Complete replacement of soybean meal with black soldier fly larvae meal in feeding program for broiler chickens from placement through to 49 days of age reduced growth performance and altered organs morphology

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ABSTRACT Black soldier fly larvae meal (BSFLM) is characterized with good nutritional and functional attributes. However, there is limited data on inclusion of BSFLM in broiler chicken rations from placement through to market weight. Therefore, we examined growth and organ responses of partial to complete replacement of soybean meal (SBM) with BSFLM in a practical feeding program. A total of 1,152 d-old male Ross × Ross 708 chicks were allocated to 48 pens and assigned one of six diets (n = 8). The diets were: a basal corn-SBM diet (0%BSFLM), 4 diets in which SBM in 0%BSFLM was replaced with BSFLM at 12.5, 25, 50, and 100% and a final diet (0 + AGP) in which 0%BSFLM was treated with coccidiostat (70 mg Narasin/kg) and antibiotic (55 mg Bacitracin Methylene Disalicylate/kg). For energy fortification, soy oil was used for 0%BSFLM diets and black soldier fly oil in the other diets. Body weight, feed intake (FI), BW gain (BWG), and mortality-corrected feed conversion ratio (FCR) were reported. Organ weights were recorded on d 24 and 49. On d 10, birds fed diets 12.5, 25, and 0 + AGP had higher BWG than birds fed diets 0, 50, and 100 ($P < 0.01$), and birds fed diet 100 had lower BWG than birds fed diets 0 or 50 ($P < 0.01$). Birds fed diets 50 and 100 had lower BWG than birds fed all other diets on d 24 and 49 ($P < 0.05$). Overall (d 0 −49), BSFLM linearly ($P < 0.01$) decreased BW, BWG, and FI and increased FCR and mortality. The overall BWG of 50 and 100% BSFLM birds was 92 and 81% of birds fed 0%BSFLM, repectively and coresponding overall FI was 96 and 90%. An increase in gizzard, small intestine, pancreas, and liver relative weights were observed with increasing BSFLM inclusion ($P < 0.01$). The data indicated that lower levels of BSFLM could provide some growth-promoting effects commensurate to antibiotics in the starter phase. However, replacing SBM with greater amounts (≥50) of BSFLM reduced growth and increased organ size.

Key words: broiler chickens, black soldier fly larvae meal, growth performance, soybean meal replacement

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INTRODUCTION

Black soldier fly larvae meal (**BSFLM**) has been identified as a possible source of protein and energy in broiler chicken diet which can be a replacement for common plant-based proteins, such as soybean meal (**SBM**; Makkar et al., 2014). The BSFLM is a dense source of protein, fat, metabolizable energy (**AME**), phosphorus, and fiber (Mwaniki and Kiarie, 2018). As part of the fiber and fat components, BSFLM also contains high concentrations of chitin and medium-chain fatty acids (**MCFA**), such as lauric and myristic acid, which are thought to improve both gut and immune health in broiler chickens through prebiotic and antimicrobial properties (Dörper et al., 2020). These properties could reduce the reliance on antibiotics and coccidiostats in the poultry industry (Bean-Hodgins and Kiarie, 2021). In addition to the nutritional and functional benefits of BSFLM, its use in broiler feed could help reduce the environmental footprint of poultry feed production and close the gap in a circular food economy (Barrera and Hertel, 2021) as black soldier fly larvae can consume

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food waste and convert it into high-quality protein and energy (Ravi et al., 2020).

However, within the current literature, growth performance responses have been variable for the inclusion of BSFLM in broiler diets. Previous researchers that included BSFLM below ~30% replacement of SBM observed no change or improved growth performance of broilers, including BW, feed intake (**FI**), and feed conversion ratio (**FCR**) (Cullere et al., 2016; Dabbou et al., 2018; Onsongo et al., 2018; Schiavone et al., 2019; de Souza Vilela et al., 2021). However, some studies implementing BSFLM levels greater than ~30% replacement of SBM, more notably with levels above ~50%, resulted in reduced growth performance through lower BW, FI, and FCR as compared to lower inclusion rates (Dabbou et al., 2018; Onsongo et al., 2018; Velten et al., 2018; Schiavone et al., 2019; Murawska et al., 2021). Additionally, results on organ weights and organ histology when BSFLM is included in higher amounts (>50% inclusion) are also variable. Some researchers did not observe any changes in organ weights or organ tissue histology (Onsongo et al., 2018; Velten et al., 2018; Schiavone et al., 2019), whereas Murawska et al. (2021) observed an increase in liver weight when replacing 75% SBM with BSFLM. Changes to organ weight are indicative of further health concerns, and therefore can aid in deducing information on the impact of BSFLM on broiler chickens.

