

Effects of Dietary Supplementation of Black Soldier Fly (*Hermetia illucens*) Larvae Oil on Broiler Health

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Insects are a potential source of proteins and fats which can be incorporated into diets of broiler chickens. Accordingly, black soldier fly larvae oil (BSFLO) needs to be tested as an appropriate fat source to produce healthy chickens for consumers. Therefore, the objective of the present study was to evaluate the effects of the replacement of soybean oil (SBO) with BSFLO in broiler diets on intestinal health and blood profiles. A total of 210 one-day-old male broilers were randomly allocated to three dietary treatments (10 replicates of seven birds per group): a control diet and two experimental diets in which SBO was replaced with 50% (50 BSFLO) or 100% (100 BSFLO) BSFLO. At the end of the study (35 days), 18 birds (six broilers per treatment) were slaughtered to determine the intestinal morphology, digestibility, and volatile fatty acid (VFA) profile. Blood samples were collected from 24 randomly selected birds (eight broilers per treatment) to determine the blood profiles. BSFLO supplementation positively affected villus height but did not affect digestibility. BSFLO showed no adverse effects on the VFA and blood profiles. In conclusion, the results of this study suggest that SBO can be replaced by BSFLO without any adverse effects on broiler health.

Key words: blood profile, digestibility, morphology, soybean oil, volatile fatty acid

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Introduction

According to an investigation, the world population will reach 9.1 billion by 2050, with concomitant issues related to food and feed (Wise, 2013). There is already competition to secure food and feed, such as soybean, which is commonly used as an animal feed. However, the high price and limited supply of soybean are major problems which intensify as the competition with human demand increases (Schiafone *et al.*, 2017b). In addition, managing organic waste resulting from the increase in the human population is another problem (Surendra *et al.*, 2016). To manage such waste, insects are efficient converters of organic waste to biofuel (Makkar *et*

al., 2014; Surendra *et al.*, 2016; Spranghers *et al.*, 2017) and have consequently been investigated as a promising solution to many global concerns (Newton *et al.*, 2005).

Black soldier fly (BSF, *Hermetia illucens*) larvae have the ability to decompose organic waste to animal feed as a dietary source (Sheppard *et al.*, 1994; Newton *et al.*, 2005; Surendra *et al.*, 2016). In fact, insects are a natural diet for birds, and BSF larvae (BSFL) contain 42% protein and 35% fat sources in their body to nourish them during the pupal and adult stages (Newton *et al.*, 2005; Lock *et al.*, 2016). The fatty acid (FA) composition of BSFL reflects the FA composition of their feed, and these larvae predominantly comprise saturated fatty acids (SFAs), such as lauric and palmitic acids (Makkar *et al.*, 2014; Cullere *et al.*, 2019; Kim *et al.*, 2020a).

The effects of using different FAs (saturated vs. unsaturated) from various sources of oil have been reported; as such, their combination showed positive effects on the intestinal morphology, nutrient digestibility, and serum lipid profile without negative effects on growth performance and meat quality (Khatun *et al.*, 2018). Therefore, the use of SFA-rich BSFL oil (BSFLO) with unsaturated fatty acid

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(UFA)-rich soybean oil (SBO) may positively affect the intestinal morphology and blood profile. Recently, Kim *et al.* (2020b) reported that dietary BSFLO had no negative effect on short-chain fatty acids (SCFAs) in cecal and ileal digesta. However, limited information is available regarding the effects of BSFLO on volatile fatty acids (VFAs).

The use of BSFLO as an alternative to SBO in broiler diets has been studied, and this replacement has been reported to show no negative effects on growth performance. Moreover, it is a valuable alternative dietary fat source to support productivity (Schiaivone *et al.*, 2018; Cullere *et al.*, 2019; Kim *et al.*, 2020b). Although BSFLO is a promising alternative to SBO, further research is needed to investigate the effects of BSFLO substitution on the overall health of broilers, so that consumers can be assured of healthy chicken meat. Therefore, the objective of the present study was to evaluate whether BSFLO is an appropriate fat source for broilers.

