Black soldier fly larvae oil as an alternative fat source in broiler nutrition

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ABSTRACT The present study was conducted to investigate growth performance, carcass characteristics, short-chain fatty acids, fatty acid composition in abdominal fat, and serum parameters in broiler chickens fed diets containing corn oil, coconut oil, or black soldier fly larvae (**BSFL**) oil at the level of 50 g per kg of diet during the 30-day-feeding period. A total 450 one-day-old male broiler chicks (Ross 308) were randomly allocated to one of 3 dietary groups. Each treatment had 10 replicates with 15 chicks per replicate. Feed conversion ratio was decreased in the coconut and BSFL oil group compared with the corn oil group. Dietary BSFL oil increased ileal weight-to-length ratio at day 30 after hatch. Dietary BSFL oil increased significantly ileal branched-chain fatty acid ($P < 0.05$) and moderately total short-chain fatty acid in 15-day-old broilers ($P = 0.074$). At day 30, ileal propionate was highest in the coconut oil group but cecal propionate was highest ($P < 0.05$) in the BSFL oil group. Fatty acid composition of abdominal fat was affected by dietary fat sources. Especially, chickens fed diets containing coconut oil or BSFL oil had higher contents ($P < 0.05$) of saturated fatty acid being dominant in lauric and myristic acids compared with those fed on corn oil. On the other hand, the reverse trend was noted ($P < 0.05$) as to poly-unsaturated fatty acids being dominant in corn oil compared with coconut oil and BSFL oil. Coconut oil vs. corn oil significantly increased total and high-density lipoprotein cholesterol. Finally, BSFL oil vs. corn oil significantly increased total antioxidant capacity in chickens. It is concluded that dietary BSFL oil improves feed conversion ratio and increases the incorporation of medium-chain fatty acids into abdominal fat pad and serum antioxidant capacity in broiler chickens.

Key words: black soldier fly larvae oil, broiler chicken, growth performance, fatty acid composition, gut health

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INTRODUCTION

Insects are becoming value-added food resources of human diets in many countries (Borrelli et al., 2017) and also used as an alternative to conventional protein ingredients such as fish meal or soybean meal for livestock (Loponte et al., 2017; Bovera et al., 2018; Cutrignelli et al., 2018). Black soldier fly (*Hermetia illucens*) is a popular biorecycling organism being able to convert the large quantities of organic, otherwise pollutants, substrates in food or animal wastes to edible protein and fats during their growth (Zheng et al., 2012; Li et al., 2016). Black soldier fly larvae (**BSFL**) contains up to 40% of protein rich in essential amino acids, more than 28% of lipids, and minerals such as Ca and P (Makkar et al., 2014; Wang and Shelomi, 2017). In contrast to the nutritional advantage, potential danger of insects used as animal feeds has also been reported because of the accumulation of heavy metals and toxins when insects were grown on the contaminated substrates (Wang and Shelomi, 2017).

Kierończyk et al. (2018) suggested that the use of insect oil, such as *Tenebrio molitor* and *Zophobas morio*, could replace soybean oil in broiler chicken nutrition without compromising their growth performance and nutrient digestibility. In addition, Li et al. (2016) reported that dietary BSFL oil increased omega-3 fatty acid deposition in muscles but lowered intraperitoneal fat deposition in juvenile carp. BSFL oil is rich in medium-chain fatty acids such as lauric acid (C12:0), which is similar to coconut oil (Li et al., 2016; Ushakova et al., 2016). Coconut oil is the only plant-origin oil where about 50% of the fatty

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acid composition is lauric acid (C12:0) (Dayrit, 2014). Wang et al. (2015) showed that medium-chain fatty acids may be advantageous in abdominal fat reduction because of their preferential use in energy utilization over long-chain saturated or unsaturated fatty acids. In addition, medium-chain fatty acids including lauric acid are known for their antimicrobial effects on gut bacteria (Zeitz et al., 2015; Schiavone et al., 2017). Thus, it can be postulated that insect oils rich in lauric acid could impact on growth performance and gut health in fast-growing broiler chickens (Schiavone et al., 2018). It is reported that fatty acid composition in broilers' diet determines those in meats and internal organs and is closely correlated with the fat storage and metabolism (Taulescu et al., 2010; Khatun et al., 2018). Schiavone et al. (2017) found that partial or total replacement of soybean oil with BSFL oil altered the fatty acid profile of broiler chickens. However, knowledge about the suitability of BSFL oil as a poultry feed ingredient is still limited. Based on the functionality of medium-chain fatty acids–rich insect oils being antimicrobials and modifying body fatty acid composition, it is postulated that dietary BSFL oil could improve growth performance and gut health, alter the fatty acid composition of abdominal fat pads, and enhance meat quality in broiler chickens. It has been reported that dietary fat sources increased meat quality in broiler chickens (Zeitz et al., 2015; Khatun et al., 2018; Cullere et al., 2019).

In this study, we used 3 fat sources including corn oil, coconut oil, and BSFL oil to see their effects on growth performance, carcass characteristic, volatile fatty acid production, body fatty acid composition, and serum parameters in broiler chickens.

Table 1. Ingredients and nutrient composition of the basal diet (as-fed).

Ingredients	g/100 of diet
Corn, 8.8% CP	59.35
Soybean meal, 44.8% CP	24.50
Corn gluten meal, 60% CP	6.00
Oil[1]	5.00
L-Lysine-HCl, 78%	0.35
DL-methionine, 99%	0.40
Dicalcium phosphate	1.60
L-threonine	0.08
Choline chloride, 50%	0.20
Salt	0.30
Limestone	1.60
Sodium bicarbonate	0.22
Vitamin premix[2]	0.20
Mineral premix[3]	0.20
Total	100.00
Calculated nutrient composition	
AMEn, kcal/kg	3,248
Dry matter, %	89.5
Crude protein, %	20.4
Lysine, %	1.19
Met + cys, %	1.06
Threonine, %	0.81
Calcium, %	1.02
Nonphytate phosphorus, %	0.46

[1]The 3 treatments were generated by adding 3 types of oil (corn oil, coconut oil, and black soldier fly larvae oil).

[2]Vitamin premix provided following nutrients per kg of diet: vitamin A, 24,000 IU; vitamin D_3, 6,000 IU; vitamin E, 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}, 0.04 mg; niacin, 80 mg; pantothenic acid, 20 mg; folic acid, 2 mg. biotin, 0.3 mg.