The hypothesis of the study was that broilers will experience a quadratic effect on growth performance where (1) feeding BSFLM will have commensurate growth responses to antibiotic growth promoting (**AGP**) diet via the functional properties of BSFLM and where (2) high levels of BSFLM will depress growth performance due to decreased feed intake. The objective was to examine the influence of partial to complete replacement of SBM with BSFLM in a broiler chicken feeding program from placement through d 49 of age on growth performance and organ weights.

MATERIALS AND METHODS

Animal care and use protocols are approved by the University of Guelph Animal Care and Use Committee (AUP#4403) and birds were cared for in accordance with the Canadian Council on Animal Care guidelines (Canadian Council on Animal Care, 2009).

Birds and Housing

A total of 1,152 d old (male) Ross x Ross 708 broiler chicks were obtained from a commercial hatchery (Maple Leaf Foods, New Hamburg, ON, Canada). The chicks were allocated to 48-floor pens (24 birds per pen) according to hatch weight. The pens were in 4 separate environmentally controlled rooms with 12 pens each, providing 4.27 m^2 of area per pen and fresh wood shavings. The room temperature was set to the breeder recommendation of 32°C on d 0 and gradually decreased to 21°C by d 30 (Aviagen, 2014). Birds were exposed to fluorescent lighting with 23 h of light (20+ lux) for the first 4 d and then 16 h light: 8 dark (10−15 lux) light cycle for the remainder of the experiment in accord with Arkell Poultry Research Station standard operating procedures.

Experimental Diets

Partially defatted BSFLM (12.7% crude fat and 52.5% CP, as-fed) and black soldier fly larvae oil (**BSFLO**) were obtained from a commercial manufacturer and vendor (Enterra feed Corp., Vancouver, BC, Canada). The meal was a dry, ground product derived from black soldier fly (Hermetia illucens) larvae reared on preconsumer recycled food collected from local farms, food processors, and grocery stores. Coefficients for standardized ileal digestible (**SID**) amino acids and AME values for BSFLM were obtained from published literature (de Marco et al., 2015; Schiavone et al., 2017; Mwaniki and Kiarie, 2018) and the other feedstuffs from Evonik Aminodat 5.0 for diet formulation (Evonik Nutrition and Care GmbH., 2016). The AME value of BSFLO was assumed to be equivalent to that of soybean oil (**SBO**) as research has shown no effect on growth performance when SBO is completely replaced with BSFLO (Kim et al., 2020; Heuel et al., 2021). Diets were formulated for a 3-phase feeding program: Starter; d 0 to 10, Grower: d 11 to 24, and Finisher; d 25 to 49 (Tables 1−3) and met or exceeded specifications for Ross x Ross 708 (Aviagen, 2014). In each phase, a 0%BSFLM corn-SBM-SBO basal diet was formulated with no prebiotic, probiotic, anticoccidial, or antimicrobial growth-promoting substances added. Four BSFLM diets were formulated based on the basal diet by replacing SBM with increasing proportions of BSFLM: 12.5, 25, 50, and 100%. The 0%BSFLM diet was fed without or with (0 + AGP) coccidiostat (Monteban 100, 70 mg Narasin/kg, Elanco Canada Ltd, Guelph, ON) and antibiotic growth promoter (BMD 110 G, 55 mg Bacitracin Methylene Disalicylate/kg, Zoetis Canada Inc., Kirkland, QC). The 0%BSFLM diets used SBO and the BSFLM diets used BSFLO for dietary energy fortification. The diets were mixed separately, with flushing between diets and were prepared in mash form. Representative samples of the experimental diets, BSFLM, SBM, BSFLO, and SBO were collected and immediately shipped for chemical analyses and another portion was stored at −20°C for future analyses.

Experimental Procedures

The 6 diets were allocated to 48-floor pens in 4 rooms (12 pens per room) in a completely randomized block (room) design (n = 8) and fed for 49 d. Birds had free access to water and feed throughout the experiment. The BW and FI were recorded at the end of each phase (d 0, 10, 24, and 49) on a pen basis, and mortalities were recorded throughout the trial for calculation of

Table 1. Composition of starter diets, as fed basis.