Materials and Methods

Birds, Husbandry, and Diets

This study was conducted at the poultry facility of the National Institute of Animal Science of South Korea and was approved by the Institutional Animal Care and Use Committee of the Rural Development Administration (No. NIAS-

2019-1710). A total of 210 one-day-old male broiler chicks (Ross 308) were randomly allocated to three dietary treatments (10 replicates of seven birds per group). On day 21, the chicks were randomly allocated to individual cages with a feeder and drinker. The diets provided included a control diet (CON) based on corn and soybean meal (SBM), and an experimental diet with partial (50%) or total (100%) replacement of SBO with BSFLO (50 BSFLO and 100 BSFLO diets, respectively). The experimental diets were provided in three phases: starter (days 1 to 7), grower (days 7 to 21), and finisher (days 21 to 35) phases. Chromic oxide (Cr_2O_3) was added to all diets at a dose of $2\text{ g}\cdot\text{kg}^{-1}$ as an indigestible marker to determine ileal digestibility. The diets were formulated to meet or exceed the minimum NRC (1994) requirements for the three phases (Table 1). The chicks had *ad libitum* access to feed and water throughout the trial. Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated for all experimental periods (data not shown).

FA Profile of Diets

Lipid extraction from the experimental diets was performed using the chloroform:methanol (1:2) method. The samples were transmethylated using a methanolic solution of H_2SO_4 (4%) to determine fatty acid methyl esters (FAMES)

Table 1. Composition (%) of the experimental broiler diets containing different levels of black soldier fly larvae oil (BSFLO)

Item	Starter			Grower			Finisher		
	CON	50 BSFLO	100 BSFLO	CON	50 BSFLO	100 BSFLO	CON	50 BSFLO	100 BSFLO
Ingredients (%)									
Corn	53.05	53.05	53.05	59.00	59.00	59.00	61.80	61.80	61.80
Soybean meal (44%)	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Corn gluten meal	6.35	6.35	6.35	4.00	4.00	4.00	1.60	1.60	1.60
Wheat bran	3.15	3.15	3.15	—	—	—	—	—	—
Soybean oil	3.00	1.50	—	3.00	1.50	—	3.00	1.50	—
BSFLO	—	1.50	3.00	—	1.50	3.00	—	1.50	3.00
Dicalcium phosphate	1.75	1.75	1.75	1.50	1.50	1.50	1.45	1.45	1.45
Limestone	1.25	1.25	1.25	1.15	1.15	1.15	1.05	1.05	1.05
Vitamin-mineral premix ¹⁾	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-lysine (78%)	0.40	0.40	0.40	0.25	0.25	0.25	0.10	0.10	0.10
DL-methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.15	0.15	0.15
Salt	0.35	0.35	0.35	0.40	0.40	0.40	0.35	0.35	0.35
Calculated composition									
Metabolizable energy ($\text{kcal}\cdot\text{kg}^{-1}$)	3,031	3,031	3,031	3,106	3,106	3,106	3,152	3,152	3,152
Methionine (%)	0.53	0.53	0.53	0.50	0.50	0.50	0.43	0.43	0.43
Lysine (%)	1.42	1.42	1.42	1.26	1.26	1.26	1.11	1.11	1.11
Calcium (%)	0.96	0.96	0.96	0.91	0.91	0.91	0.86	0.86	0.86
Total phosphorus (%)	0.77	0.77	0.77	0.70	0.70	0.70	0.65	0.65	0.65
Analyzed composition									
Crude protein (%)	24.63	24.48	23.28	22.15	22.77	23.14	22.71	22.03	21.37
Crude fat (%)	3.63	5.39	5.93	6.76	7.51	7.70	4.68	4.44	5.22

CON=control diet; 50 BSFLO and 100 BSFLO=BSFLO groups in which the soybean oil was replaced by 50% and 100% of the black soldier fly larvae oil, respectively.

