators, and altered signaling pathways, thereby contributing to cardiovascular disease and diabetes mellitus [87]. High SFA concentrations in meat are linked to increased risk of cardiovascular disease, obesity, and other chronic health conditions [88, 89]. In the present study, dietary inclusion of BSFL oil increased SFA concentrations and n-3 PUFAs, particularly eicosapentaenoic acid (EPA) and DHA, while reducing n-6/n-3 ratio. The n-3 PUFAs include α-linolenic acid, EPA, and DHA [90]. Among them, EPA and DHA are long-chain fatty acids

(LCFAs) with beneficial health effects, including anti-atherogenic, anti-thrombotic and anti-inflammatory [91, 92]. In addition, a high n-6/n-3 ratio has been associated with an increased risk of chronic diseases [91]. Therefore, dietary inclusion of different fat sources exerts both positive effects and negative effects on fatty acid profile of breast meat. Soybean oil increased PUFA concentrations but also increased the susceptibility of meat to oxidation. Tallow supplementation resulted in higher MUFA concentrations; however, it also led to an elevated n-6/n-3 ratio. The BSFL oil contributed to greater SFA concentrations while lowering the n-6/n-3 ratio. Thus, each fat source presents both advantages and disadvantages, and BSFL oil may be considered a potential alternative in light of the functional properties of the meat fatty acid profile.

In current study, dietary inclusion of tallow increased serum TG levels compared with the other fat sources. This finding is consistent with the results of Fascina et al. [3], who observed that inclusion of 4% tallow in diets increased serum TG levels compared with diets containing 4% soybean oil. This result may be attributed to the high concentrations of LCFAs in tallow. Tallow is mainly composed of LCFA such as palmitic acid, stearic acid, and oleic acid [93, 94]. The main storage form of LCFAs is TG, which consists of three LCFAs esterified to a glycerol [95]. Thus, LCFAs serve as essential substrates for TG synthesis and lipid metabolism [96]. In our study, the fatty acid profile of tallow showed high concentrations of palmitic acid, stearic acid, and oleic acid, which may explain the elevated serum TG levels observed in broiler chickens. The AST is widely used as an indicator of liver cell damage. However, it also plays an essential role in amino acid metabolism and serves as a critical intermediary in cellular energy production [97, 98]. Previous studies have reported that dietary BSFL oil did not significantly affect serum AST levels in poultry [53, 54, 99]. In contrast, our results showed that dietary inclusion of BSFL oil increased serum AST levels in broiler chickens. This discrepancy may be associated with the distinct metabolic characteristics of MCFAs. Dietary MCFAs are digested and absorbed in the small intestine and transported as free fatty acids via the portal vein to the liver [100]. Moreover, MCFAs are oxidized independently of the carnitine shuttle, which enables rapid and continuous oxidation [58]. This process constitutes MCFAs a prime source of acetyl-CoA, thereby promoting ketogenesis [100, 101]. The BSFL oil is rich in MCFA, such as lauric acid [102], which was also identified as the predominant fatty acid in our analysis. Also, no significant differences were observed in ALT levels and liver MDA concentrations among treatments. In our study, ALT levels ranged from 4.0 to 4.4 U/L, and liver MDA concentrations ranged from 0.31 to 0.33 nmol/mg. These levels fall within the physiological ranges previously reported in healthy broiler chickens. Dabbou et al. [103] and Chen et al. [104] reported that ALT levels of 2.0 to 8.0 U/L, while Yeom et al. [105] and Chen et al. [106] observed MDA concentrations of 0.3 to 0.6 nmol/mg. Therefore, the metabolic characteristics of MCFAs in BSFL oil may have contributed to the elevated serum AST levels, which may reflect enhanced hepatic metabolic processes rather than apparent liver damage. Dietary inclusion of BSFL oil had less serum uric acid levels than soybean oil and tallow diets. This finding may also be related to the independent oxidation of MCFAs. Unlike LCFAs, MCFA are directly activated in the mitochondria matrix without the carnitine-dependent transport system [100]. This unregulated oxidation renders MCFA a rapid source of acetyl-CoA, thereby promoting ketogenesis [100, 101]. Ketone bodies have been reported to influence glycogen phosphorylase, the enzyme responsible for glycogen breakdown [107], thereby linking lipid-derived energy metabolism with carbohydrate utilization [107, 108]. In addition, uric acid is produced as the end of purine catabolism [109], with purine pools derived from exogenous and endogenous sources. Therefore, the unregulated characteristics of MCFA oxidation in BSFL oils may have reduced the utilization of protein catabolism for energy, thereby decreasing purine degradation and serum uric acid levels.

Intestinal morphology is closely related to the nutrient absorption capacity of the small intestine. Short villi and deep crypts reflect a compromised intestinal structure, which can impair nutrient absorption and negatively affect growth performance of monogastric animals

[49, 110]. Chen et al. [104] demonstrated that 100% replacement of soybean oil with BSFL oil did not significantly affect small intestine morphology. Similarly, Schiavone et al. [4] observed that replacing 50% and 100% of soybean oil with BSFL fat in diet did not result in significant intestinal morphological changes. In the present study, jejunal morphology did not differ among dietary fat sources. This result may be explained by the fact that all diets were formulated to be isoenergetic and isonitrogenous, minimizing potential differences in intestinal structure [111]. In addition, negative effects on intestinal morphology are generally observed under stress conditions, such as heat stress or pathogen exposure [112, 113], but such stressor were absent in the present trial. Therefore, these results indicate that dietary inclusion of soybean oil, tallow, and BSFL oil does not alter jejunal morphology in broiler chickens, which may be attributed to the balanced composition of the diets and the absence of stressors that typically induce morphological variation.

The maintenance and optimization of intestinal barrier in broiler chickens have important implications for their health and performance [114]. The intestinal epithelium functions as a selectively permeable barrier, allowing transport of beneficial nutrients while preventing the entry of harmful substances [115, 116]. Intestinal permeability can be influenced by intestinal microflora, stress, disease, and toxins [117]. In particular, stress has been associated with detrimental effects on gut health, including increased visceral perception, elevated intestinal permeability, and adverse impacts on the intestinal microbiota [118, 119]. Previous studies have demonstrated that stress conditions negatively affect intestinal permeability in broiler chickens [35, 120]. As a result, increased intestinal permeability has been linked to impaired health, performance, immune activation, and lameness [121]. In contrast, dietary fat sources did not significantly affect jejunal permeability in the present study. This result may be attributed to the absence of stress conditions such as heat stress, high stocking density, and immune challenges during the experimental period [34, 122]. Moreover, stress indicators such as the H:L ratio and feather CORT concentrations were not significantly different among dietary fat sources, suggesting that the birds were raised under non-stressful conditions. Therefore, our findings suggest that replacing soybean oil and tallow with BSFL oil does not impair jejunal barrier integrity in broiler chickens, supporting the view that BSFL oil may serve as a safe alternative fat source under normal rearing conditions.

In conclusion, dietary inclusion of different fat sources, including soybean oil, tallow, and BSFL oil did not have adverse effects on growth performance, relative organ weight, liver antioxidant capacity, stress indicators, jejunal morphology, jejunal permeability, and animal welfare assessment. Soybean oil contributed to higher levels of PUFAs in breast meat, whereas tallow increased MUFAs such as stearic and palmitic acids. BSFL oil exhibited a high content of SFAs as well as n-3 PUFAs in breast meat. Therefore, our findings suggest that BSFL oil can be utilized as an alternative to fat sources in broiler diets, while modifying the fatty acid profile of breast meat.

ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. RS-2024-00465159). This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. RS-2023-00210634).