	Starter phase, d 0−10					
	SBM replacement with BSFLM, %					
Item	0 + AGP[1]	0	12.5	25	50	100
Corn	33.7	33.8	35.8	37.8	42.0	46.5
Wheat	20.0	20.0	20.0	20.0	20.0	20.0
Soybean meal	32.6	32.6	27.4	22.2	11.6	-
Pork meal	4.00	4.00	4.00	4.00	4.00	4.00
BSFLM[2]	-	-	4.10	8.10	16.3	25.3
BSFLO[2]	-	-	4.66	3.80	1.99	-
Soy oil	5.54	5.54	-	-	-	-
L-Lysine HCL	0.35	0.35	0.41	0.48	0.61	0.76
DL-Methionine	0.38	0.38	0.40	0.43	0.48	0.53
L-Threonine	0.18	0.18	0.20	0.22	0.26	0.30
L-Tryptophan	-	-	-	0.01	0.07	0.13
Limestone	0.68	0.68	0.6	0.52	0.36	0.18
Monocalcium phosphate	1.17	1.17	1.16	1.14	1.10	1.06
Salt	0.23	0.23	0.21	0.18	0.14	0.09
Sodium bicarbonate	0.09	0.09	0.10	0.10	0.12	0.13
Vitamin and trace minerals premix[3]	1.00	1.00	1.00	1.00	1.00	1.00
Antibiotic	0.05	-	-	-	-	-
Coccidiostat	0.07	-	-	-	-	-
Total	100	100	100	100	100	100
Calculated provisions						
AMEn, kcal/kg	3,086	3,086	3,086	3,086	3,086	3,086
Crude fat, %	7.70	7.70	7.40	7.20	6.70	6.10
Linoleic acid, %	3.90	3.90	3.50	3.10	2.20	1.20
Crude protein, %	23.0	23.0	23.0	23.0	23.0	23.0
SID[4] Lys, %	1.28	1.28	1.28	1.28	1.28	1.28
SID Met, %	0.67	0.67	0.69	0.70	0.73	0.77
SID Met + Cys, %	0.95	0.95	0.95	0.95	0.95	0.95
Calcium, %	0.96	0.96	0.96	0.96	0.96	0.96
Available P, %	0.48	0.48	0.48	0.48	0.48	0.48
Sodium, %	0.16	0.16	0.16	0.16	0.16	0.16

[1]Coccidiostat (Monteban 100, 70 mg Narasin/kg, Elanco Canada Ltd, Guelph, ON) and antibiotic (BMD 110 G, 55 mg Bacitracin Methylene Disalicylate/kg, Zoetis Canada Inc., Kirkland, QC).

[2]BSFLM; black soldier fly larvae meal, BSFLO; black solider fly larvae oil.

[3]Provided per kg of premix: vitamin A (retinol), 880 KIU; vitamin D_3 (cholecalciferol), 330 KIU; vitamin E, 4,000 IU; vitamin K_3 (menadione), 330 mg; vitamin B_1 (thiamin), 400 mg; vitamin B_2 (riboflavin), 800 mg; vitamin B_3 (niacin), 5,000 mg; vitamin B_5 (pantothenic acid), 1,500 mg; vitamin B_6 (pyridoxine), 300 mg; vitamin B_9 (folic acid), 100 mg; vitamin B_{12} (cyanocobalamin), 1200 mcg; biotin, 200 mcg; choline, 60,000 mg; Fe, 6000 mg; Cu, 1000 mg; I, 1 mg, Se, 30 mg.

[4]SID: standardized ileal digestible.

mortality corrected FCR. The BWG was calculated from recorded BW on a pen basis at the end of each phase. On d 24 and 49, two birds per pen were randomly selected and sacrificed via cervical dislocation for gizzard, small intestine, pancreas, spleen, liver, and bursa weights.

Sample Processing and Laboratory Analyses

The BSFLM, SBM, and diet samples were finely ground (CBG5 Smart Grind, Applica Consumer Products Inc., Shelton, CT) and thoroughly mixed prior to analyses. Samples were submitted to a commercial lab (SGS Canada Inc, Guelph, ON, Canada) for dry matter, crude protein, crude fat, starch, and minerals analyses (AOAC International, 2005; Methods 930.15, 935.11, 920.39, 920.40, 935.12). Feed samples were analyzed for gross energy (**GE**) using a bomb calorimeter (IKA Calorimeter System C 6000; IKA Works, Wilmington, NC) using benzoic acid as the calibration standard. Neutral detergent fiber (**NDF**) concentrations were determined using ANKOM 200 Fibre Analyzer (ANKOM Technology, Fairport, NY) using the methodology described by Van Soest et al. (1991). Chitin concentration was determined in the BSFLM samples by subtracting the ADF-linked protein from the ash-free ADF as described by Marono et al. (2015). Fatty acid concentration in BSFLO and SBO samples was determined in a commercial lab (Activation Laboratories, Ancaster, ON) according to O'Fallon et al. (2007). The amino acid contents of BSFLM, SBM, and diet samples were also analyzed by acid hydrolysis and oxidative hydrolysis (AOAC International, 2005; Methods 994.12), followed by ultra-performance liquid chromatography (UPLC; Waters Corporation, Milford, MA).

Calculations and Statistics

Mortality corrected FCR is an adjusted FCR value which accounts for dead birds removed from the pens throughout the trial. Mortality corrected FCR was calculated using the following equations.

1. Mortality BWG = [($deadBW_1$ − BW/bird/pen at the start of phase) + ($deadBW_2$ − BW/bird/pen at the start of phase) + ...]