¹⁾Supplied per kilogram of diet: vitamin A, 1,600,000 IU; vitamin D₃, 300,000 IU; vitamin E, 800 IU; vitamin K₃, 132 mg; vitamin B₁, 97 mg; vitamin B₂, 500 mg; vitamin B₆, 200 mg; vitamin B₁₂, 1.2 mg; nicotinic acid, 2,000 mg; pantothenic acid, 800 mg; folic acid, 60 mg; choline chloride, 35,000 mg; Mn, 12,000 mg; Zn, 9,000 mg; Fe, 4,000 mg; Cu, 500 mg; I, 250 mg; Co, 100 mg; Se, 50 mg.

Table 2. Fatty acid profile (% of total fatty acid methyl esters) of the experimental diets

Item	Starter			Grower			Finisher		
	CON	50 BSFLO	100 BSFLO	CON	50 BSFLO	100 BSFLO	CON	50 BSFLO	100 BSFLO
C12:0 (Lauric)	0.15	17.08	26.23	0.23	17.32	26.48	1.13	18.15	27.39
C14:0 (Myristic)	0.14	2.17	3.39	0.18	2.27	3.52	0.19	2.31	3.63
C16:0 (Palmitic)	9.87	11.86	12.72	10.40	12.58	13.86	10.78	12.65	13.97
C16:1	0.11	1.38	1.83	0.13	1.58	2.01	0.12	1.62	2.08
C18:0 (Stearic)	2.74	2.61	2.49	3.02	2.85	2.73	3.15	2.97	2.79
C18:1 n-9 (Oleic)	1.85	6.72	12.85	2.17	7.87	13.28	2.24	8.13	13.73
C18:2 n-6 (Linoleic)	55.48	30.68	13.91	57.5	32.53	14.78	56.23	32.26	14.92
C18:3 n-3 (Linolenic)	4.96	3.28	2.57	5.18	3.08	1.59	5.26	3.21	1.64
SFA	12.90	33.72	44.83	13.83	35.02	46.59	15.25	36.08	47.78
MUFA	1.96	8.10	14.68	2.30	9.45	15.29	2.36	9.75	15.81
PUFA	60.44	33.96	16.48	62.68	35.61	16.37	61.49	35.47	16.56
SFA:UFA	0.20	0.80	1.43	0.21	0.77	1.47	0.23	0.79	1.47

CON=control diet; 50 BSFLO and 100 BSFLO=BSFLO groups in which the soybean oil was replaced by 50% and 100% of the black soldier fly larvae oil, respectively; SFA=saturated fatty acid; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; UFA=unsaturated fatty acid.

using gas chromatography (7890A; Agilent Technologies, Wilmington, DE, USA). The results were expressed as a percentage (%) of total detected FAMES. The FA composition of the diets is presented in Table 2.

Digestibility Trial and Calculation

At the end of the study (35 days), 54 birds (18 broilers per treatment) were selected based on the average final body weight (BW) and slaughtered by cutting the carotid artery. The intestinal tract was excised, and the ileum (from Meckel's diverticulum to the ileocolic junction) was flushed to remove the contents. The ileal digesta from three birds was pooled into a sample ($n=18/\text{treatment}$) and collected to obtain sufficient samples for determining apparent ileal digestibility (AID) of dry matter (DM), crude protein (CP), ether extract (EE), and gross energy.

Chemical analyses of DM, CP, EE, and energy were based on previously described methods (AOAC, 2005). Frozen ileal digesta was freeze-dried and then ground through a 1 mm screen (Cyclotec 1093; Foss Tecator AB, Hoganas, Sweden) before chemical analysis. The diets and ileal digesta samples were analyzed for DM and the moisture content was measured by drying the samples at 105°C for 24 h (AOAC, 2005). In diets and ileal digesta samples, the energy was measured by bomb calorimetry (Parr 6300; Parr Instrument, Moline, USA) and CP of the samples was analyzed according to the Kjeldahl method (VAPO45; Gerhardt Ltd., Germany). Cr_2O_3 concentration of the diets and ileal digesta samples was determined by graphite furnace atomic absorption spectrometry. The AID of DM, CP, EE, and energy was calculated for each diet based on a previously described method (Equation 1) (Stein *et al.*, 2007).