[3]Mineral premix provided following nutrients per kg of diet: Fe, 176 mg; Cu, 145.2 mg; Zn, 120 mg; Mn, 132 mg; I, 1.98 mg; Co, 0.66 mg; Se, 0.44 mg.

MATERIALS AND METHODS

All animal care procedures were approved by the Institutional Animal Care and Use of Committee of Konkuk University (KU18028).

Birds, Diets, and Experimental Design

A total of 450 one-day-old feather-sexed male broiler chicks (Ross 308) were purchased from a local hatchery, weighed individually upon arrival, randomly divided into 30 floor pens with rice husk as a bedding material, and assigned to one of 3 dietary treatments. Each treatment had 10 replicates with 15 birds per pen. Experimental diets (Table 1), in mash form, were formulated by mixing a corn and soybean meal-based diet with 3 different oils (corn oil, coconut oil, and BSFL oil) to reach 50 g per kg of diet. BSFL oil was provided by Foody Worm, Inc. (Cheongju-si, Chungcheongbuk-do, South Korea), and fatty acid profiles of the diets and fat sources were analyzed and presented (Table 2). Temperature of the facility was initially set at 34°C during the first week, then gradually decreased to reach 24°C at 21 D, and maintained constant thereafter. Feed and water were provided ad libitum throughout the 30-day-feeding trial, and light was provided 23 h/D. The 30-day-feeding trial was considered sufficient to elucidate the biological effect of insect oils, if any, in broiler chickens as reported elsewhere (Kierończyk et al., 2018). Body weight and feed intake by pen were monitored at the beginning and 15 and 30 D of experiment. Mortality was recorded daily to calculate the mortality-adjusted feed conversion ratio.

Sampling

On day 15 and 30, one bird per pen was randomly selected and euthanized with overdose of carbon dioxide. Immediately after euthanasia, blood was taken via cardiac puncture. Serum samples were obtained by gentle centrifugation (200 × *g*) for 15 min and stored at −20°C before analysis. Immediately after blood sampling, internal organs (i.e., liver, spleen, abdominal fat, and bursa of Fabricius), small intestine, and a pair of ceca were excised. Internal organs were weighed and discarded except for abdominal fat. Each segment of the small intestine was emptied by gentle pressure, and the length and weight were recorded to calculate the relative length and weight of each segment. Special care was paid to collect aseptically ileal and cecal digesta which were maintained on ice and prepared for counting *Clostridium perfringens* and short-chain fatty acids (**SCFAs**) on the day of the sampling. On day 30, right breast and leg meats were sampled, weighed, and stored at 4°C until the measurement of meat quality.

Table 2. Analyzed fatty acid composition (% of total fatty acid methyl esters) of oils and experimental diets.

	Oils			Experimental diets[1]		
Fatty acid	Corn oil	Coconut oil	BSFL oil	Corn oil	Coconut oil	BSFL oil
Lauric acid (C12:0)	0.06	53.98	37.55	0.10	30.43	21.43
Myristic acid (C14:0)	0.07	20.97	6.73	0.13	12.17	3.94
Palmitic acids (C16:0)	11.80	10.86	15.60	13.87	12.15	14.77
Stearic acid (C18:0)	2.26	3.60	3.90	2.66	3.32	3.33
Heneicosylic acid (C21:0)	0.02	0.05	0.14	0.04	0.06	0.03
Palmitoleic acid (C16:1 ω7)	0.12	0.06	2.53	0.17	0.09	1.57
Elaidic acid (C18:1 ω9)	8.92	1.32	3.48	7.15	3.27	3.77
Oleic acid (C18:1 ω9)	22.84	5.82	14.40	37.00	12.00	17.56
Gondoic acid (C20:1 ω9)	0.44	0.06	0.21	0.07	0.09	0.31
Linoleic acid (C18:2 ω6)	51.50	1.76	12.72	36.29	24.10	30.25
Linolenic acid (C18:3 ω3)	0.90	0.04	1.51	1.17	1.13	2.00
Arachidonic acid (C20:4 ω6)	0.03	0.03	0.07	0.04	0.03	0.02
Eicosadienoic acid (C20:2 ω6)	0.09	0.15	0.08	0.12	0.07	0.07
Other fatty acids	0.94	1.31	1.09	1.20	1.10	0.96
Saturated fatty acids	15.15	90.76	65.01	17.99	59.23	44.44
Monounsaturated fatty acids	32.33	7.25	20.62	44.39	15.45	23.22
Polyunsaturated fatty acids	52.52	1.99	14.37	37.62	25.32	32.34

Abbreviation: BSFL, black soldier fly larvae.
[1]Experimental diets were produced by adding oils (i.e., corn oil, coconut oil, and black soldier fly larvae oil) into a base diet to reach 50 g/kg of diet.

Meat Quality Assay

The refrigerated right breast and leg meats sampled on day 30 were used to measure meat quality on the following day of the sampling. The pH was measured at 2 different sites of the *pectoralis major* and thigh muscles using a pH meter (Testo 205; Testo AG, Lenzkirch, Germany). The color of raw breast and leg meats was measured at 3 different sites using a portable spectrophotometer (CM-2600d; Konica Minolta, Ramsey, NJ). The International Commission on Illumination lightness (L*), redness (a*), and yellowness (b*) components were obtained from the Specular Component Excluded mode readings. For water-holding capacity, meat samples were packaged in a plastic bag under vacuum, cooked in a water bath at 80°C for 30 min as described by Huang et al. (2017), cooled, and removed off residual moisture using paper towel before reweighing. Water-holding capacity (cooking loss) was calculated as the percentage of weight lost by the sample.

Clostridium perfringens *Counts in Ileal and Cecal Digesta*

Approximately 1 g of cecal and ileal digesta was mixed in 9 mL of cold distilled water and serially 10-fold diluted from 10^{-4} to 10^{-6} (for the cecal samples) or 10^{-3} to 10^{-5} (for the ileal samples). The dilutions were then spiral-plated on reinforced clostridial agar (Reinforced Clostridial medium; BD Difco) and incubated in the anaerobic cabinet at 37°C for 24 h. The number of characteristic black colonies was counted and expressed as $\log_{10}$ colony forming unit (cfu) per g of digesta.