Table 2. Composition of grower diets, as fed basis.

Item	Grower phase, d 11−24					
	SBM replacement with BSFLM, %					
	0 + AGP[1]	0	12.5	25	50	100
Corn	40.0	40.2	41.8	43.5	47.0	50.6
Wheat	20.0	20.0	20.0	20.0	20.0	20.0
Soybean meal	27.2	27.2	22.8	18.5	9.70	-
Pork meal	5.00	5.00	5.00	5.00	5.00	5.00
BSFLM[2]	-	-	3.40	6.8	13.6	21.1
BSFLO[2]	-	-	3.70	2.97	1.47	-
Soy oil	4.43	4.43	-	-	-	-
L-Lysine HCL	0.31	0.31	0.37	0.42	0.54	0.66
DL-Methionine	0.33	0.33	0.35	0.37	0.42	0.46
L-Threonine	0.16	0.16	0.17	0.18	0.22	0.25
L-Tryptophan	-	-	-	-	0.05	0.11
Limestone	0.42	0.42	0.35	0.29	0.15	-
Monocalcium phosphate	0.73	0.73	0.71	0.70	0.67	0.64
Salt	0.23	0.23	0.22	0.20	0.16	0.12
Sodium bicarbonate	0.06	0.06	0.07	0.07	0.09	0.10
Vitamin and trace minerals premix[3]	1.00	1.00	1.00	1.00	1.00	1.00
Antibiotic	0.05	-	-	-	-	-
Coccidiostat	0.07	-	-	-	-	-
Total	100	100	100	100	100	100
Calculated provisions						
AMEn, kcal/kg	3,100	3,100	3,100	3,100	3,100	3,108
Crude fat, %	6.90	6.90	6.70	6.60	6.10	5.80
Linoleic acid, %	3.40	3.40	3.10	2.70	2.00	1.20
Crude protein, %	21.5	21.5	21.5	21.5	21.5	21.5
SID[4] Lys, %	1.15	1.15	1.15	1.15	1.15	1.15
SID Met, %	0.61	0.61	0.63	0.64	0.66	0.69
SID Met + Cys, %	0.87	0.87	0.87	0.87	0.87	0.87
Calcium, %	0.87	0.87	0.87	0.87	0.87	0.87
Available P, %	0.44	0.44	0.44	0.44	0.44	0.44
Sodium, %	0.16	0.16	0.16	0.16	0.16	0.16

[1]Coccidiostat (Monteban 100, 70 mg Narasin/kg, Elanco Canada Ltd, Guelph, ON) and antibiotic (BMD 110 G, 55 mg Bacitracin Methylene Disalicylate/kg, Zoetis Canada Inc., Kirkland, QC).
[2]BSFLM; black soldier fly larvae meal, BSFLO; black solider fly larvae oil.
[3]Provided per kg of premix: vitamin A (retinol), 880 KIU; vitamin D_3 (cholecalciferol), 330 KIU; vitamin E, 4,000 IU; vitamin K_3 (menadione), 330 mg; vitamin B_1 (thiamin), 400 mg; vitamin B_2 (riboflavin), 800 mg; vitamin B_3 (niacin), 5,000 mg; vitamin B_5 (pantothenic acid), 1,500 mg; vitamin B_6 (pyridoxine), 300 mg; vitamin B_9 (folic acid), 100 mg; vitamin B_{12} (cyanocobalamin), 1200 mcg; biotin, 200 mcg; choline, 60,000 mg; Fe, 6000 mg; Cu, 1000 mg; I, 1 mg, Se, 30 mg.
[4]SID: standardized ileal digestible.

2. Survivors BWG = (Total pen BW at the end of the current phase) − (Total pen BW at the end of the previous phase)
3. Mortality corrected FCR = Total FI / (Survivors BWG + Mortality BWG)

All the data were analyzed with the following statistical model using the SAS Studio OnDemand for Academics software (2022):

$$Y = \mu + trmt_i + block_j + e_{ij}$$

where Y represents the outcome, μ is the mean of the given observations (fixed effect), $trmt_i$ is the effect of the inclusion level of BSFLM (fixed effect), $block_j$ is the effect of block design, hence the variation between the four rooms (random effect), and e_{ij} accounts for the experimental error (random effect). The Proc GLIMMIX procedure was used to obtain the LS means and Tukey Kramer's post-hoc test was applied. The Proc IML procedure was used to obtain contrast coefficients (SAS Institute Inc, 2022). Coefficients were used in contrast statements applied to the 0%BSFLM and BSFLM diets to identify linear and quadratic relationships resulting from the BSFLM inclusion. A P-value of ≤ 0.05 was considered significant.