REFERENCES

1. Smith K, Watson AW, Lonnie M, Peeters W, Oonincx D, Tsoutsoura N, Simon-Miquel G, Szepe K, Cochetel N, Pearson AG, Witard O, Salter AM, Bennett M, Corfe BM. Meeting the global protein supply requirements of a growing and ageing population. Eur. J. Nutr. 2024;63:1425-1433. https://doi.org/10.1007/s00394-024-03358-2
2. Ravindran V, Abodollahi MR, Bootwalla SM. Nutrient analysis, metabolizable energy, and digestible amino acids of soybean meals of different origins for broilers. Poult. Sci. 2014;93:2567-2577. http://dx.doi.org/ 10.3382/ps.2014-04068
3. Fascina VB, Carrijo AS, Souza KMR, Garcia AML, Kiefer C, Sartori JR. Soybean oil and beef tallow in starter broiler diets. Braz. J. Poult. Sci. 2009;11:249-256. https://doi.org/10.1590/S1516-635X2009000400006
4. Schiavone A, Dabbou S, De Marco M, Cullere M, Biasato I, Biasibetti E, Capucchio MT, Bergagna S, Dezzutto D, Meneguz M, Gai D, Dalle Zotte A, Gasco L. Black soldier fly larva fat inclusion in finisher broiler chicken diet as an alternative fat source. Animals. 2018;12:2032-2039. https://doi.org/10.1017/S1751731117003743
5. Environmental and Health Risk Assessment and Management (ERASM). Environmental Fact Sheet (#21): Beef tallow. ERASM; 2017 [cited 2025 Dec 1]. https://www.erasm.org/wp-content/uploads/2022/07/21-ERASM-Environmental-Fact-Sheet-Beef-Tallow.pdf
6. Okur N. The effects of soy oil, poultry fat and tallow with fixed energy: protein ratio on broiler performance. Arch. Anim. Breed. 2020;63:91-101. https://doi.org/10.5194/aab-63-91-2020
7. Bovera F, Loponte R, Pero ME, Cutrignelli MI, Calabrò S, Musco N, Vassalotti G, Panettieri V, Lombardi P, Piccolo G, Di Meo C, Siddi G, Fliegerova K, Moniello G. Laying performance, blood profiles, nutrient digestibility and inner organs traits of hens fed an

insect meal from *Hermetia illucens* larvae. Res. Vet. Sci. 2018;120:86-93. https://doi.org/10.1016/j.rvsc.2018.09.006

8. Liu X, Chen X, Wang H, Yang Q, ur Rehman K, Ki W, Cai M, Li Q, Mazza L, Zhang J, Yu Z, Zheng L, Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. PLos ONE 2017;12:e0182601. https://doi.org/10.1371/journal.pone.0182601
9. Shorstkii I, Alles MC, Parniakov O, Smetana S, Aganovic K, Sonsin M, Toepfl S, Heinz V. Optimization of pulsed electric field assisted drying process of black soldier fly (*Hermetia illucens*) larvae. Dry. Technol. 2020;40:595-603. https://doi.org/10.1080/07373937.2020.1819825
10. Li S, Ji H, Zhang B, Tian J, Zhou J, Yu H. Influence of black soldier fly (*Hermetia illucens*) larvae oil on growth performance, body composition, tissue fatty acid composition and lipid deposition in juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquaculture. 2016;465:43-52. https://doi.org/10.1016/j.aquaculture.2016.08.020
11. Lock ER, Arsiwalla T, Waagbø R. Insect larvae meal as an alternative source of nutrients inthe diet of Atlantic salmon (*Salmo salar*) postsmolt. Aquac. Nutr. 2016;22:1202-1213. https://doi.org/10.1111/anu.12343
12. Benzertiha A, Kierończyk B, Rawski M, Mikołajczak Z, Urbański A, Nogowski L, Józefiak D. Insect fat in animal nutrition- A review. Ann. Anim. Sci. 2020;20:1217-1240. https://doi.org/ 10.2478/aoas-2020-0076
13. Zeitz JO, Fennhoff J, Kluge H, Stangl GI, Eder K. Effects of dietary fats rich in lauric and myristic acid on performance, intestinal morphology, gut microbes, and meat quality in broilers. Poult. Sci. 2015;94:2404-2413. https://doi.org/10.3382/ps/pev191
14. Schiavone A, Cullere M, De Marco M, Meneguz M, Biasato I, Bergagna S, Dezzutto D, Gai F, Dabbou S, Gasco L, Dalle Zotte A. Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens L.*) fat in broiler diets: effect on growth

performances, feed-choice, blood traits, carcass characteristics and meat quality. Ital. J. Anim. Sci. 2017;16: 93-100. http://dx.doi.org/10.1080/1828051X.2016.1249968

15. Kierończyk B, Sypinewski J, Rawski M, Czekała W, Świątkiewicz S, Józefiak D. From waste to sustainable feed material: The effect of *Hermetia Illucens* oil on the growth performance, nutrient digestibility, and gastrointestinal tract morphometry of broiler chickens. Ann. Anim. Sci. 2020;20:157-177. https://doi.org/10.2478/aoas-2019-0066

16. Riekkinen K, Väkeväinen K, Korhonen J. The effect of substrate on the nutrient content and fatty acid composition of edible insects. Insects. 2022;13:590. https://doi.org/10.3390/insects13070590

17. Hoc B, Genva M, Fauconnier ML, Lognay G, Francis F, Caparros Megido C. About lipid metabolism in *Hermetia illucens* (L. 1758): on the origin of fatty acids in prepupae. Sci. Rep. 2020;10:11916. https://doi.org/10.1038/s41598-020-68784-8

18. Kierończyk B, Kaczmarek SA, Hejdysz M, Szymkowiak P, Aslam MR, Rawski M, Kołodziejski PA, Mikuła R, Stuper-Szablewska K, Józefiak D. Implementation of a metabolizable energy regression model for black soldier fly larvae fat in broiler chicken diets: effect on growth performance, nutrient digestibility, and selected physiological indices. J. Anim. Feed Sci. 2024;33:493-503. https://doi.org/10.22358/jafs/187805/2024

19. Kierończyk B, Rawski M, Stuper-Szablewska K, Józefiak D. First report of the apparent metabolisable energy value of black soldier fly larvae fat used in broiler chicken diets. Animal. 2022;16:100656. https://doi.org/10.1016/j.animal.2022.100656

20. Rostagno HS, Albino LFT, Hannas MI, Donzele JL, Sakomura NK, Perazzo FG, Saraiva A, de Abreu MLT, Rodrigyes PB, de Oliveira RF, Barreto SLT, Brito CO. Brazilian Tables for Poultry and Swine: Feedstuff Composition and Nutritional Requirements. 4th rev. ed. Universidade Federal de Viçosa; 2017.

21. Aviagen. Ross Boiler: Nutrition specifications [Internet]. Aviagen Inc.; 2022 [cited 2025 Dec 1].

https://aviagen.com/assets/Tech_Center/Ross_Broiler/RossBroilerNutritionSpecifications2022-EN.pdf

22. Lee SJ, Lee SY, Kim GD, Kim GB, Jin SK, Hur SJ. Effects of self-carbon dioxide-generation material for active packaging on pH, water-holding capacity, meat color, lipid oxidation and microbial growth in beef during cold storage. J. Sci. Food Agric. 2017;97:3642-3648. https://doi.org/10.1002/jsfa.8223

23. Lee HJ, Yim DG, Jo C. Effect of plasma-activated organic acids against Salmonella Typhimurium and Escherichia coli O157: H7 inoculated on pork loin and its quality characteristics. Innov. Food Sci. Emerg. 2023;88:103455. https://doi.org/10.1016/j.ifset.2023.103455

24. Folch J, Lees M, Sloane stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 1957;226:497-509. https://doi.org/10.1016/S0021-9258(18)64849-5

25. Diaz GJ, Squires EJ, Julian RJ. The use of selected plasma enzyme activities for the diagnosis of fatty liver-hemorrhagic syndrome in laying hens. Avian Dis. 1999;43:768-773. https://doi.org/10.2307/1592746

26. Choi YI, Ahn HJ, Lee BK, Oh ST, An BK, Kang CW. Nutritional and hormonal induction of fatty liver syndrome and effects of dietary lipotropic factors in egg-type male chicks. Asian-Australas. J. Anim. Sci. 2012;25:1145-1152. https://doi.org/10.5713/ajas.2011.11418

27. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis. 18th ed. rev. 2. AOAC International; 2007.