SCFA Analysis

Approximately 1 g of ileal and cecal digesta was homogenized in 9 mL of cold distilled water; the homogenate was added to 0.05 mL of saturated $HgCl_2$, 1 mL of 25% H_3PO_4, and 0.2 mL of 2% pivalic acid and centrifuged at 1,000 × *g* at 4°C for 20 min; and 1 mL of supernatant was used to measure the concentrations of SCFAs in cecal and ileal samples by gas chromatography (6,890 Series GC System; HP, Palo Alto, CA) as described by Van Der Wielen et al. (2000).

Fatty Acid Composition Analysis

In brief, a 1-g sample was extracted with a chloroform-methanol (2:1, vol/vol) mixture according to the method of Folch et al. (1957). Then, the extracted fat was converted to fatty acid methyl esters with boron trifluoride in methanol (Wang et al., 2013). The fatty acid methyl esters obtained were separated and analyzed by gas chromatography (HP 7890 series GC system; Agilent technologies, Santa Clara, CA).

Serum Biochemical Parameters

Serum samples were analyzed for glutamic pyruvic transaminase (**GPT**), glutamic oxaloacetic transaminase (**GOT**), high-density lipoprotein (**HDL**) cholesterol, total cholesterol, triglyceride, and uric acid using an automatic blood chemical analyzer (Film DRI CHEM 7000i; Fuji film, Tokyo, Japan).

Antioxidant and Immune Markers

Serum total antioxidant capacity (**TAC**) was analyzed using QuantiChrom antioxidant assay kit (BioAssay Systems, Hayward, CA) and expressed by Trolox equivalents (Sies, 1997; Prior et al., 2005). Malondialdehyde (**MDA**) was measured using the OxiSelect TBARS Assay kit (Cell Biolabs, Inc., San Diego, CA). Nitric oxide in serum samples was determined as described by Ndazigaruye et al. (2019) using the Griess reagent (Sigma-Aldrich, St. Louis, MO).

Statistical Analysis

Each pen was considered as an experimental unit. Data were checked for normality using PROC UNIVARIATE (version 9.4; SAS Institute Inc., Cary, NC) and were analyzed by one-way ANOVA using the PROC GLM (version 9.4; SAS Institute Inc., Cary, NC). Duncan's multiple range test was used to determine means and differences among treatments. The significance level was preset at $P < 0.05$, and tendency was declared at $P < 0.10$.

RESULTS

Fatty Acid Composition of the Fat Sources

Fat sources were added into a corn and soybean meal base diet at the level of 50 g per kg of diet. The analyzed experimental diets had similar total fat, protein, and ash contents but differed greatly in their fatty acid composition (Table 2). As expected, corn oil is rich in oleic and linoleic acids and coconut oil in lauric and myristic acids. BSFL oil had lesser amounts of lauric and myristic acids but had greater amounts of oleic and linoleic acids than the coconut oil.

Growth Performance

Dietary fat sources did not affect live body weights of broiler chickens at 15 ($P = 0.985$) and 30 D ($P = 0.125$). None of fat sources affected body weight gain ($P = 0.183$) and feed intake ($P = 0.665$) in broiler chickens during days 1 to 30 (Table 3). Feed conversion ratio was decreased ($P < 0.05$) in the coconut oil and BSFL oil groups compared with that in the corn oil group.

Length and Weight of Small Intestine

On day 15, relative weight of each segment of small intestine was not affected ($P > 0.05$) by fat sources (Table 4). Coconut oil significantly lowered relative ileal weight compared with the corn oil and BSFL oil at day 30. Dietary fat sources failed ($P > 0.05$) to affect relative length of each segment of small intestine at days 15 and 30. Relative ileal length was numerically decreased by 10.5 and 10.3% in coconut oil and 7.9 and 9.7% in BSFL oil on days 15 ($P = 0.156$) and 30 ($P = 0.151$) compared with the corn oil. The weight-to-length ratios of each segment of intestine were not affected by dietary fat sources in 15-day-old broiler chickens. On the other hand, ileal weigh-to-length ratio significantly elevated ($P < 0.05$) in BSFL oil vs. corn oil and coconut oil in 30-day-old broilers.

Organ Weights

Relative organ weights at day 15 were not affected ($P > 0.05$) by dietary fat sources (Table 5). On day 30, coconut oil decreased ($P = 0.073$) the relative weight of liver by 9.1 and 5.9% compared with the corn oil and BSFL oil, respectively. Relative weight of spleen was lighter ($P = 0.066$) in the coconut oil group by 21.5 and 25.4% than in the corn oil and BSFL oil groups. On the other hand, dietary fat sources did not affect pancreas, bursa, and abdominal fat at day 30.

Breast and Thigh Meat Qualities

Dietary fat sources did not affect the absolute and relative yields of breast and thigh meats (Table 6). The pH of the breast meat was lowest ($P = 0.022$) in the coconut oil compared with that in the corn oil and BSFL oil. Addition of corn oil into the broilers diet did not affect L* value but lowered a* ($P = 0.153$) and b* values ($P = 0.009$) compared with coconut oil and BSFL oil. None of meat traits in thigh meats was affected by fat sources.

Perfringens Counts in Ileal and Cecal Digesta

C. perfringens counts in ileal and cecal digesta were not affected by dietary fat sources (Table 7). Although not significant, BSFL oil vs. corn oil increased ($P = 0.196$) ileal *C. perfringens* count by 8.6% at day 15 but lowered ($P = 0.220$) ileal *C. perfringens* count by 13.7% at day 30.

Table 3. Effect of dietary oil sources on growth performance in broiler chickens (1–30 D)[1].

Item	Experimental diets[2]			SEM	*P* value
	Corn oil	Coconut oil	BSFL oil		
Initial body weight (g/bird)	45.68	45.66	45.69	0.13	0.989
Body weight at 15 D (g/bird)	417.28	418.71	417.53	6.14	0.985
Body weight at 30 D (g/bird)	1,383.87	1,479.23	1,453.79	32.94	0.125
Body weight gain (g/day/bird)	45.43	48.05	48.11	1.14	0.183
Feed intake (g/day/bird)	71.70	69.93	71.31	1.42	0.655
Feed conversion ratio (g:g)	1.58[a]	1.46[b]	1.49[b]	0.03	0.021

[a-b]Means without a common superscript letter differ ($P < 0.05$).
Abbreviations: BSFL, black soldier fly larvae; SEM, standard error of the means.
[1]All means are average of 10 replicates per treatment.
[2]Experimental diets were produced by adding oils (i.e., corn oil, coconut oil, and black soldier fly larvae oil) into a base diet to reach 50 g/kg of diet.