RESULTS

Analyzed Chemical Composition

Black solider fly larvae meal had a greater concentration of crude protein, crude fat, phosphorus, calcium, magnesium, and sodium on a DM basis (Table 4). The BSFLM also had a greater concentration of starch and NDF than SBM, whereas SBM had a greater concentration of potassium. Additionally, of the indispensable amino acids, BSFLM had greater concentrations of His, Val, and Met, whereas SBM was greater in Arg and Trp. Of the dispensable amino acids, BSFLM was greater in Ala, Gly, and Pro, and SBM was greater in Asp, Cys, and Glu. The fatty acid profile of BSFLO and SBO are presented in Table 5. The BSFLO and SBO had some key differences in fatty acid profile, where BSFLO had greater total saturated fats and medium chain fatty acids (**MCFA**) such as lauric and myristic acid, as well as palmitic and palmitoleic acid. Conversely, SBO contained higher polyunsaturated fatty acids such as stearic, linoleic, and linolenic acid. Within phase, the concentration for GE, CP, and crude fat were comparable; however, DM, starch, and NDF increased with the level of BSFLM,

Table 3. Composition of finisher diets, as fed basis.

	Finisher phase, d25−49					
	SBM replacement with BSFLM, %					
Item	0 + AGP[1]	0	12.5	25	50	100
Corn	45.8	45.9	47.2	48.4	50.9	53.1
Wheat	20.0	20.0	20.0	20.0	20.0	20.0
Soybean meal	20.6	20.6	17.3	14.1	7.40	-
Pork meal	6.00	6.00	6.00	6.00	6.00	6.00
BSFLM[2]	-	-	2.60	5.10	10.3	16.1
BSFLO[2]	-	-	4.30	3.86	2.77	1.75
Soy oil	4.95	4.95	-	-	-	-
L-Lysine HCL	0.33	0.33	0.37	0.41	0.50	0.59
DL-Methionine	0.31	0.31	0.33	0.34	0.38	0.41
L-Threonine	0.16	0.16	0.17	0.18	0.20	0.23
L-Tryptophan	-	-		-	0.04	0.08
Limestone	0.18	0.18	0.13	0.08	-	-
Monocalcium phosphate	0.33	0.33	0.31	0.30	0.33	0.52
Salt	0.23	0.23	0.21	0.20	0.17	0.14
Sodium bicarbonate	0.06	0.06	0.06	0.07	0.07	0.08
Vitamin and trace minerals premix[3]	1.00	1.00	1.00	1.00	1.00	1.00
Antibiotic	0.05	-	-	-	-	-
Coccidiostat	0.07	-	-	-	-	-
Total	100	100	100	100	100	100
Calculated provisions						
AMEn, kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200
Crude fat, %	7.80	7.80	7.60	7.50	7.20	7.10
Linoleic acid, %	3.80	3.80	3.50	3.30	2.70	2.20
Crude protein, %	19.5	19.5	19.5	19.5	19.5	19.5
SID[4] Lys, %	1.03	1.03	1.03	1.03	1.03	1.03
SID Met, %	0.57	0.57	0.58	0.59	0.61	0.63
SID Met + Cys, %	0.80	0.80	0.80	0.80	0.80	0.80
Calcium, %	0.79	0.79	0.79	0.79	0.81	0.89
Available P, %	0.40	0.40	0.40	0.40	0.41	0.45
Sodium, %	0.16	0.16	0.16	0.16	0.16	0.16

[1]Coccidiostat (Monteban 100, 70 mg Narasin/kg, Elanco Canada Ltd, Guelph, ON) and antibiotic (BMD 110 G, 55 mg Bacitracin Methylene Disalicylate/kg, Zoetis Canada Inc., Kirkland, QC).

[2]BSFLM; black soldier fly larvae meal, BSFLO; black solider fly larvae oil.

[3]Provided per kg of premix: vitamin A (retinol), 880 KIU; vitamin D_3 (cholecalciferol), 330 KIU; vitamin E, 4,000 IU; vitamin K_3 (menadione), 330 mg; vitamin B_1 (thiamin), 400 mg; vitamin B_2 (riboflavin), 800 mg; vitamin B_3 (niacin), 5,000 mg; vitamin B_5 (pantothenic acid), 1,500 mg; vitamin B_6 (pyridoxine), 300 mg; vitamin B_9 (folic acid), 100 mg; vitamin B_{12} (cyanocobalamin), 1200 mcg; biotin, 200 mcg; choline, 60,000 mg; Fe, 6000 mg; Cu, 1000 mg; I, 1 mg, Se, 30 mg.

[4]SID: standardized ileal digestible.

Table 4. Analyzed composition of black soldier larvae meal (BSFLM) and soybean meal (SBM) used in the study, DM basis.