28. Samour J, Howlett JC. Hematology analyses. In: Samour J, editor. Avian Medicine. Elsevier; 2008. p. 35-52.

29. Bortolotti GR, Marchant T, Blas J, Cabezas S. Tracking stress: localisation, deposition and stability of corticosterone in feathers. J. Exp. Biol. 2009;212:1477-1482. https://doi.org/10.1242/jeb.022152
30. Sabry MIE, El-Ghany WAA. A Mathematical model for calculating intestinal villus Surface area in broiler chickens. Int. J. Vet. Sci. 2021;10:317–322. https://doi.org/10.47278/journal.ijvs/2021.062
31. Goo D, Kim JH, Choi HS, Park GH, Han GP, Kil DY. Effect of stocking density and sex on growth performance, meat quality, and intestinal barrier function in broiler chickens. Poult. Sci. 2019;98:1153-1160. http://dx.doi.org/10.3382/ps/pey491
32. Kim HY, Kim JH, Han GP, Kil DY. Increasing concentrations of dietary threonine, tryptophan, and glycine improve growth performance and intestinal health with decreasing stress responses in broiler chickens raised under multiple stress conditions. Anim. Nutr. 2024;18:145-153. https://doi.org/10.1016/j.aninu.2024.03.018
33. Gabler NK, Spencer JD, Webel DM, Spurlock ME. In utero and postnatal exposure to long chain (n-3) PUFA enhances intestinal glucose absorption and energy stores in weanling pigs. J. Nutr. 2007;137:2351-2358. https://doi.org/10.1093/jn/137.11.2351
34. Goo D, Kim JH, Park GH, Reyes JBD, Kil DY. Effect of heat stress and stocking density on growth performance, breast meat quality, and intestinal barrier function in broiler chickens. Animals. 2019;9:107. https://doi.org/10.3390/ani9030107
35. Kim HY, Kil DY, Kim JH. Effects of dietary concentration of threonine on growth performance, meat quality, antioxidant capacity, stress biomarker, and intestinal barrier function in broiler chickens raised under multiple stress conditions. Anim. Feed Sci. Technol. 2025;23:116303. https://doi.org/10.1016/j.anifeedsci.2025.116303
36. Welfare Quality®. Welfare Quality® assessment protocol for poultry (broilers, laying hens). Welfare Quality® Consortium; 2009.
37. Steel RGD, Torrie JH, Dicky DA. Principles and procedures of statistics, a biometrical approach. 3rd ed. McGraw Hill; 1997.

38. Meydani SN, Lichtenstein AH, White PJ, Goodnight SH, Elson CE, Woods M, Gorbach SL, Schaefer EJ. Food use and health effects of soybean and sunflower oils. J. Am. Coll. Nutr. 1991;10:406-428. http://dx.doi.org/10.1080/07315724.1991.10718168

39. Aydin R. Conjugated linoleic acid: chemical structure, sources and biological properties. Turk. J. Vet. Anim. Sci. 2005;29:189-195.

40. Ivanov DS, Lević JD, Sredanović SA, Fatty acid composition of various soybean products. Food Feed Res. 2011;37:65-70.

41. Whelan J, Fritsche K. Linoleic acid. Adv. Nutr. 2013;4:311-312. https://doi.org/10.3945/an.113.003772

42. Cherian G. Nutrition and metabolism in poultry: role of lipids in early diet. J. Anim. Sci. Biotechnol. 2015;6:28. https://doi.org/10.1186/s40104-015-0029-9

43. Calder PC. Eicosanoids. Essays Biochem. 2020;64:423-441. https://doi.org/10.1042/EBC20190083

44. Klingenberg IL, Knabe DA, Smith SB. Lipid metabolism in pigs fed beef tallow or high-oleic acid sunflower oil. Comp. Biochem. Psysiol. B Biochem. Mol. Biol. 1995;110:183-192. https://doi.org/10.1016/0305-0491(94)00116-C

45. Karacor K, Cam M. Effects of oleic acid. Med. Sci. Discov. 2015;2:125-132. https://doi.org/ 10.17546/msd.25609

46. Terés S, Barceló-Coblijn G, Benet M, Álvarez R, Bressani R, Halver JE, Escribá PV. Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. Proc. Natl. Acad. Sci. 2008;105:13811-13816. https://doi.org/10.1073/pnas.0807500105

47. Massimo C, Lucio T, Jesus MA, Giovanni L, Caramia GM. Extra virgin olive oil and oleic acid. Nutr. Clín. Diet. Hosp. 2009;29:12-24.

48. Liland NS, Biancarosa I, Araujo P, Biemans D, Bruckner CG, Waagbø R, Torstensen BE, Lock EJ. Modulation of nutrient composition of black soldier fly (Hermetia illucens) larvae

by feeding seaweed-enriched media. PLoS One 2017;12:e0183188. https://doi.org/10.1371/journal.pone.0183188

49. Kim B, Bang HT, Kim KH, Kim MJ, Jeong JY, Chun JL, Ji SY. Effects of dietary supplementation of black soldier fly (*Hermetia illucens*) larvae oil on broiler health. J. Poult. Sci. 2021;58:222-229. https://doi.org/10.2141/jpsa.0200070

50. Dayrit FM. The properties of lauric acid and their significance in coconut oil. J. Am. Oil. Chem. Soc. 2015;92:1-15. https://doi.org/10.1007/s11746-014-2562-7

51. Omonijo FA, Kim S, Guo T, Wang Q, Gong J, Lahaye L, Bodin JC, Nyachoti M, Liu S, Yang C. Development of novel microparticles for effective delivery of thymol and lauric acid to pig intestinal tract. J. Agric. Food Chem. 2018;66:9608-9615. https://doi.org/10.1021/acs.jafc.8b02808

52. Thacker PA, Campbell GL, Xu Y. Composition and nutritive value of acidulated fatty acids, degummed canola oils and tallow as energy sources for starting broiler chicks. Anim. Feed Sci. Technol. 1994;46:251-260. https://doi.org/10.1016/0377-8401(94)90143-0

53. Kim YB, Kim DH, Jeong SB, Lee JW, Kim TH, Lee HG, Lee KW. Black soldier fly larvae oil as an alternative fat source in broiler nutrition. Poult. Sci. 2020;99:3133-3143. https://doi.org/10.1016/j.psj.2020.01.018

54. Sypniewski J, Kierończyk B, Benzertiha A, Mikołajczak Z, Pruszyńska-Oszmałek E, Kołodziejski P, Sassek M, Rawski M, Czekała W, Józefiak D. Replacement of soybean oil by *Hermetia illucens* fat in turkey nutrition: effect on performance, digestibility, microbial community, immune and physiological status and final product quality. Br. Poult. Sci. 2020;61:294-302. https://doi.org/10.1080/00071668.2020.1716302

55. Kappally S, Shirwaikar A, Shirwaikar A. Coconut oil – A review of potential applications. Hygeia. J. D. Med. 2015;7:34-41. https://doi.org/10.15254/H.J.D.Med.7.2015.149