Table 4. Effect of dietary oil sources on relative weight and length of the small intestine in broiler chickens[1].

Item	Experimental diets[2]			SEM	*P* value
	Corn oil	Coconut oil	BSFL oil		
Relative weight, g/100 g BW					
Day 15					
Duodenum	1.02	1.03	1.02	0.05	0.975
Jejunum	1.71	1.79	1.69	0.08	0.671
Ileum	1.04	1.09	1.01	0.06	0.632
Day 30					
Duodenum	0.56	0.53	0.57	0.04	0.671
Jejunum	1.12	1.04	1.01	0.08	0.590
Ileum	0.92[a]	0.77[b]	0.93[a]	0.04	0.012
Relative length, cm/100 g BW					
Day 15					
Duodenum	5.37	4.93	4.99	0.19	0.223
Jejunum	12.10	11.77	11.90	0.41	0.850
Ileum	12.64	11.31	11.64	0.49	0.156
Day 30					
Duodenum	1.92	1.82	1.83	0.08	0.679
Jejunum	4.75	4.55	4.27	0.24	0.395
Ileum	4.88	4.38	4.40	0.20	0.151
Weight:length, cm/g					
Day 15					
Duodenum	0.19	0.21	0.21	0.01	0.508
Jejunum	0.14	0.15	0.14	0.01	0.513
Ileum	0.08	0.10	0.09	0.01	0.224
Day 30					
Duodenum	0.29	0.29	0.31	0.01	0.592
Jejunum	0.24	0.23	0.24	0.01	0.937
Ileum	0.19[b]	0.18[b]	0.21[a]	0.01	0.003

[a-b]Means without a common superscript letter differ ($P < 0.05$).

Abbreviations: BSFL, black soldier fly larvae; BW, body weight; SEM, Standard error of the means.

[1]All means are average of 10 pens per treatment.

[2]Experimental diets were produced by adding oils (i.e., corn oil, coconut oil, and black soldier fly larvae oil) into a base diet to reach 50 g/kg of diet.

Concentration of SCFA in Ileal and Cecal Digesta

Concentrations of SCFAs in ileal and cecal contents are presented in Tables 8 and 9. BSFL oil increased acetate ($P = 0.275$), butyrate ($P = 0.127$), valerate ($P = 0.074$), branched-chain fatty acids ($P = 0.034$), and SCFAs ($P = 0.074$) in ileal digesta at day 15 compared with the corn oil and coconut oil. At day 30, dietary fat source did not affect ileal total SCFAs except for ileal propionate which was lowered ($P = 0.042$) in the BSFL oil group compared with that in the corn oil and coconut oil groups. SCFAs were higher in cecal vs. ileal digesta at all ages. Dietary fat sources did not affect cecal SCFAs except for isobutyrate being the highest ($P = 0.099$) at day 15 and propionate being the lowest ($P = 0.010$) at day 30 in coconut oil compared with BSFL oil.

Table 5. Effect of dietary oil sources on relative organ weights (g/100 g body weight) in broiler chickens[1].

Item	Experimental diets[2]			SEM	*P* value
	Corn oil	Coconut oil	BSFL oil		
Day 15					
Liver	3.44	3.59	3.44	0.12	0.628
Spleen	0.10	0.11	0.09	0.01	0.547
Pancreas	0.48	0.50	0.50	0.02	0.863
Bursa	0.25	0.24	0.29	0.02	0.213
Abdominal fat	1.12	1.08	1.11	0.08	0.951
Day 30					
Liver	2.83	2.58	2.74	0.08	0.073
Spleen	0.14	0.11	0.14	0.01	0.066
Pancreas	0.30	0.32	0.33	0.02	0.398
Bursa	0.18	0.21	0.21	0.02	0.495
Abdominal fat	1.29	1.34	1.28	0.06	0.799

Abbreviations: BSFL, black soldier fly larvae; SEM, standard error of the means.

[1]All means are average of 10 replicates per treatment.

[2]Experimental diets were produced by adding oils (i.e., corn oil, coconut oil, and black soldier fly larvae oil) into a base diet to reach 50 g/kg of diet.

Fatty Acid Composition of Abdominal Fat

Fatty acid composition of abdominal fat in 15-day-old broiler chickens is shown in Table 10. Significant increase in saturated and monounsaturated fatty acids such as lauric acid, myristic acid, palmitic acid, and palmitoleic acid was noted ($P < 0.05$) in chickens fed either coconut oil or BSFL oil compared with the corn oil. On the other hand, unsaturated fatty acids including oleic acid, linoleic acid, and arachidonic acid were higher ($P < 0.05$) in the corn oil than in coconut oil and BSFL oil. Linolenic acid was highest in BSFL oil and lowest in coconut oil. Fatty acid composition of abdominal fat in 30-day-old broiler chickens is shown in Table 11. Fatty acid composition of abdominal fat in day 30 was comparable to that detected in day 15. Palmitoleic acid was highest ($P = 0.001$) in BSFL oil compared with that in corn oil and coconut oil. Heneicosanoic acid and arachidonic acid were lowest ($P < 0.05$) in coconut oil compared with corn oil and BSFL oil.

Serum Parameters

Dietary fat sources did not affect serum parameters including antioxidant and immune markers at day 15 (Table 12). Coconut oil vs. BSFL oil numerically increased HDL cholesterol by 18.5%, but the difference was not disclosed between treatments. At 30 D, BSFL oil lowered ($P < 0.05$) HDL cholesterol and total cholesterol in serum samples compared with the coconut oil. Dietary BSFL oil vs. corn oil increased ($P = 0.034$) TAC. However, dietary fat sources did not affect serum levels of GPT, GOT, triglyceride, uric acid, nitric oxide, and MDA.