$$\text{AID} = \left[1 - \left(\frac{\text{Nutrient}_{\text{digesta}}}{\text{Nutrient}_{\text{diet}}} \right) \times \left(\frac{\text{Cr}_{\text{diet}}}{\text{Cr}_{\text{digesta}}} \right) \right] \times 100, \quad (1)$$

where AID represents the AID of each nutrient. $\text{Nutrient}_{\text{digesta}}$ is the amount of nutrients in the ileal digesta, $\text{Nutrient}_{\text{diet}}$ is

the amount of nutrients in the diet, Cr_{diet} is the Cr_2O_3 concentration of the diet, and $\text{Cr}_{\text{digesta}}$ is the Cr_2O_3 concentration of the ileal digesta.

Intestinal Morphology

Intestinal morphology was determined based on the methods of Shen *et al.* (2009). Briefly, 4 cm segments of the central part of the ileum (six broilers per treatment) were washed and fixed in 10% buffered formalin. The fixed samples were dehydrated and embedded in paraffin. The samples were cut into transverse sections (three per sample) of 5 μm thickness using a rotary microtome (Leica RM 2245, Tokyo, Japan) and fixed on a glass slide. The slides were then stained with hematoxylin and eosin and scanned (NanoZoomer Digital Pathology System; Hamamatsu Co., Bridgewater, NJ). All measurements were conducted using the associated Nano Zoomer Digital Pathology (NDP) slide-viewing software. Fifteen straight and integrated villi (five per section) and their associated crypts were selected and measured. The intestinal morphological measurements included villus height (VH), crypt depth (CD), villus width, and villus area. The captured images were analyzed using ImageJ software (National Institute of Health; Bethesda, MD, USA) and the average values of the measurements were calculated for each bird.

VFA Analysis

At 35 days, after slaughter, the cecal digesta samples were collected and stored at -80°C until analysis. VFA concentrations were determined by gas chromatography (6890N; Agilent Technologies; Waldbronn, Germany) using a capillary column (15 m \times 0.53 mm \times 0.5 μm ; Supelco Inc., Bellefonte, PA, USA) according to the method described by Metzler-Zebeli *et al.* (2015). Approximately 1 g of cecal digesta was thawed and diluted with 1 mL of distilled water, and the resulting solution was centrifuged. After centrifugation (10 min at 5,000 $\times g$), the supernatant was transferred, and 200 μL of 25% metaphosphoric acid was added. The

samples were centrifuged (10 min at $5,000\times g$) and the clear supernatant was analyzed for the concentrations of acetate, propionate, butyrate, and valerate.

Hematological Analysis

Blood samples were collected from 24 birds (eight broilers per treatment) and placed in serum-separating tubes. The tubes were left to clot at room temperature for 1 h to obtain the serum and then centrifuged at $1,800\times g$ for 15 min. The serum samples were frozen at -80°C until analysis. Glucose, cholesterol, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), amylase, lipase, total protein, albumin, globulin, calcium, phosphorus, and total bilirubin were measured using a chemistry analyzer (Catalyst Dx, IDEXX Labs Inc., Westbrook, USA).

Statistical Analysis

Statistical analysis of data was performed using SAS, version 9.4 (SAS, 2009). For AID data, a pooled ileal digesta sample from three birds was considered as an experimental unit (replicate) for statistical analysis. For intestinal morphology, VFA concentrations, and blood parameters, the experimental unit was an individual bird. The data were analyzed using a general linear model (GLM). The results are presented as mean \pm standard error of the mean (SEM). Statistical differences among treatments were determined using Tukey's multiple comparison test (SAS, 2009). Significance and tendency were declared at $P < 0.05$ and $0.05 < P < 0.10$, respectively.