56. Aas M, Bremer J. Short-chain fatty acid activation in rat liver. A new assay procedure for the enzymes and studies on their intracellular localization. Biochim. Biophys. Acta. 1968.164:157–166.
57. Boomgaarden I, Vock C, Klapper M, Döring F. Comparative analyses of disease risk genes belonging to the acyl-CoA synthetase medium-chain (ACSM) family in human liver and cell lines. Biochem. Genet. 2009;47:739-748. https://doi.org/10.1007/s10528-009-9273-z
58. Schönfeld P, Wojtczak L. Short- and medium-chain fatty acids in energy metabolism: the cellular perspective. J. Lipid Res. 2016;57:943-954. https://doi.org/10.1194/jlr.R067629
59. Wang J, Wang X, Li J, Chen Y, Yang W, Zhang L. Effects of dietary coconut oil as a medium-chain fatty acid source on performance, carcass composition and serum lipids in male broilers. Asian-Australas. J. Anim. Sci. 2015;28:223-230. http://dx.doi.org/10.5713/ajas.14.0328
60. Aslam MR, Kierończyk B, Rawski M, Szymkowiak P, Stuper-Szablewska K, Kołodziejski P, Mikuła R, Dankowiakowska A, Józefiak D. Insect fat influences broiler performance, meat quality, and the cecal microbiota similarly to plant oils rather than animal fats. Sci. Rep. 2025;15:18086. https://doi.org/10.1038/s41598-025-02820-3
61. Poorghasemi M, Seidavi A, Qotbi AAA, Laudadio V, Tufarelli V. Influence of dietary fat source on growth performance responses and carcass traits of broiler chicks. Asian-Australas. J. Anim. Sci. 2013:26:705-710. http://dx.doi.org/10.5713/ajas.2012.12633
62. Azman MA, Çerçi İH, Birben N. Effects of various dietary fat sources on performance and body fatty acid composition of broiler chickens. Turk. J. Vet. Anim. Sci. 2005;29:811-819.
63. Teixeira A, Rodrigues S. Meat quality, brands and consumer trends. In: Lorenzo JM, Munekata PES, Barba FJ, Toldrá F. editors. More than beef, pork and chicken – the production, processing, and quality traits of other sources of meat for human diet. Springer; 2019. p. 21-30. https://doi.org/10.1007/978-3-030-05484-7

64. Sari TV, Zalukhu P, Mirwandhono RE. Water content, pH and cooking loss of broiler meat with garlic-based herbs solution on drinking water. E3S Web conf. 2021;332:01011. https://doi.org/10.1051/e3sconf/202133201011
65. Aberle ED, Forrest JC, Hedrick HB, Judge MD, Merkel RA, Gerrard DE, Mills EW. Principles of meat science. 4th ed. Kendall/Hunt Publishing Company; 2001.
66. West GE, Larue B, Touil C, Scott SL. The perceived importance of veal meat attributes in consumer choice decisions. Agribusiness. 2001;17:365-382. https://doi.org/10.1002/agr.1022
67. Mir NA, Rafiq A, Kumar F, Singh V, Shukla V. Determinants of broiler chicken meat quality and factors affecting them: a review. J. Food Sci. Technol. 2017;54:2997-3009. https://doi.org/10.1007/s13197-017-2789-z
68. Lawrie RA. Lawrie's meat science. 5th ed. Pergamon Press; 1991.
69. Cullere M, Schiavone A, Dabbou S, Gasco L, Dalle Zotte A. Meat quality and sensory traits of finisher broiler chickens fed with black soldier fly (*Hermetia Illucens* L.) larvae fat as alternative fat source. Animals. 2019;9:140. https://doi.org/10.3390/ani9040140
70. Bianchi M, Ferioli F, Petracci M, Caboni MF, Cavani C. The influence of dietary lipid source on quality characteristics of raw and processed chicken meat. Eur. Food Res. Technol. 2009;229:339-348. https://doi.org/10.1007/s00217-009-1060-x
71. Sosa DAT, Fogliano V. Potential of insect-derived ingredients for food applications. In: Shields VDC, editor. Insects Physiology and Ecology. InTech; 2017. p. 215-231 http;//dx.doi.org/10.5772/67318
72. Lorenzo JM, Gómez M. Shelf life of fresh foal meat under MAP, overwrap and vacuum packaging conditions. Meat Sci. 2012;92:610-618. https://doi.org/10.1016/j.meatsci.2012.06.008

73. Kim B, Bang HT, Kim KH, Kim MJ, Jeong JY, Chun JL, Ji SY. Evaluation of black soldier fly larvae oil as a dietary fat source in broiler chicken diets. J. Anim. Sci. Technol. 2020;62:187-197. https://doi.org/10.5187/jast.2020.62.2.187

74. Domínguez R, Pateiro M, Gagaoua M, Barba FJ, Zhang W, Lorenzo JM. A comprehensive review on lipid oxidation in meat and meat products. Antioxidants. 2019;8:429. https://doi.org/10.3390/antiox8100429

75. Cortinas L, Barroeta A, Villaverde C, Galobart J, Guardiola F, Baucells MD. Influence of the dietary polyunsaturation level on chicken meat quality: Lipid oxidation. Poult. Sci. 2005;84:48-55. https://doi.org/10.1093/ps/84.1.48

76. Gutteridge JMC. The use of standards for malonyldialdehyde. Anal. Biochem. 197;69:518-526. https://doi.org/10.1016/0003-2697(75)90155-4

77. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic. Biol. Med. 1991;11:81-128. https://doi.org/10.1016/0891-5849(91)90192-6

78. Aubourg SP. Interaction of malondialdehyde with biological molecules — new trends about reactivity and significance. Int. J. Food Sci. Technol. 1993;28:323-335. https://doi.org/10.1111/j.1365-2621.1993.tb01278.x

79. Russo GL. Dietary n − 6 and n − 3 polyunsaturated fatty acids: From biochemistry to clinical implications in cardiovascular prevention. 2009;77:937-946. https://doi.org/10.1016/j.bcp.2008.10.020

80. Maraschiello C, Sárraga C, García Regueiro JA. Glutathione peroxidase activity, TBARs, and α-tocopherol in meat from chicken fed different diets. J. Agric. Food Chem. 1999;47:867–872. https://doi.org/10.1021/jf980824o

81. Iso H, Sato S, Umemura U, Kudo M, Koike K, Kitamura A, Imano H, Okamura T, Naito Y, Shimamoto T. Linoleic Acid, other fatty acids, and the risk of stroke. Stroke. 2002;33:2086-2093. https://doi.org/10.1161/01.STR.0000023890.25066.50