DISCUSSION

The present study aimed to evaluate BSFL oil as a fat source or a functional feed supplement to modify fatty acid composition and physiological responses in broiler chickens. Edible insects have been acknowledged in human and animal nutrition because of their nature being effectively converting organic materials present in food wastes to biologically available body composition (e.g., protein, fats) (Meneguz et al., 2018). Among the edible insects, black soldier fly has been most studied as it has advantages such as positive Ca:P ratio and higher

Table 6. Effect of dietary oil sources on meat characteristics in broiler chickens[1].

Item	Experimental diets[2]			SEM	*P* value
	Corn oil	Coconut oil	BSFL oil		
Breast meat					
Weight, g/100 g BW	7.08	6.87	7.19	0.44	0.870
Fresh weight, g	100.19	102.02	105.98	6.94	0.835
pH	5.70[a]	5.64[b]	5.73[a]	0.02	0.022
L* (lightness)	48.87	48.77	47.98	0.60	0.522
a* (redness)	3.20	4.24	4.23	0.42	0.153
b* (yellowness)	14.73[b]	17.30[a]	17.07[a]	0.59	0.009
Cooking loss, %	21.15	23.14	20.98	1.10	0.319
Leg meat					
Weight, g/100 g BW	6.93	6.98	6.72	0.11	0.204
Fresh weight, g	98.04	104.2	99.05	2.92	0.296
pH	6.03	6.07	6.11	0.05	0.559
L* (lightness)	51.59	52.47	52.19	0.92	0.793
a* (redness)	9.67	9.26	8.97	0.55	0.669
b* (yellowness)	18.04	19.29	19.07	0.50	0.184
Cooking loss, %	28.72	28.06	27.95	0.90	0.810

[a-b]Means without a common superscript letter differ ($P < 0.05$).

Abbreviations: BSFL, black soldier fly larvae; BW, body weight; SEM, standard error of the means.

[1]All means are average of 10 replicates per treatment.

[2]Experimental diets were produced by adding oils (i.e., corn oil, coconut oil, and black soldier fly larvae oil) into a base diet to reach 50 g/kg of diet.

amounts of minerals (Finke, 2015). It has been reported that BSFL contain on average 40.8 ± 3.8% protein, 28.6 ± 8.6% fat, 7.0% fiber, 20.6 ± 6.0% ash, 6 to 8% lysine, 5 to 8% Ca, and 0.6 to 1.5% P (Makkar et al., 2014; Wang and Shelomi, 2017). In addition to protein and fat, BSFL and its oil contain biological active components that exhibit antioxidant, antimicrobial, and immune-modulating properties in animals (Mlcek et al., 2014; Zdybicka-Barabas et al., 2017; Lee et al., 2018; Vogel et al., 2018; Rabani et al., 2019). BSFL oil contains medium-chain fatty acids, being lauric acid dominant, which are known to exhibit antibacterial activity (Zeitz et al., 2015; Schiavone et al., 2017), modulate lipid metabolism (Taulescu et al., 2010; Khatun et al., 2018), and improve growth performance in livestock and chickens (Schiavone et al., 2018). It is thus expected that dietary BSFL oil, as a source of functional fatty acids, would affect lipid metabolism including alteration in fatty acid composition as well as improve performance and health of the chicken, which prompted us to test those expectation.

Table 7. Effects of dietary oil sources on *Clostridium perfringens* counts (log cfu/g digesta) in broiler chickens[1].

Item	Experimental diets[2]			SEM	*P* value
	Corn oil	Coconut oil	BSFL oil		
Ileum					
day 15	5.38	5.07	5.84	0.25	0.196
day 30	5.66	5.63	4.89	0.35	0.220
Cecum					
day 15	6.89	6.93	6.75	0.14	0.635
day 30	7.72	7.51	7.63	0.11	0.384

Abbreviations: BSFL, black soldier fly larvae; SEM, standard error of the means.

[1]All means are average of 10 replicates per treatment.

[2]Experimental diets were produced by adding oils (i.e., corn oil, coconut oil, and black soldier fly larvae oil) into a base diet to reach 50 g/kg of diet.

Substituting corn oil with coconut oil or BSFL oil did not affect daily feed intake but nonsignificantly increased body weight gain by on average 5.8 and 6.0% and improved feed conversion ratio by on average 7.6 and 5.7%. It is thus clear from this study that moderate, but not significant, increase in daily weight gain by coconut oil or BSFL oil is not related to concomitant increase in feed intake. Our observation that fat sources did not affect feed intake corroborates with earlier findings

Table 8. Effect of dietary oil sources on concentrations (mM/g digesta) of ileal short-chain fatty acids in broiler chickens[1].

Item	Experimental diets[2]			SEM	*P* value
	Corn oil	Coconut oil	BSFL oil		
Day 15					
Acetate	3.56	3.61	4.54	0.47	0.275
Propionate	0.71	0.77	0.72	0.05	0.686
Isobutyrate	0.48	0.47	0.53	0.03	0.374
Butyrate	0.45	0.52	0.79	0.12	0.127
Isovalerate	0.40	0.39	0.48	0.04	0.249
Valerate	0.37	0.35	0.42	0.02	0.074
Lactate	0.46	0.50	0.61	0.08	0.381
BCFA[3]	1.25[b]	1.21[b]	1.43[a]	0.06	0.034
SCFA[3]	6.43	6.61	8.09	0.53	0.074
Day 30					
Acetate	4.49	6.79	4.69	0.88	0.146
Propionate	1.20[a,b]	1.30[a]	0.93[b]	0.10	0.042
Isobutyrate	0.64	0.51	0.61	0.07	0.350
Butyrate	0.59	0.52	0.51	0.04	0.359
Isovalerate	0.37	0.38	0.37	0.02	0.896
Valerate	0.40	0.34	0.36	0.03	0.232
Lactate	0.39	0.35	0.37	0.04	0.777
BCFA[3]	1.41	1.23	1.34	0.08	0.261
SCFA[3]	8.07	10.17	7.84	0.92	0.162

[a-b]Means without a common superscript letter differ ($P < 0.05$).

Abbreviations: BCFA, branched-chain fatty acid; BSFL, black soldier fly larvae; SCFA, short-chain fatty acid; SEM, standard error of the means.

[1]All means are average of 10 replicates per treatment.

[2]Experimental diets were produced by adding oils (i.e., corn oil, coconut oil, and black soldier fly larvae oil) into a base diet to reach 50 g/kg of diet.