Results

Composition and FA Profile of the Experimental Diets

The experimental diets had similar nutrient compositions (Table 1), but they substantially differed in their FA profiles (Table 2). The concentration of SFAs in the diets increased but that of total UFAs decreased with BSFLO supplementation. With an increase in the proportion of BSFLO, the concentration of lauric acid (C12:0) increased. However, the concentration of linoleic acid (C18:2 n-6) was lower in the 100 BSFLO group than in the CON group.

Intestinal Morphology and Digestibility

As presented in Table 3 and Fig. 1, BSFLO substitution improved the microscopic structure of the ileum. Partial replacement (50%) of SBO with BSFLO increased the VH compared with the other treatments ($P < 0.05$). However, no significant differences were observed in the CD, VH:CD, villus width, and villus area among the treatments. The AID of DM, CP, and energy was not significantly different among the treatments (Table 4). However, the AID of EE tended to be higher in the 50 BSFLO group than in the CON group ($P < 0.10$).

VFA Profile

Dietary BSFLO supplementation did not significantly influence the absolute VFA concentration (Table 5). However, the relative concentration of acetate decreased ($P < 0.05$) and that of butyrate increased with BSFLO supplementation ($P < 0.01$). There were no significant differences in the relative concentrations of propionate and valerate.

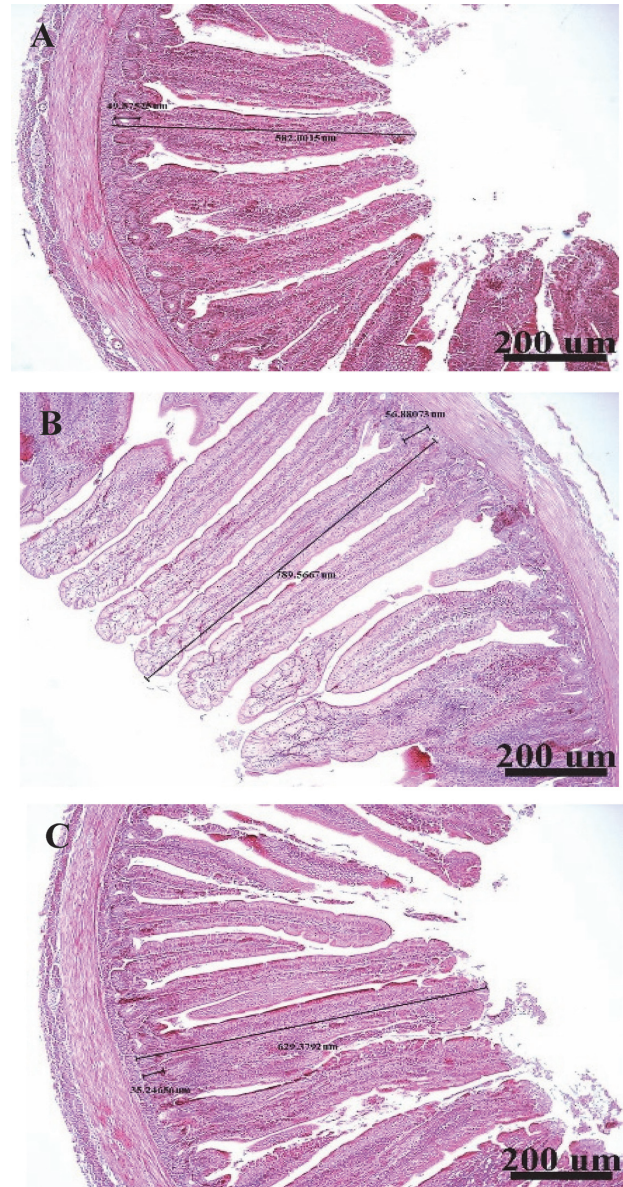


Fig. 1. Morphological observation of the villi in the ileum of broilers in the (A) CON, (B) 50 BSFLO, and (C) 100 BSFLO groups. CON=control diet; 50 BSFLO and 100 BSFLO=BSFLO groups in which the soybean oil was replaced by 50% and 100% of the black soldier fly larvae oil, respectively.