Item	BSFLM	SBM
Dry matter, %	95.42	88.22
Crude protein, %	55.1	51.4
Crude fat, %	13.3	2.64
Starch, %	3.73	1.29
Chitin, %	6.67	0.00
Neutral detergent fiber, %	19.7	6.90
Gross energy, kcal/kg	5,341	4,746
Calcium, %	1.51	0.14
Phosphorus, %	0.70	0.62
Potassium, %	1.36	2.41
Magnesium, %	0.36	0.31
Sodium, %	0.18	0.05
Indispensable Amino Acids, %		
Arg	2.75	3.55
His	1.73	1.38
Ile	1.99	1.97
Leu	3.60	3.79
Lys	2.75	2.86
Met	0.80	0.61
Phe	2.73	2.65
Thr	1.95	1.84
Trp	0.72	1.40
Val	3.14	2.35
Dispensable Amino Acids, %		
Ala	3.38	2.13
Asp	4.91	5.77
Cys	0.50	0.77
Glu	5.45	7.54
Gly	2.30	1.75
Pro	3.54	2.76
Ser	2.36	2.61
Tyr	1.78	1.61

Table 5. Analyzed composition of black soldier larvae oil (BSFLO) and soybean oil (SBO) used in the study, as fed basis.

Fatty acids, % of total fat	BSFLO	SBO
Decanoic	0.73	0.00
Undecanoic	0.01	0.00
Lauric	35.32	0.00
Myristic	7.32	0.06
Myristoleic	0.24	0.00
Pentadecanoic	0.07	0.01
Palmitic	12.08	9.93
Palmitoleic	3.01	0.08
Heptadecanoic	0.10	0.10
cis-10-Heptadecenoic	0.06	0.05
Stearic	2.37	3.86
Oleic	20.02	18.83
Linolelic	13.00	55.35
Arachidic	0.11	0.29
γ-Linolenic	0.05	0.03
Gadoleic	0.17	0.19
Linolenic	2.50	8.76
Heneicosanoic	0.00	0.06
cis-11,14-Eicosadienoic	0.02	0.06
Behenic	0.03	0.31
Tricosanoic	0.02	0.00
Methyl cis-5,8,11,14-eicosatetraenoic	0.00	0.04
Lignoceric	0.01	0.10
Others	2.76	1.90
Total saturated fatty acids %	58.00	15.00
Total monounsaturated fatty acids, %	23.00	19.00
Total poly unsaturated fatty acids, %	16.00	64.00

Table 6. Analyzed chemical composition of starter diets, as fed basis.

	Starter phase, d 0−10				
	SBM Replacement with BSFLM,[2] %				
Item	0[1]	12.5	25	50	100
Dry matter, %	89.4	89.7	89.7	90.1	90.6
Gross Energy, kcal/kg	4167	4210	4260	4221	4252
Crude protein, %	22.4	23.2	21.4	23.5	23.4
Crude fat, %	6.7	7.0	7.3	5.7	6.2
Starch, %	37.8	26.6	37.9	38.6	40.7
NDF,[3] %	10.0	11.8	12.6	15.2	14.6
Calcium, %	0.73	0.80	0.83	0.91	1.02
Phosphorus, %	0.73	0.71	0.78	0.70	0.79
Potassium, %	0.89	0.89	0.87	0.77	0.67
Magnesium, %	0.17	0.18	0.18	0.18	0.19
Sodium, %	0.15	0.20	0.17	0.18	0.16
Amino acids, %					
Indispensable					
Arg	1.38	1.39	1.28	1.32	1.1
His	0.57	0.61	0.54	0.65	0.62
Ile	1.10	0.86	0.79	1.11	0.92
Leu	1.97	1.76	1.72	2.07	1.8
Lys	1.29	1.39	1.57	1.65	1.59
Met	0.55	0.59	0.68	0.76	0.77
Phe	1.34	1.12	0.67	1.31	0.96
Thr	0.87	1.07	0.98	0.99	1.04
Trp	0.47	0.46	0.44	0.37	0.32
Val	0.95	1.04	1.02	1.14	1.14
Dispensable					
Ala	1.01	1.10	1.17	1.34	1.34
Asp	2.11	2.17	2.18	2.11	1.84
Cys	0.44	0.46	0.42	0.41	0.38
Glu	3.17	1.32	3.20	3.08	2.57
Gly	0.80	0.88	0.80	1.01	0.98
Pro	1.32	1.43	1.36	1.61	1.57
Ser	1.06	1.07	1.07	1.11	0.98
Tyr	0.43	0.53	0.17	0.64	0.41

[1]Same analyzed nutrient composition as diet 0%BSFLM + AGP (Coccidiostat (Monteban 100, 70 mg Narasin/kg, Elanco Canada Ltd, Guelph, ON) and antibiotic (BMD 110 G, 55 mg Bacitracin Methylene Disalicylate/kg, Zoetis Canada Inc., Kirkland, QC)).
[2]BSFLM; black soldier fly larvae meal.
[3]NDF; neutral detergent fibre.

and potassium decreased (Tables 6−8). One notable difference is that the CP concentration in diet 0 in the finisher phase was lower than expected. Tables 6 to 8 also presented concentrations of the amino acid in the starter, grower, and finisher phases, respectively.