[3]BCFA (isobutyrate + valerate + isovalerate); SCFA (acetate + propionate + butyrate + isobutyrate + isovalerate + valerate + lactate).

Table 9. Effect of dietary oil sources on concentrations (mM/g digesta) of cecal short-chain fatty acids in broiler chickens[1].

Item	Experimental diets[2]			SEM	*P* value
	Corn oil	Coconut oil	BSFL oil		
Day 15					
Acetate	46.45	40.96	47.34	2.92	0.262
Propionate	5.17	4.66	4.84	0.49	0.754
Isobutyrate	0.50	0.67	0.58	0.05	0.099
Butyrate	21.27	20.66	21.73	2.56	0.958
Isovalerate	0.56	0.82	0.68	0.10	0.189
Valerate	1.00	0.96	0.82	0.09	0.336
Lactate	0.51	0.58	0.41	0.08	0.334
BCFA[3]	2.06	2.44	2.08	0.17	0.228
SCFA[3]	75.46	69.30	76.40	4.69	0.517
Day 30					
Acetate	69.85	74.07	71.44	6.26	0.891
Propionate	10.20[a,b]	7.43[b]	11.88[a]	0.96	0.010
Isobutyrate	1.01	1.05	1.01	0.09	0.925
Butyrate	15.48	20.51	15.38	2.24	0.198
Isovalerate	1.15	1.07	1.24	0.15	0.724
Valerate	1.30	1.42	1.47	0.15	0.711
Lactate	0.50	0.44	0.37	0.08	0.564
BCFA[3]	3.45	3.54	3.72	0.32	0.836
SCFA[3]	99.48	106.00	102.79	8.33	0.859

[a-b]Means without a common superscript letter differ ($P < 0.05$).

Abbreviations: BCFA, branched-chain fatty acid; BSFL, black soldier fly larvae; SCFA, short-chain fatty acid; SEM, standard error of the means.

[1]All means are average of 10 replicates per treatment.

[2]Experimental diets were produced by adding oils (i.e., corn oil, coconut oil, and black soldier fly larvae oil) into a base diet to reach 50 g/kg of diet.

[3]BCFA, (isobutyrate + valerate + isovalerate); SCFA, (acetate + propionate + butyrate + isobutyrate + isovalerate + valerate + lactate).

(Poorghasemi et al., 2013; Khatun et al., 2018) that feed intake was not altered in chickens fed different fat sources in diets. It is tentatively concluded that medium-chain fatty acid–rich fats (e.g., coconut oil and BSFL oil) vs. corn oil could facilitate improvement in nutrient digestion and absorption or altered body composition in chickens (Baltić et al., 2017; Spranghers et al., 2017) as manifested by improved feed conversion ratio. In contrast to our finding, no apparent effect of dietary insect oils or fats rich in medium-chain fatty acids on growth performance in broiler chickens was reported (Shokrollahi et al., 2014; Wang et al., 2015; Zeitz et al., 2015; Schiavone et al., 2017). A clear explanation on discrepancy between the previous results and our present study is not readily available. Difference in age, breed, facility, and diet composition in addition to the large variation in fatty acid compositions of insect oils (Danieli et al., 2019; Gao et al., 2019; Kawasaki et al., 2019) may in part explain the discrepancies.

The positive association between body weight and intestinal weight and length is reported (Yang et al., 2013), emphasizing the important role of intestine in digestion and absorption in poultry (Ferrer et al., 2003; Parsaie et al., 2007; Aziza et al., 2014). The latter is postulated as dietary fats can influence lipid composition of the brush border membrane of the jejunum and the nutrient transporters present in the membrane (Ferrer et al., 2003), which prompted us to measure intestinal parameters. Different fat sources did not significantly affect intestinal length and weight, and the weight-to-length ratio in all ages except for ileal parameter. At day 30, coconut oil did not affect ileal length but lowered ileal weight without affecting the weight-to-length ratio. On the other hand, BSFL oil did not affect ileal weight and length but significantly increased the weight-to-length ratio. Our study shows that fats rich in medium-chain fatty acids vs. corn oil affected distal intestine at later days. Further studies are warranted to confirm whether fats rich in medium-chain fatty acids would affect the microstructures of each segment of the small intestine. Contrary results were reported as to the intestinal development. For

Table 10. Effect of dietary oil sources on fatty acid composition (% of total fatty acid methyl esters) of abdominal fat in 15-day-old broiler chickens[1].

Fatty acid	Experimental diets[2]			SEM	*P* value
	Corn oil	Coconut oil	BSFL oil		
Lauric acid (C12:0)	0.36[c]	15.70[a]	11.44[b]	0.30	<0.001
Myristic acid (C14:0)	0.56[c]	7.55[a]	3.80[b]	0.15	<0.001
Palmitic acids (C16:0)	21.07[b]	22.12[a,b]	22.46[a]	0.37	0.032
Stearic acid (C18:0)	4.28	3.95	4.10	0.17	0.410
Heneicosylic acid (C21:0)	0.17[a]	0.07[b]	0.09[b]	0.01	<0.001
Palmitoleic acid (C16:1 ω7)	5.06[b]	7.18[a]	6.97[a]	0.39	0.001
Elaidic acid (C18:1 ω9)	7.39	6.89	6.75	0.67	0.779
Oleic acid (C18:1 ω9)	25.77[a]	21.11[c]	23.42[b]	0.73	0.001
Gondoic acid (C20:1 ω9)	0.20	0.17	0.21	0.02	0.393
Linoleic acid (C18:2 ω6)	32.95[a]	13.92[c]	18.82[b]	0.71	<0.001
Linolenic acid (C18:3 ω3)	1.09[b]	0.69[c]	1.30[a]	0.03	<0.001
Arachidonic acid (C20:4 ω6)	0.19[a]	0.12[c]	0.15[b]	0.01	<0.001
Eicosadienoic acid (C20:2 ω6)	0.33	0.19	0.19	0.06	0.200
Other fatty acids	0.58	0.35	0.32	0.15	0.435
Saturated fatty acids	27.01[c]	49.73[a]	42.21[b]	0.58	<0.001
Monounsaturated fatty acids	38.42	35.35	37.34	0.85	0.050
Polyunsaturated fatty acids	34.57[a]	14.92[c]	20.45[b]	0.75	<0.001

[a-c]Means without a common superscript letter differ ($P < 0.05$).