Hematological Parameters

The effects of BSFLO supplementation on blood parameters are summarized in Table 6. BSFLO supplementation tended to increase ($P < 0.10$) the level of cholesterol. The concentration of lipase was significantly higher ($P < 0.05$) in the CON group than in the 50 and 100 BSFLO groups. However, no significant differences were observed in the other parameters among treatments.

Table 3. Effects of dietary black soldier fly larvae oil supplementation level on the ileal morphology of broiler chickens at 35 days of age

Item	Dietary treatments			SEM	P value
	CON	50 BSFLO	100 BSFLO		
Villus height (μm)	653.75 ^b	774.20 ^a	698.88 ^{ab}	29.13	0.032
Crypt depth (μm)	51.00	56.96	58.46	5.69	0.626
Villus height:Crypt depth	13.32	14.14	12.64	1.22	0.692
Villus width (μm)	106.63	100.87	88.46	8.76	0.351
Villus area (μm^2)	58863.09	59054.36	52820.49	5634.85	0.679

SEM=standard error of the mean; CON=control diet; 50 BSFLO and 100 BSFLO=BSFLO groups in which the soybean oil was replaced by 50% and 100% of the black soldier fly larvae oil, respectively. Values with different superscripts (a and b) in the same row are significantly different ($P<0.05$).

Table 4. Apparent ileal digestibility of nutrients in broiler diets supplemented with black soldier fly larvae oil

Item	Dietary treatments			SEM	P value
	CON	50 BSFLO	100 BSFLO		
Dry matter (%)	63.65	64.96	61.71	1.27	0.270
Crude protein (%)	59.31	57.56	62.40	1.42	0.126
Ether extract (%)	77.17	81.58	78.87	1.19	0.098
Energy (%)	63.47	65.60	66.15	1.36	0.396

SEM=standard error of the mean; CON=control diet; 50 BSFLO and 100 BSFLO=BSFLO groups in which the soybean oil was replaced by 50% and 100% of the black soldier fly larvae oil, respectively.

Table 5. Volatile fatty acid (VFA) levels in the cecal contents of broiler chickens

Parameter	Dietary treatments			SEM	P value
	CON	50 BSFLO	100 BSFLO		
Absolute value ($\text{mmol}\cdot\text{L}^{-1}$)					
Acetate	94.13	83.55	90.94	5.13	0.377
Propionate	6.61	5.85	5.48	0.66	0.522
Butyrate	20.82	22.07	25.03	1.65	0.235
Valerate	1.78	1.75	1.59	1.12	0.554
Total VFAs	123.00	113.24	123.05	6.89	0.535
Relative value (% of total VFAs)					
Acetate	76.59 ^a	73.71 ^b	73.96 ^b	0.62	0.015
Propionate	5.43	5.22	4.43	0.57	0.464
Butyrate	16.85 ^b	19.48 ^a	20.29 ^a	0.59	0.004
Valerate	1.38	1.57	1.31	0.10	0.257

SEM=standard error of the mean; CON=control diet; 50 BSFLO and 100 BSFLO=BSFLO groups in which the soybean oil was replaced by 50% and 100% of the black soldier fly larvae oil, respectively. Values with different superscripts (a and b) in the same row are significantly different ($P<0.05$).

Discussion

Lauric acid belongs to the family of medium-chain fatty acids (MCFAs), composed of six to 12 carbon atoms. It is commonly found in coconut oil in the triglyceride form (Suzuki, 2013). MCFAs show antimicrobial effects against gut microbiota and other pathogens by assisting in the infiltration of antimicrobial compounds into the cytoplasm (Kim and Rhee, 2016; Spranghers *et al.*, 2018). It has also

been shown that natural antibiotics in BSFL can be used as an alternative for improving gut health (Sheppard *et al.*, 2007; Zeitz *et al.*, 2015; Spranghers *et al.*, 2018).