Growth Performance

Both the d 10 BW and starter BWG decreased linearly and quadratically as the inclusion level of BSFLM increased ($P < 0.01$) (Table 9). A quadratic decrease occurred where birds fed diets 12.5 and 25 had higher BW than diets 0, 50, and 100 ($P < 0.01$), and a linear decrease occurred where diet 100 resulted in lower BW than diets 0 and 50 ($P < 0.01$). On d 10, birds fed diets 12.5 and 25 had BW not different from birds fed the 0 + AGP diet, whereas birds fed diets 0, 50, and 100 had lower BW ($P < 0.01$). The FI also decreased linearly and quadratically as the level of BSFLM increased ($P < 0.01$), where birds fed diets 0, 12.5, 25, and 50 had greater FI than birds fed diet 100, and birds fed diet 12.5 had greater FI than birds fed diets 50 and 100 ($P < 0.01$). Compared to birds fed the 0 + AGP diet, only birds fed diet 100 had lower FI ($P < 0.05$). The FCR was lowest for birds fed diet 0 + AGP ($P < 0.01$) and greatest for birds fed diets 0 and 50 ($P < 0.01$), while all other treatments were intermediate.

On d 24, BW decreased linearly and quadratically as the level of BSFLM increased ($P < 0.01$), where birds fed diets 0, 12.5, and 25 had greater BW than birds fed diets 50 and 100 ($P < 0.01$). However, birds fed diets 12.5 and 25 had intermediate BW compared to birds fed the 0 + AGP diet and diet 0. The BWG during the grower phase decreased linearly as birds fed diets 0, 12.5, and 25 had greater BWG than birds fed diets 50 and 100 ($P < 0.01$); no differences were observed for BWG for birds fed diets 0 + AGP, 0, 12.5, and 25. The FI decreased linearly with increasing BSFLM level as birds fed diets 0 and 12.5 had greater FI than birds fed diet 100 ($P < 0.01$), and birds fed diets 25 and 50 were intermediate to birds fed diets 0, 12.5, and 100. The FCR increased linearly and quadratically with increasing BSFLM inclusion level as birds fed diet 12.5 had the lowest FCR and birds fed diet 100 had the greatest FCR ($P < 0.01$) with all other diets intermediate.

On d 49, BW decreased linearly as the dietary inclusion level of BSFLM increased, where birds fed diets 0, 12.5, and 25 had greater BW than birds fed diets 50 and 100 ($P < 0.01$). Birds fed diet 0 + AGP had the greatest BW versus all other treatment groups ($P < 0.01$). The

Table 7. Analyzed chemical composition of grower diets, as fed basis.

Item	Grower, d 11–24				
	SBM replacement with BSFLM[2], %				
	0[1]	12.5	25	50	100
Dry matter, %	89.1	89.2	89.2	89.8	89.9
Gross Energy, kcal/kg	4191	4132	4189	4210	4184
Crude protein, %	19.2	19.4	20.9	21.5	20.6
Crude fat, %	7.1	6.6	5.7	5.9	5.6
Starch, %	39.6	39.6	42.1	42.7	44.7
NDF,[3] %	12.2	11.7	14.1	14.2	15.7
Calcium, %	0.60	0.65	0.65	0.70	0.69
Phosphorus, %	0.56	0.54	0.58	0.59	0.56
Potassium, %	0.86	0.80	0.75	0.70	0.58
Magnesium, %	0.16	0.17	0.16	0.19	0.19
Sodium, %	0.14	0.16	0.17	0.15	0.14
Amino acids, %					
Indispensable					
Arg	1.28	1.20	1.19	1.13	0.99
His	0.54	0.51	0.54	0.55	0.52
Ile	0.86	1.01	0.94	0.96	0.75
Leu	1.71	1.94	1.85	1.87	1.64
Lys	1.12	1.28	1.37	1.34	1.38
Met	0.58	0.57	0.62	0.70	0.73
Phe	1.15	1.23	1.14	1.19	0.83
Thr	0.84	0.89	0.97	0.90	0.90
Trp	0.45	0.43	0.44	0.38	0.36
Val	0.87	0.87	0.89	0.91	0.95
Dispensable					
Ala	0.96	1.07	1.13	1.25	1.30
Asp	1.89	1.98	1.96	1.84	1.71
Cys	0.46	0.44	0.44	0.35	0.35
Glu	2.93	3.04	2.98	2.75	2.49
Gly	0.79	0.80	0.82	0.99	0.91
Pro	1.27	1.34	1.37	1.51	1.45
Ser	0.98	1.00	1.01	0.97	0.93
Tyr	0.43	0.47	0.48	0.41	0.35

[1]Same analyzed nutrient composition as diet 0%BSFLM + AGP (Coccidiostat (Monteban 100, 70 mg Narasin/kg, Elanco Canada Ltd, Guelph, ON) and antibiotic (BMD 110 G, 55 mg Bacitracin Methylene Disalicylate/kg, Zoetis Canada Inc., Kirkland, QC)).
[2]BSFLM: black soldier fly larvae meal.
[3]NDF: neutral detergent fibre.