Abbreviations: BSFL, black soldier fly larvae; SEM, standard error of the means.

[1]All means are average of 10 replicates per treatment.

[2]Experimental diets were produced by adding oils (i.e., corn oil, coconut oil, and black soldier fly larvae oil) into a base diet to reach 50 g/kg of diet.

Table 11. Effect of dietary oil sources on fatty acid composition (% of total fatty acid methyl esters) of abdominal fat in 30-day-old broiler chickens[1].

	Experimental diets[2]				
Fatty acid	Corn oil	Coconut oil	BSFL oil	SEM	*P* value
Lauric acid (C12:0)	0.08[c]	15.43[a]	12.33[b]	1.04	<0.001
Myristic acid (C14:0)	0.45[c]	7.80[a]	4.07[b]	0.53	<0.001
Palmitic acids (C16:0)	19.51	18.24	20.96	1.23	0.324
Stearic acid (C18:0)	3.97	3.48	3.99	0.24	0.269
Heneicosylic acid (C21:0)	0.21[a]	0.12[b]	0.19[a]	0.02	0.019
Palmitoleic acid (C16:1 ω7)	3.84[b]	4.70[b]	6.11[a]	0.39	0.001
Elaidic acid (C18:1 ω9)	10.12[a]	7.14[b]	7.70[b]	0.64	0.007
Oleic acid (C18:1 ω9)	23.01[a]	17.29[b]	21.89[a]	1.19	0.007
Gondoic acid (C20:1 ω9)	0.27[a,b]	0.24[b]	0.32[a]	0.02	0.035
Linoleic acid (C18:2 ω6)	36.50[a]	13.60[c]	20.08[b]	1.00	<0.001
Linolenic acid (C18:3 ω3)	1.13[b]	0.66[c]	1.38[a]	0.04	<0.001
Arachidonic acid (C20:4 ω6)	0.17[a]	0.12[b]	0.17[a]	0.02	0.040
Eicosadienoic acid (C20:2 ω6)	0.40[a]	0.22[b]	0.38[a]	0.04	0.003
Other fatty acids	0.34[a,b]	0.23[b]	0.43[a]	0.05	0.030
Saturated fatty acids	24.55[b]	45.28[a]	41.97[a]	2.95	<0.001
Monounsaturated fatty acids	37.24[a]	29.36[b]	36.02[a]	1.90	0.019
Polyunsaturated fatty acids	38.20[a]	14.61[c]	22.01[b]	1.02	<0.001

[a-c]Means without a common superscript letter differ ($P < 0.05$).

Abbreviations: BSFL, black soldier fly larvae; SEM, standard error of the means.

[1]All means are average of 10 replicates per treatment.

[2]Experimental diets were produced by adding oils (i.e., corn oil, coconut oil, and black soldier fly larvae oil) into a base diet to reach 50 g/kg of diet.

example, dietary mealworms increased intestinal weight (Ballitoc and Sun, 2013), but dietary BSFL did not affect intestinal length and weight in broiler chickens (Cutrignelli et al., 2018). None of internal organs was significantly affected by dietary fat sources. However, coconut oil partially decreased relative liver and spleen weights by 9.1 and 5.9%, respectively, and 21.5 and 25.4% compared with the corn oil and BSFL oil groups in 30-day-old chickens. Nonsignificant, but clear reduction of spleen weight in chickens fed the diet containing coconut oil needs further scrutiny whether it is an indicator of impaired spleen function. In addition, the

Table 12. Effect of dietary oil sources on serum characteristics in broiler chickens[1].

	Experimental diets[2]				
Item	Corn oil	Coconut oil	BSFL oil	SEM	*P* value
Day 15					
GPT, U/L	3.78	4.20	4.10	0.29	0.591
GOT, U/L	130.9	135.6	140.0	4.24	0.353
HDL, mg/dL	81.89	92.89	78.38	4.21	0.063
HDL, % total	67.63	67.68	64.59	1.97	0.493
TCHO, mg/dL	122.0	134.1	122.0	4.13	0.106
TG, mg/dL	230.6	289.0	209.0	33.92	0.273
UA, mg/dL	12.98	11.43	13.59	0.91	0.338
NO, μmol/L	24.75	33.83	19.46	4.47	0.102
TAC, mmol/L	0.78	0.82	0.77	0.06	0.828
MDA, μmol/L	19.02	22.84	18.53	2.79	0.497
Day 30					
GPT, U/L	3.90	3.60	3.40	0.24	0.351
GOT, U/L	180.0	150.5	173.6	14.72	0.344
HDL, mg/dL	58.50[b]	74.10[a]	53.75[b]	4.44	0.012
HDL, % total	61.68	63.83	58.28	2.52	0.386
TCHO, mg/dL	87.25[b]	115.0[a]	97.13[b]	3.97	<0.001
TG, mg/dL	138.4	123.5	117.4	15.81	0.647
UA, mg/dL	10.88	11.06	10.92	0.81	0.987
NO, μmol/L	11.92	12.36	11.21	1.12	0.765
TAC, mmol/L	1.13[b]	1.21[a,b]	1.32[a]	0.05	0.034
MDA, μmol/L	11.37	14.74	10.00	3.11	0.570

[a-b]Means without a common superscript letter differ ($P < 0.05$).

Abbreviations: BSFL, black soldier fly larvae; GPT, glutamic pyruvic transaminase; GOT, glutamic oxaloacetic transaminase; HDL, high-density lipoprotein cholesterol; MDA, malondialdehyde; NO, nitric oxide; SEM, standard error of the means; TAC, total antioxidant capacity; TCHO, total cholesterol; TG, triglyceride; UA, uric acid.

[1]All means are average of 10 replicates per treatment.

[2]Experimental diets were produced by adding oils (i.e., corn oil, coconut oil, and black soldier fly larvae oil) into a base diet to reach 50 g/kg of diet.

decreasing effect of medium-chain fatty acid on abdominal fat (Shokrollahi et al., 2014; Wang et al., 2015) was not observed in this study, which corroborates with previous studies (Rondelli et al., 2004; Potença et al., 2008).