In the present study, the substitution of SBO with BSFLO in a broiler diet showed positive effects on intestinal morphology. Intestinal morphology is related to the absorptive capacity of nutrients in the small intestine. Villus atrophy and deep crypts can lead to poor nutrient absorption and, consequently, poor growth performance (Xu *et al.*, 2003;

Table 6. Effects of dietary black soldier fly larvae oil supplementation level on the blood parameters of broiler chickens

Parameter	Dietary treatments			SEM	P value
	CON	50 BSFLO	100 BSFLO		
Glucose (mg·dL ⁻¹)	267.50	252.75	263.50	9.25	0.517
Cholesterol (mg·dL ⁻¹)	75.37	90.25	95.00	5.85	0.068
Alkaline phosphatase (U·L ⁻¹)	375.50	314.37	393.87	56.49	0.589
Gamma-glutamyltransferase (U·L ⁻¹)	10.00	10.50	11.37	1.56	0.821
Amylase (U·L ⁻¹)	297.87	266.25	277.50	37.75	0.836
Lipase (U·L ⁻¹)	185.57 ^a	111.00 ^b	122.50 ^b	19.89	0.042
Total protein (g·dL ⁻¹)	2.96	3.01	2.98	0.11	0.954
Albumin (g·dL ⁻¹)	1.25	1.27	1.27	0.03	0.859
Globulin (g·dL ⁻¹)	1.71	1.73	1.71	0.08	0.971
Albumin:Globulin	0.72	0.73	0.76	0.02	0.588
Calcium (mg·dL ⁻¹)	9.25	9.26	9.32	0.26	0.976
Phosphorus (mg·dL ⁻¹)	6.02	6.51	6.32	0.41	0.711
Total bilirubin (mg·dL ⁻¹)	0.33	0.35	0.32	0.06	0.968

SEM=standard error of the mean; CON=control diet; 50 BSFLO and 100 BSFLO=BSFLO groups in which the soybean oil was replaced by 50% and 100% of the black soldier fly larvae oil, respectively.

Values with different superscripts (a and b) in the same row are significantly different ($P<0.05$).

Oliver *et al.*, 2013). Generally, MCFAs are absorbed more rapidly and hydrolyzed more efficiently than long-chain fatty acids (LCFAs) in the proximal intestine, and they are transported to the liver via the portal blood (Jenkins and Thompson, 1993; Belghit *et al.*, 2019). It was expected that lauric acid in BSFLO may affect intestinal morphology because of its ability to stimulate cell renewal (Zeitz *et al.*, 2015), and it was observed that the 50 BSFLO diet increased VH compared with the CON diet. Several studies have shown that the use of BSFLO did not affect intestinal morphology in monogastric animals (Schiavone *et al.*, 2018; Spranghers *et al.*, 2018). The reason for this is unclear, but MCFAs in BSFLO may be used as an energy source for the proliferation of enterocytes and intestinal mucosa (Zentek *et al.*, 2012; Zeitz *et al.*, 2015). MCFAs are more polar and have a shorter carbon atom chain length than other lipids, and these characteristics also affect their absorption (Zeitz *et al.*, 2015; Belghit *et al.*, 2019). Due to its positive effects on VH, the substitution of BSFLO was expected to affect AID. However, the change in VH did not affect AID. Even though the use of BSFLO positively affected VH, it did not translate to nutrient absorption (Saenphoom *et al.*, 2013).

The use of BSFLO with an elevated proportion of MCFAs in the diets modulated the proportion of acetate and butyrate. As expected, the use of BSFLO did not affect the absolute concentrations of SCFAs. Several studies have reported the effects of BSFL meal (BSFLM) as a protein source in broiler diets (Cuttrignelli *et al.*, 2018; Dabbou *et al.*, 2018; Schiavone *et al.*, 2019). Cuttrignelli *et al.* (2018) reported that the absolute concentrations of acetate, butyrate, and total VFAs were increased by a BSFLM diet; however, BSFLO did not affect the concentration of VFAs in the present study. BSFLM contains an indigestible substance, chitin, as a potential prebiotic, and chitin increases the production of VFAs in the cecum (Cuttrignelli *et al.*, 2018; Kawasaki *et al.*, 2019).