82. Funk CD. Prostaglandins and leukotrienes: Advances in eicosanoid biology. Science. 2001;294:1871-1875. https://doi.org/10.1126/science.294.5548.1871
83. Greene ER, Huang S, Serhan CN, Panigrahy D. Regulation of inflammation in cancer by eicosanoids. Prostaglandins Other Lipid Mediat. 2011;96:27-36. https://doi.org/10.1016/j.prostaglandins.2011.08.004
84. Kris-Etherton PM, Pearson TA, Wan Y, Hargrove RL, Moriarty K, Fishell V, Etherton TD. High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. Am. J. Clin. Nutr. 1999;70:1009-1015. https://doi.org/10.1093/ajcn/70.6.1009
85. Roche HM. Olive oil, high-oleic acid sunflower oil and CHD. Br. J. Nutr. 2001;85:3-4.
86. Rebolé A, Rodríguez ML, Ortiz LT, Alzueta C, Centeno C, Viveros A, Brenes A, Arija I. Effect of dietary high-oleic acid sunflower seed, palm oil and vitamin E supplementation on broiler performance, fatty acid composition and oxidation susceptibility of meat. Br. Poult. Sci. 2006;47:581-591. https://doi.org/10. 1080/00071660600939727
87. Balta I, Stef L, Pet I, Iancu T, Stef D, Corcionivoschi N. Essential fatty acids as biomedicines in cardiac health. Biomedicines 2021;9:1466. https://doi.org/10.3390/biomedicines9101466
88. Katidi A, Xypolitaki K, Vlassopoulos A, Kapsokefalou M. Nutritional quality of plant-based meat and dairy imitation products and comparison with animal-based counterparts. Nutrients. 2023;15:401. https://doi.org/10.3390/nu15020401
89. Yazdanparast S, Mohammadi-Naseabadi F, Hashmati A, Rezazadeh R, Taheri M, Esfarjani F. Comprehensive assessment of fatty acid profiles of meat products to develop action plan strategies for healthier products. Sci. Res. 2025;15:23188. https://doi.org/10.1038/s41598-025-04749-z
90. Calder PC. Mechanisms of action of (n-3) fatty acids. J. Nutr. 2012;142:592S-599S. https://doi.org/10.3945/jn.111.155259

91. Givens DI, Kliem KE, Gibbs RA. The role of meat as a source of *n-3* polyunsaturated fatty acids in the human diet. Meat Sci. 2006;74:209-218. https://doi.org/10.1016/j.meatsci.2006.04.008

92. Kolanowski W, Jaworska D, Weißbrodt J. Importance of instrumental and sensory analysis in the assessment of oxidative deterioration of omega-3 long-chain polyunsaturated fatty acid-rich foods. J. Sci. Food Agric. 2007;87:181-191. https://doi.org/10.1002/jsfa.2733

93. Segura N, da Silva RC, Soares FASM, Gioielli LA, Jachmanián I. Valorization of beef tallow by lipase-catalyzed interesterification with high oleic sunflower oil. J. Am. Oil Chem. Soc. 2011;88:1945-1954. https://doi.org/ 10.1007/s11746-011-1876-y

94. Loften JR, Linn JG, Drackley JK, Jenkins TC, Soderholm CG, Kertz AF. *Invited review*: Palmitic and stearic acid metabolism in lactating dairy cows. J. Dairy Sci. 2014;97:4661-4674. http://dx.doi.org/ 10.3168/jds.2014-7919

95. Bruss MD, Khambatta CF, Ruby MA, Aggarwal I, Hellerstein MK. Calorie restriction increases fatty acid synthesis and whole body fat oxidation rates. Am. J. Physiol. Endocrinol. Metab. 2010;298:E108-E116.

96. Li Y, Mei H, Liu Y, Li Z, Qamar H, Yu M, Ma X. Dietary supplementation with rutin alters meat quality, fatty acid profile, antioxidant capacity, and expression levels of genes associated with lipid metabolism in breast muscle of Qingyuan partridge chickens. Foods. 2023;12:2302. https://doi.org/10.3390/foods12122302

97. Otto-Ślusarczyk D, Graboń W, Mielczarek-Puta M. Aspartate aminotransferase--key enzyme in the human systemic metabolism. Postepy Hig. Med. Dosw. 2016;70:219-230. https://doi.org/10.5604/17322693.1197373

98. Leal KW, do Rego Leal ML, Klein GS, Brunett ALR, Deolindo GL, Justen CE, Baldissera MD, Santos TL, Zanini D, de Araujo RC, da Silva AS. Role of microencapsulated essential oil and pepper resin in the diet of cows in the third lactation phase on immunological pathways. Vet. Sci. 2025;12:344. https://doi.org/10.3390/vetsci12040344

99. Srikha T, Pootthachaya P, Puangsap W, Pintaphrom N, Somparn N, Boonkum W, Cherdthong A, Tengjaroenkul B, Wongtangtintharn S. Effects of black soldier fly larvae oil on growth performance, blood biochemical parameters, carcass quality, and metabolomics profile of breast muscle of Thai Native Chickens. Animals. 2024;14:3098. https://doi.org/10.3390/ani14213098

100. Pereyra AS, McLaughlin KL, Buddo KA, Ellis JM. Medium-chain fatty acid oxidation is independent of L-carnitine in liver and kidney but not in heart and skeletal muscle. Am. J. Physiol. Gastrointest Liver Physiol. 2023;325:G287-294. https://doi.org/10.1152/ajpgi.00105.2023

101. Frost SC, Wells MA. A comparison of the utilization of medium and long-chain fatty acids for oxidation and ketogenesis in the suckling rat: *In vivo* and *in vitro* studies. Arch. Biochem. Biophys. 1981;211:537-546. https://doi.org/10.1016/0003-9861(81)90488-4

102. Lee KS, Lee MG, Jeong K, Yun EY, Goo TW. Medium-chain fatty acids extracted from black soldier fly (*Hermetia illucens*) larvae prevents high-fat diet-induced obesity in vivo in C57BL/6J mice. Animals. 2025;15:1384. https://doi.org/10.3390/ani15101384

103. Dabbou S, Lauwaerts A, Ferrocino I, Biasato I, Sirri F, Zampiga M, Bergagna S, Pagliasso G, Gariglio M, Colombino E, Garcés Narro C, Gai F, Capucchio MT, Gasco L, Cocolin L, Schiavone A. Modified black soldier fly larva fat in broiler diet: Effects on performance, carcass traits, blood parameters, histomorphology features and gut microbiota. Animals. 2021;11;1837. https://doi.org/10.3390/ani11061837

104. Chen X, Jin J, Hou F, Song B, Li Z, Zhao Y. Effects of black soldier fly larvae oil on growth performance, immunity and antioxidant capacity, and intestinal function and microbiota of broilers. J. Appl. Poult. Res. 2022;31:100292. https://doi.org/10.1016/j.japr.2022.100292

105. Yeom GL, Lee HN, Kim YB, Park JY, Park GY, Shin JW, Park S, Park G, Jang S, Choi Y, Choi J, Kim JH. Research note: Effects of dietary supplementation of *Abelmoschus manihot* L. flower extract on growth performance, antioxidant capacity, intestinal health,

and welfare in broiler chickens. Poult. Sci. 2026;105;106358. https://doi.org/10.1016/j.psj.2025.106358

106. Chen YP, Gu YF, Zhao HR, Zhou YM. Dietary squalene supplementation alleviates diquat-induced oxidative stress and liver damage of broiler chickens. Poult. Sci. 2021;100;100919. https://doi.org/10.1016/j.psj.2020.12.017

107. Sitko S. The role of exogenous ketones in road cycling: Evidence, mechanisms, and performance claims. Physiologia. 2024;4:433-444. https://doi.org/10.3390/physiologia4040029

108. Dearlove DJ, Faull OK, Clarke K. Context is key: exogenous ketosis and athletic performance. Curr. Opin. Physiol. 2019;10:81-89. https://doi.org/10.1016/j.cophys.2019.04.010

109. Wen S, Arakawa H, Tamai I. Uric acid in health and disease: From physiological functions to pathogenic mechanisms. Pharmacol. Ther. 2024;256:108615. https://doi.org/10.1016/j.pharmthera.2024.108615

110. Oliver WT, Wells JE. Lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology in nursery pigs. J. Anim. Sci. 2013;91:3129-3136. https://doi.org/10.2527/jas2012-5782

111. Danek-Majewska A, Kwiecień M, Samolińska W, Kowalczyk-Pecka D, Nowakowicz-Dębek B, Winiarska-Mieczan A. Animals. 2022;12:1767. https://doi.org/10.3390/ani12141767