Different fats did not affect thigh meat but affected breast meat traits. Especially, the pH of the breast meat was decreased in chickens fed diet containing coconut oil compared with those fed on corn oil or BSFL oil. Nonetheless, the negative relation between fresh meat lightness and pH (Qiao et al., 2001) was not observed in this study. Of interest, fats rich in medium-chain fatty acids vs. corn oil significantly increased b* values. Increased breast meat yellowness has been postulated with an increase in carotenoid contents (da Silva et al., 2017) or lipid contents (Zhao et al., 2018) in broiler chickens. Nonetheless, all observed values as to pH, meat colors, and cooking loss of breast and thigh meats are within the acceptable range in meat characteristics (Cullere et al., 2019). Analysis of nutrients and fatty acid composition or antioxidant components in breast and leg meats might clearly address those observed different findings on meat quality by fat sources.

In this study, the indicators to evaluate gut health and physiology in response to different fat sources were monitored with *C. perfringens* counts and concentration of SCFAs in addition to intestine development. The genus *Clostridium* includes potentially pathogenic species, such as *C. perfringens*, known to play a role in the development of dysbacteriosis (Ranjitkar et al., 2016), and the viable count of *C. perfringens* was influenced by lipid source (Knarreborg et al., 2002). In addition, it is reported that SCFA or medium-chain vs. long-chain fatty acids exhibit inhibitory activities against intestinal microbiota and alter intestinal SCFAs in livestock (Rinttilä and Apajalahti, 2013; Baltić et al., 2017). In contrast to our expectation, dietary fat sources did not affect *C. perfringens* counts in ileal and cecal digesta at all ages, although BSFL oil vs. corn oil numerically lowered them in ileal, but not cecal, digesta by 13.7% at day 30, showing nonsignificant, moderate antimicrobial activity of BSFL oil in broilers. As to SCFAs, dietary fats affected more ileal SCFAs than cecal SCFAs. In addition, dietary BSFL oil vs. corn oil increased total ileal BCFAs and SCFAs by 14.4 and 25.9%, respectively, at younger ages. It is not clearly understood why BSFL oil–induced increase in ileal BCFAs at day 15 was not noted at day 30. Among SCFAs, propionate was most affected, being lowest in BSFL oil in ileal digesta and in coconut oil in cecal digesta. It is favorably assumed that dietary fats rich in medium-chain fatty acids could affect gut microbiota and their metabolites to some extent although higher ileal concentration of BCFAs and SCFAs, which is considered an indicator of improved gut health, did not coincide with lowering ileal *C. perfringens* counts. In any events, microbiome and metabolomic studies will help to elucidate our not-fully addressed results how dietary fats inconsistently affect *C. perfringens* counts and SCFAs depending on the age of chickens and the segment of intestine.

It is well reported that dietary fats influence the fatty acid composition of meats and adipose tissues in broilers (Crespo and Esteve-Garcia, 2001; Londok et al., 2017; Skřivan et al., 2018), incorporating diet-origin fatty acid into tissues. As expected, fatty acid profiles in the abdominal fat reflected those in dietary fat sources, and no age effect was noted which indicates that fatty acid composition of abdominal fat, once altered, maintained constant throughout the experimental period. BSFL oil significantly increased medium-chain fatty acids (i.e., lauric acid and myristic acid) compared with corn oil and unsaturated fatty acids (i.e., oleic acid, linoleic acid) compared with coconut oil. In addition, it significantly increased linolenic acid content at all ages among fat sources used which are considered beneficial in edible meats being enriched with omega-3 fatty acids. In line with our study, Cullere et al., 2019 reported that increasing BSFL oil significantly increased lauric acid, myristic acid, and palmitic acid contents in tissues of the chicken breast and leg meats. Our study reveals that BSFL oil can be used to effectively incorporate significant amounts of medium-chain fatty acid and moderate amounts of essential fatty acids (e.g., linoleic and linolenic acids) in broiler chickens.

Different fat sources did not affect the indicators of liver functions (i.e., GPT and GOT) (Manterys et al., 2016; Keum et al., 2018), which indicates that BSFL oil did not induce toxic or adverse effect on liver functions. As to serum cholesterol, perplexed results were emerged from this study. It is reported that fats rich in medium-chain vs. long-chain fatty acids are known to decrease total or HDL cholesterol in chickens (Wang et al., 2015; Khatibjoo et al., 2018). In contrast, we found that total and HDL cholesterol was higher by on average 31.8 and 27.0% in coconut oil–fed chickens than corn-oil fed ones. However, BSFL oil did not affect total and HDL cholesterol compared with corn oil. It is not clear how coconut oil, but not BSFL oil, affected cholesterol metabolism which warrants further studies. In line with our study, dietary BSFL oil did not affect blood parameters in chickens (Schiavone et al., 2017), juvenile carp (Li et al., 2016), and rabbits (Gasco et al., 2019). Thus, it seems that BSFL oil acts differently to coconut oil in lipid metabolism although both are rich in medium-chain fatty acids. It should be kept in mind that BSFL oil vs. coconut oil has higher amount of linolenic acid (Table 2) which is known to have hypocholesterolemic effect (Lorenzo et al., 2014). Thus, it may be likely that BSFL oil would disturb or curb medium-chain fatty acid–induced increase in serum cholesterol. As for the antioxidant markers, we found that dietary BSFL oil did not affect serum MDA contents as oxidative stress index but increased serum TAC, an indicator of antioxidant activity, in broiler chickens. It may well be likely that increase in antioxidant capacity in BSFL oil–fed chickens would be in part related to the higher amounts of saturated fatty acids but lesser amounts of

unsaturated fatty acids compared with those fed on corn oil. Alternatively, transfer of insect-origin antioxidant components into the BSFL oil as reported elsewhere (Di Mattia et al., 2019) may explain the observed antioxidant capacity by the insect oil.

In conclusion, dietary fats rich in medium-chain fatty acids (i.e., BSFL and coconut oil) vs. corn oil improved feed conversion ratio in broiler chickens and had no adverse effect on organ weights and intestine development. On the other hands, BSFL and coconut oils were effective in increasing percentages of medium-chain fatty acids in adipose tissue of chickens. Finally, BSFL oil increased breast meat yellowness and altered intestinal SCFAs and serum parameters. Collectively, our study suggests that BSFL oil can be used as the functional fat ingredient to enrich medium-chain fatty acids in edible tissues, affect gut health, and increase antioxidant capacity in broiler chickens.

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