However, in the present study, BSFLO was not expected to change the proportion of acetate and butyrate. The use of coconut oil (which contains high concentrations of MCFAs) increased the butyrate proportion without altering the concentrations of acetate and butyrate as well as the total amount of VFAs compared with the use of other oils with different FA compositions (Dohme *et al.*, 2000; Matsuba *et al.*, 2019). Butyrate is rapidly absorbed by colonocytes as an energy source and is the most important SCFA in terms of the development of intestinal epithelial cells (Ritzhaupt *et al.*, 1998; Pryde *et al.*, 2002; Guilloteau *et al.*, 2010). It remains unclear why the proportions of acetate and butyrate were altered. However, it has been suggested that changes in microbial composition due to the antimicrobial effects of lauric acid or the rapid absorption of MCFAs altered the microbial composition and, consequently, fermentation in the cecum (Dohme *et al.*, 2000; Hanczakowska, 2017; Matsuba *et al.*, 2019).

MCFAs in BSFLO affected the blood parameters by decreasing the concentration of serum lipase. MCFAs have a lower weight and smaller molecular size than LCFAs, facilitating their rapid hydrolysis and absorption into the portal vein (Baltić *et al.*, 2017; Hanczakowska, 2017). Pancreatic enzymes are not necessary for the absorption pathway of MCFAs (Shah and Limketkai, 2017). For this reason, MCFAs are more effectively digested in young animals that have an undeveloped digestive system than older animals (Baltić *et al.*, 2017). MCFAs, with shorter chain lengths, are hydrolyzed faster, and the lipase activity decreases as the proportion of LCFAs decreases (Hanczakowska, 2017). Thus, a high proportion of MCFAs and a low content of LCFAs in BSFLO can be attributed to the decrease in serum lipase concentration.

There was concern that a high level of SFAs with the addition of BSFLO might alter the cholesterol level. How-

ever, substitution with BSFLO did not affect the serum cholesterol concentration, as reported previously (Schiaivone *et al.*, 2017a, 2018). No other study has demonstrated a reduction in cholesterol in chicken meat (Dabbou *et al.*, 2018; Cullere *et al.*, 2019). However, in the present study, cholesterol concentration tended to increase with an increase in BSFLO proportion. Although it is difficult to explain this result, supplementation of a high proportion of SFAs in broiler diets has been shown to increase the cholesterol level in the blood, breasts, and thigh muscles (Duraismy *et al.*, 2013; Khatun *et al.*, 2018). A high proportion of SFAs in BSFLO diets may affect the cholesterol level in the blood.

We observed no negative effects of partial (50%) or total replacement (100%) of SBO with BSFLO on growth performance (data not shown). Moreover, the chickens remained healthy without clinical signs during the experiment, as verified by the blood parameters. The serum levels of total protein, globulin, and albumin are the criteria for evaluating the health of broilers, because these parameters are associated with disease or stress (Gomez-Bautista *et al.*, 1986; Attia *et al.*, 2017). The low albumin-to-globulin ratio also indicates improved disease resistance (Marono *et al.*, 2017). In the present study, these parameters were not affected by any treatment, indicating that BSFLO had no negative effects on broiler health.

In conclusion, the present study showed that the substitution of SBO with BSFLO in broiler diets had positive effects on intestinal morphology. The higher VH in broilers fed the 50 BSFLO diet did not affect AID. The findings of this study indicate that BSFLO can be used to substitute SBO in broiler diets without any adverse effects on VFA levels, hematological characteristics, or health parameters. These findings suggest that BSFLO can be a suitable fat source in broiler diets.

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Conflict of Interest

The authors declare no conflict of interest.

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