112. Quinteiro-Filho WM, Ribeiro A, Ferraz-de-Paula V, Pinheiro ML, Sakai M, Sá LRM, Ferreira AJP, Palermo-Neto J. Poult. Sci. 2010;89:1905-1914. https://doi.org/10.3382/ps.2010-00812

113. Shekhu NA, Abdo JM. Impact of pathogenic bacterial challenges on growth performance, gut morphology, serum biochemistry, and meat quality in broiler chickens. Pak. J. Life. Soc. Sci. 2025;23:7214-7224. https://doi.org/10.57239/PJLSS-2025-23.1.00561

114. Gilani S, Chrystal PV, Barekatain R. Current experimental models, assessment and dietary modulations of intestinal permeability in broiler chickens. Anim. Nutr. 2021;7:801-811. https://doi.org/10.1016/j.aninu.2021.03.001
115. Kraehenbuhl JP, Pringault E, Neutra MR. Review article: Intestinal epithelia and barrier functions. Aliment. Pharmacol. Ther. 1997;11:3-9. https://doi.org/10.1111/j.1365-2036.1997.tb00803.x
116. Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. J. Allergy Clin. Immunol. 2009;124:3-20. https://doi.org/10.1016/j.jaci.2009.05.038
117. Bischoff SC, Barvara G, Buurman W, Ockhuizen T, Schulzke JD, Serino M, Tilg H, Waston A, Wells JM. Intestinal permeability – a new target for disease prevention and therapy. BMC Gastroenterology. 2014;14:189. https://doi.org/10.1186/s12876-014-0189-7
118. Alhenaky A, Abdelqader A, Abuajamieh M, Al-Fataftah AR. The effect of heat stress on intestinal integrity and *Salmonella* invasion in broiler birds. J. Therm. Biol. 2017;70:9-14. https://doi.org/10.1016/j.jtherbio.2017.10.015
119. Mazzoni M, Zampiga M, Clavenzani P, Lattanzio G, Tagliavia C, Sirri F. Effect of chronic heat stress on gastrointestinal histology and expression of feed intake-regulatory hormones in broiler chickens. Animal. 2022;16:100600. https://doi.org/10.1016/j.animal.2022.100600
120. Barekatain R, Inhuber V, Sharma N, Nowland T, Van TTH, Moore RJ, Cadogan D. Intestinal barrier function, caecal microbiota and growth performance of thermoneutral or heat stressed broiler chickens fed reduced crude protein diets supplemented with guanidinoacetic acid. Poult. Sci. 2025;104:104792. https://doi.org/10.1016/j.psj.2025.104792

121. Gilani S, Howarth GS, Kitessa SM, Forder REA, Tran CD, Hughes RJ. New biomarkers for intestinal permeability induced by lipopolysaccharide in chickens. Anim. Prod. Sci. 2016;56: 1984-1997. https://doi.org/10.1071/AN15725

122. Zhang Y, Rehman H, Khattak F, Tariq M, Khan BN, Chaman S, Riaz A, Omer MO, Ali A, Nisa QU, Ali MM, Saleem G. Immunomodulatory and growth-promoting effects of *Rauwolfia serpentina* root powder in broiler chicks challenged with *Salmonella* Gallinarum. Front. Vet. Sci. 2025;12:1534347. https://doi.org/10.3389/fvets.2025.1534347

Table 1 Composition and nutrient content of the experimental diets (as-fed basis)

	Starter and grower diets (0 to 3 wk), %			Finisher diets (4 to 5 wk), %		
Items	Soybean oil	Tallow	BSFL oil	Soybean oil	Tallow	BSFL oil
Ingredient, %						
Corn	54.40	55.15	54.25	61.96	62.73	61.80
Soybean meal, 46% CP	34.62	31.73	34.99	28.59	25.81	28.87
Corn gluten meal	3.69	5.64	3.45	2.87	4.76	2.69
Oil	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.30	0.30	0.30	0.30	0.30	0.30
MCP	1.70	1.75	1.70	1.30	1.32	1.30
Limestone	1.10	1.12	1.11	0.80	0.83	0.80
98.5% Met	0.30	0.29	0.30	0.27	0.26	0.27
55% Lys H_2SO_4	0.25	0.37	0.23	0.25	0.36	0.23
98.5% Thr	0.00	0.02	0.00	0.00	0.00	0.00

50% choline	0.09	0.08	0.12	0.11	0.08	0.19
$NaHCO_3$	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix[1)]	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix[2)]	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated energy and nutrient content[3)]						
AME_n, kcal/kg	3,013	3,013	3,013	3,100	3,100	3,100
CP, %	22.26	22.26	22.26	19.50	19.50	19.50
Digestible Lys, %	1.25	1.25	1.25	1.08	1.08	1.08
Digestible Met + Cys, %	0.96	0.96	0.96	0.86	0.86	0.86
Digestible Met, %	0.67	0.67	0.67	0.60	0.60	0.60
Digestible Thr, %	0.84	0.85	0.85	0.75	0.73	0.75
Digestible Trp, %	0.23	0.22	0.23	0.20	0.19	0.20
Total calcium, %	0.85	0.85	0.85	0.65	0.65	0.65

Available phosphorus, %	0.46	0.46	0.46	0.36	0.36	0.36

1) Provided per kilogram of the complete diet: vitamin A (from vitamin A acetate), 12,000 IU; vitamin D_3, 4,000 IU; vitamin E (from DL-α-tocopheryl acetate), 80 mg; vitamin K_3, 4 mg; vitamin B_1, 4 mg; vitamin B_2, 10 mg; vitamin B_6, 6 mg; vitamin B_{12}, 20 µg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 200 µg; niacin, 60 mg.

2) Provided per kilogram of the complete diet: iron, 57.14 mg; copper, 16 mg; zinc, 64.29 mg; manganese, 85.71 mg; cobalt, 170 µg; selenium, 200 µg; iodine, 570 µg.

3) Calculated values from the Arbor Acres broiler nutrition specifications (Aviagen, 2022).

BSFL, black soldier fly larvae; MCP, monocalcium phosphate.

Table 2. Analyzed fatty acid concentrations (% of total fatty acid) in fat sources

Items	Fat sources		
	Soybean oil	Tallow	BSFL oil
Saturated fatty acid, %			
Caprylic acid (C8:0)	0.00	0.02	0.04
Capric acid (C10:0)	0.00	0.07	1.80
Lauric acid (C12:0)	0.01	0.20	41.82
Myristic acid (C14:0)	0.08	2.56	6.50
Pentadecanoic acid (C15:0)	0.01	0.20	0.10
Palmitic acid (C16:0)	10.60	23.40	13.15
Margaric acid (C17:0)	0.10	0.53	0.17
Stearic acid (C18:0)	4.60	11.84	2.54
Arachidic acid (C20:0)	0.40	0.01	0.02
Heneicosanoic acid (C21:0)	0.06	0.10	0.12
Behenic acid (C22:0)	0.03	0.18	0.08
Lignoceric acid (C24:0)	0.01	0.05	0.01

Monounsaturated fatty acid, %			
Myristoleic acid (C14:1)	0.00	0.64	0.20
Pentadecenoic acid (C15:1)	0.01	0.07	0.02
Palmitoleic acid (C16:1)	0.10	4.06	2.75
Heptadecenoic acid (C17:1)	0.05	0.50	0.16
Oleic acid (C18:1 n-9)	24.02	45.85	15.82
Eicosenoic acid (C20:1 n-9)	0.24	0.78	0.70
Erucic acid (C22:1 n-9)	0.03	0.10	0.01
Nervonic acid (C24:1 n-9)	-	-	-
Polyunsaturated fatty acid, %			
Linoleic acid (C18:2 n-6)	53.00	7.52	10.96
γ-Linolenic acid (C18:3 n-6)	0.77	0.10	0.10
α-Linolenic acid (C18:3 n-3)	5.75	0.33	1.20
Eicosadienoic acid (C20:2 n-6)	0.01	0.00	0.00
Dihomoγ-Linolenic acid (C20:3 n-6)	0.00	0.01	0.00
Eicosatrienoic acid (C20:3 n-3)	0.00	0.01	0.00

Arachidonic acid (C20:4 n-6)	0.00	0.20	0.22
Eicosapentaenoic acid (C20:5 n-3)	0.01	0.01	1.00
Docosadienoic acid (C22:2 n-6)	0.01	0.01	0.01
Docosapentaenoic acid (C22:5 n-3)	0.00	0.04	0.02
Docosahexaenoic acid (C22:6 n-3)	0.00	0.02	0.15
Other fatty acids, %			
SFA	15.90	39.16	66.35
UFA	84.00	60.25	33.32
UFA/SFA	5.28	1.54	0.50
MUFA	24.45	52.00	19.66
PUFA	59.55	8.25	13.66
PUFA/MUFA	2.44	6.30	0.69
n-3	5.76	0.41	2.37
n-6	53.79	7.84	11.29
n-6/n-3 ratio	9.34	19.12	4.76

BSFL, black soldier fly larvae; SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table 3. Effects of dietary supplementation of fat sources on growth performance of broiler chickens[1)]

Items	Dietary treatments[2)]			SEM	*p* -value
	Soybean oil	Tallow	BSFL oil		
BWG, g	1,624	1,607	1,589	50.0	0.889
FI, g	2,399	2,344	2,330	66.7	0.743
FCR, g/g	1.48	1.46	1.47	0.037	0.954

[1)]Data are least squares means of 5 observations per treatment.

[2)]Experimental diets were formulated by supplementing a basal diet with 3% fat sources (i.e., soybean oil, tallow, and black soldier fly larvae oil).

BSFL, black soldier fly larvae; BWG, BW gain; FI, feed intake; FCR, feed conversion ratio (FI:BWG).

Table 4. Effects of dietary supplementation of fat sources on meat quality of broiler chickens[1)]

Items		Dietary treatments[2)]			SEM	*p* -value
		Soybean oil	Tallow	BSFL oil		
Breast yield, %		18.87	19.40	19.94	0.606	0.480
pH	1 h	6.45	6.56	6.41	0.051	0.150
	24 h	5.77[a]	5.59[b]	5.61[b]	0.053	0.043
Meat color	L*	49.80[ab]	50.40[a]	48.00[b]	0.652	0.048
(CIE value)	a*	3.70[a]	3.25[b]	3.30[b]	0.080	0.006
	b*	9.48	9.37	9.83	0.641	0.881
WHC		76.19	71.89	72.59	2.388	0.421
TBARS	5 d	0.33	0.36	0.35	0.016	0.588

[a,b]Means within a variable with no common superscript differ significantly ($p < 0.05$).

[1)]Data are least squares means of 5 observations per treatment.

[2)]Experimental diets were formulated by supplementing a basal diet with 3% fat sources (i.e., soybean oil, tallow, and black soldier fly larvae oil).

BSFL, black soldier fly larvae; L*, lightness; a*, redness; b*, yellowness; WHC, water holding capacity; TBARS, thiobarbituric acid reactive substance (malondialdehyde equivalents per g of meat sample).

Table 5. Effects of dietary supplementation of fat sources on fatty acid concentrations in the breast meat of broiler chickens[1)]

Items	Dietary treatments[2)]			SEM	*p*-value
	Soybean oil	Tallow	BSFL oil		
Saturated fatty acid, %					
Caprylic acid (C8:0)	0.09[b]	0.07[b]	0.15[a]	0.011	0.001
Capric acid (C10:0)	0.12[b]	0.11[b]	0.33[a]	0.021	<0.001
Lauric acid (C12:0)	0.15[b]	0.15[b]	5.16[a]	0.318	<0.001
Myristic acid (C14:0)	0.38[c]	0.76[b]	2.20[a]	0.104	<0.001
Pentadecanoic acid (C15:0)	0.07[b]	0.11[ab]	0.12[a]	0.012	0.037
Palmitic acid (C16:0)	19.77[b]	22.00[a]	23.00[a]	0.508	0.001
Margaric acid (C17:0)	0.13[b]	0.22[a]	0.17[b]	0.012	<0.001
Stearic acid (C18:0)	7.76[b]	7.63[b]	8.56[a]	0.140	0.001
Arachidic acid (C20:0)	-	-	-	-	-
Heneicosanoic acid (C21:0)	0.19[a]	0.06[b]	0.10[ab]	0.037	0.046
Behenic acid (C22:0)	0.59[a]	0.28[c]	0.41[b]	0.044	0.002
Lignoceric acid (C24:0)	0.91	0.73	1.00	0.106	0.202

Monounsaturated fatty acid, %					
Myristoleic acid (C14:1)	1.98	0.41	1.16	0.742	0.287
Pentadecenoic acid (C15:1)	0.13	0.11	0.16	0.030	0.314
Palmitoleic acid (C16:1)	2.81^{c}	5.19^{a}	3.54^{b}	0.165	<0.001
Heptadecenoic acid (C17:1)	0.13^{c}	0.27^{a}	0.16^{b}	0.006	<0.001
Oleic acid (C18:1 n-9)	33.55^{b}	41.14^{a}	27.04^{c}	1.068	<0.001
Eicosenoic acid (C20:1 n-9)	0.32^{b}	0.73^{a}	0.49^{b}	0.057	<0.001
Erucic acid (C22:1 n-9)	0.47	0.63	0.81	0.093	0.066
Nervonic acid (C24:1 n-9)	0.31	0.34	0.34	0.073	0.906
Polyunsaturated fatty acid, %					
Linoleic acid (C18:2 n-6)	23.71^{a}	14.30^{b}	15.62^{b}	0.868	<0.001
γ-Linolenic acid (C18:3 n-6)	0.33^{a}	0.21^{b}	0.19^{b}	0.020	<0.001
α-Linolenic acid (C18:3 n-3)	1.58^{a}	0.51^{b}	0.55^{b}	0.075	<0.001
Eicosadienoic acid (C20:2 n-6)	0.03^{b}	0.09^{a}	0.06^{ab}	0.013	0.030
Dihomoγ-Linolenic acid (C20:3 n-6)	0.07^{c}	0.23^{a}	0.14^{b}	0.017	<0.001
Eicosatrienoic acid (C20:3 n-3)	0.06	0.08	0.09	0.011	0.252

Arachidonic acid (C20:4 n-6)	2.95^{b}	2.63^{b}	4.51^{a}	0.411	0.011
Eicosapentaenoic acid (C20:5 n-3)	0.17^{b}	0.11^{c}	0.46^{a}	0.013	<0.001
Docosadienoic acid (C22:2 n-6)	0.07	0.02	0.05	0.016	0.174
Docosapentaenoic acid (C22:5 n-3)	0.52^{b}	0.31^{c}	0.98^{a}	0.067	<0.001
Docosahexaenoic acid (C22:6 n-3)	0.49^{b}	0.37^{b}	1.37^{a}	0.090	<0.001
Other fatty acids, %					
SFA	30.16^{b}	32.12^{b}	41.20^{a}	0.730	<0.001
UFA	69.68^{a}	67.68^{a}	57.72^{b}	0.876	<0.001
UFA/SFA	2.31^{a}	2.11^{a}	1.44^{b}	0.059	<0.001
MUFA	39.70^{b}	48.82^{a}	33.70^{c}	1.368	<0.001
PUFA	29.98^{a}	18.86^{c}	24.02^{b}	1.660	<0.001
PUFA/MUFA	0.76^{a}	0.39^{b}	0.71^{a}	0.058	0.001
n-3	2.82^{a}	1.38^{b}	3.45^{a}	0.197	<0.001
n-6	27.16^{a}	17.48^{b}	20.57^{b}	1.344	<0.001
n-6/n-3 ratio	9.63^{b}	12.67^{a}	5.96^{c}	0.532	<0.001

$^{a-c}$Means within variable with no common superscript differ significantly ($p < 0.05$).

[1)]Data are least squares means of 5 observations per treatment.

[2)]Experimental diets were formulated by supplementing a basal diet with 3% fat sources (i.e., soybean oil, tallow, and black soldier fly larvae oil).

BSFL, black soldier fly larvae; SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table 6. Effects of dietary supplementation of fat sources on organ weight of broiler chickens[1)]

	Dietary treatments[2)]				
Items[3)]	Soybean oil	Tallow	BSFL oil	SEM	*p*-value
Liver, %	2.15	2.08	2.14	0.085	0.731
Spleen, %	0.11	0.10	0.10	0.010	0.674
bursa of Fabricius, %	0.18	0.18	0.23	0.021	0.172
Thymus, %	0.31	0.24	0.25	0.040	0.314
Abdominal fat, %	1.58	1.34	1.38	0.233	0.720

[1)]Data are least squares means of 5 observations per treatment.

[2)]Experimental diets were formulated by supplementing a basal diet with 3% fat sources (i.e., soybean oil, tallow, and black soldier fly larvae oil).

[3)]The relative organ weight was expressed as a percentage of BW.

BSFL, black soldier fly larvae.

Table 7. Effects of dietary supplementation of fat sources on serum lipid of broiler chickens[1)]

Items	Dietary treatments[2)]			SEM	*p*-value
	Soybean oil	Tallow	BSFL oil		
HDL, (mg/dL)	73.4	77.8	80.2	4.36	0.552
LDL, mg/dL	20.6	16.5	19.2	1.79	0.270
TC, mg/dL	110.2	124.4	116.8	5.66	0.246
TG, mg/dL	22.6[b]	29.5[a]	26.3[ab]	1.57	0.014
AST, U/L	241[b]	268[ab]	333[a]	22.9	0.038
ALT, U/L	4.0	4.0	4.4	0.20	0.300
Creatinine, mg/dL	0.08	0.08	0.07	0.011	0.759
Uric acid, mg/dL	5.38[a]	6.70[a]	2.46[b]	0.909	0.018

[a,b]Means within a variable with no common superscript differ significantly ($p < 0.05$).

[1)]Data are least squares means of 5 observations per treatment.

[2)]Experimental diets were formulated by supplementing a basal diet with 3% fat sources (i.e., soybean oil, tallow, and black soldier fly larvae oil).

BSFL, black soldier fly larvae; HDL, high density lipoprotein; LDL, low density lipoprotein; TC, total cholesterol; TG, triglyceride; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 8. Effects of dietary supplementation of fat sources on liver antioxidant capacity of broiler chickens[1)]

Items	Dietary treatments[2)]			SEM	*p*-value
	Soybean oil	Tallow	BSFL oil		
TAC, nmol/mg protein	0.36	0.34	0.33	0.046	0.876
MDA, nmol/mg protein	0.33	0.56	0.50	0.096	0.267

[1)]Data are least squares means of 5 observations per treatment.

[2)]Experimental diets were formulated by supplementing a basal diet with 3% fat sources (i.e., soybean oil, tallow, and black soldier fly larvae oil).

BSFL, black soldier fly larvae; TAC, total antioxidant capacity; MDA, malondialdehyde.

Table 9. Effects of dietary supplementation of fat sources on liver characteristics of broiler chickens[1)]

Items		Dietary treatments[2)]			SEM	*p*-value
		Soybean oil	Tallow	BSFL oil		
Liver color	L*	23.28	25.00	23.48	0.928	0.387
	a*	13.14^{b}	16.04^{a}	14.40^{ab}	0.702	0.039
	b*	1.98	3.70	2.44	0.050	0.080
Liver hemorrhagic score		0.40	0.28	0.32	0.147	0.843
Fatty liver score		1.04	1.08	1.00	0.052	0.565
Liver fat concentrations		22.43	30.24	21.31	2.893	0.073

^{a,b}Means within a variable with no common superscript differ significantly ($p < 0.05$).

[1)]Data are least squares means of 5 observations per treatment.

[2)]Experimental diets were formulated by supplementing a basal diet with 3% fat sources (i.e., soybean oil, tallow, and black soldier fly larvae oil).

BSFL, black soldier fly larvae; L*, lightness; a*, redness; b*, yellowness.

Table 10. Effects of dietary supplementation of fat sources on stress indicator of broiler chickens[1)]

Items	Dietary treatments[2)]			SEM	p-value
	Soybean oil	Tallow	BSFL oil		
H:L ratio	0.22	0.21	0.24	0.008	0.106
Feather corticosterone	3.30	3.03	3.46	0.818	0.918

[1)]Data are least squares means of 5 observations per treatment.

[2)]Experimental diets were formulated by supplementing a basal diet with 3% fat sources (i.e., soybean oil, tallow, and black soldier fly larvae oil).

BSFL, black soldier fly larvae; H:L ratio, heterophil to lymphocyte ratio.

Table 11. Effects of dietary supplementation of fat sources on jejunal morphology of broiler chickens[1)]

Items	Dietary treatments[2)]			SEM	p-value
	Soybean oil	Tallow	BSFL oil		
Villus height, μm	1,185	1,205	1,141	18.0	0.073
Villus width, μm	138	143	151	6.8	0.431
Crypt depth, μm	133	139	131	8.2	0.749
VH:CD ratio	9.47	9.05	9.40	0.530	0.841
VSA, mm^2	0.51	0.54	0.54	0.027	0.736

[1)]Data are least squares means of 5 observations per treatment.

[2)]Experimental diets were formulated by supplementing a basal diet with 3% fat sources (i.e., soybean oil, tallow, and black soldier fly larvae oil).

BSFL, black soldier fly larvae; VH:CD, villus height to crypt depth ratio; VSA, villus surface area.

Table 12. Effects of dietary supplementation of fat sources on jejunal permeability of broiler chickens[1)]

	Dietary treatments[2)]				
Items	Soybean oil	Tallow	BSFL oil	SEM	*p*-value
PD, mV	15.3	14.7	15.0	0.36	0.468
Isc, μA/cm^2	100.2	104.3	100.8	3.45	0.349
TEER, Ω/cm^2	156.8	150.2	153.5	3.66	0.468

[1)]Data are least squares means of 5 observations per treatment.

[2)]Experimental diets were formulated by supplementing a basal diet with 3% fat sources (i.e., soybean oil, tallow, and black soldier fly larvae oil).

BSFL, black soldier fly larvae; PD, potential difference; Isc, short circuit current; TEER, transepithelial electrical resistance.

Table 13. Effects of dietary supplementation of fat sources on welfare parameters of broiler chickens[1)]

Items	Dietary treatments[2)]			SEM	*p*-value
	Soybean oil	Tallow	BSFL oil		
Gait score	0.41	0.27	0.43	0.087	0.398
Footpad dermatitis	0.25	0.18	0.21	0.072	0.775
Hock burn	0.03	0.05	0.01	0.017	0.199
Plumage cleanliness	0.01	0.02	0.01	0.009	0.531

[1)]Data are least squares means of 5 observations per treatment.

[2)]Experimental diets were formulated by supplementing a basal diet with 3% fat sources (i.e., soybean oil, tallow, and black soldier fly larvae oil).

BSFL, black soldier fly larvae.