Table 8. Analyzed chemical composition of finisher diets, as fed basis.

Item	Finisher, d 25–49				
	SBM Replacement with BSFLM[2], %				
	0[1]	12.5	25	50	100
Dry matter, %	88.9	88.6	88.5	88.8	89.2
Gross Energy, kcal/kg	4231	4238	4231	4226	4372
Crude protein, %	17.0	19.0	20.4	19.3	18.6
Crude fat, %	7.8	7.5	6.9	7.3	6.5
Starch, %	41.7	41.3	41.4	43.4	46.8
NDF,[3] %	12.3	12.7	12.2	14.5	15.2
Calcium, %	0.66	0.60	0.49	0.68	0.87
Phosphorus, %	0.55	0.55	0.47	0.57	0.63
Potassium, %	0.72	0.72	0.67	0.61	0.51
Magnesium, %	0.16	0.17	0.15	0.16	0.16
Sodium, %	0.14	0.17	0.14	0.14	0.16
Amino acids, %					
Indispensable					
Arg	1.16	1.14	1.14	1.03	0.89
His	0.49	0.50	0.51	0.50	0.49
Ile	0.85	0.82	0.70	0.63	0.59
Leu	1.75	1.77	1.60	1.48	1.39
Lys	1.06	1.17	1.40	1.22	1.28
Met	0.54	0.53	0.59	0.61	0.63
Phe	1.17	1.14	0.92	0.73	0.78
Thr	0.88	0.84	0.83	0.80	0.82
Trp	0.53	0.46	0.39	0.39	0.34
Val	0.75	0.82	0.94	0.90	0.90
Dispensable					
Ala	0.98	1.06	1.12	1.11	1.13
Asp	1.75	1.84	1.86	1.65	1.47
Cys	0.34	0.34	0.31	0.27	0.23
Glu	2.82	2.81	2.81	2.48	2.19
Gly	0.84	0.85	0.86	0.85	0.82
Pro	1.29	1.34	1.35	1.35	1.32
Ser	0.95	1.00	0.94	0.87	0.80
Tyr	0.40	0.39	0.45	0.25	0.41

[1]Same analyzed nutrient composition as diet 0%BSFLM + AGP (Coccidiostat (Monteban 100, 70 mg Narasin/kg, Elanco Canada Ltd, Guelph, ON) and antibiotic (BMD 110 G, 55 mg Bacitracin Methylene Disalicylate/kg, Zoetis Canada Inc., Kirkland, QC)).
[2]BSFLM: black soldier fly larvae meal.
[3]NDF: neutral detergent fibre.

Table 9. Effects of replacing soybean (SBM) with increasing proportions of black soldier fly larvae meal (BSFLM) on growth performance of broiler chickens.

	SBM replacement with BSFLM,[3] %								Responses to BSFLM[2]	
Item	0+AGP[1]	0	12.5	25	50	100	SEM	*P*-value	Linear	Quadratic
Placement BW, g/bird	45.5	45.6	45.6	45.6	45.5	45.7	0.11	0.420	-	-
Starter, d 0–10										
BW, g/bird	265.4^{a}	245.6^{b}	259.9^{a}	261.3^{a}	244.2^{b}	203.0^{c}	2.99	<0.001	<0.001	<0.001
BW gain, g/bird	220.0^{a}	200.2^{b}	214.5^{a}	215.9^{a}	198.9^{b}	157.4^{c}	2.91	<0.001	<0.001	<0.001
Feed intake, g/bird	236.7^{ab}	239.2^{ab}	244.7^{a}	235.0^{ab}	230.0^{b}	117.3^{c}	4.11	<0.001	<0.001	<0.001
FCR	1.082^{b}	1.197^{a}	1.146^{ab}	1.092^{b}	1.162^{a}	1.132^{ab}	0.02	<0.001	0.149	0.081
Grower, d 11–24										
Final BW, g/bird	973.3^{a}	904.7^{b}	926.6^{ab}	921.8^{ab}	818.0^{c}	669.2^{d}	19.82	<0.001	<0.001	0.008
BW gain, g/bird	709.6^{a}	660.8^{a}	668.3^{a}	662.2^{a}	575.5^{b}	467.9^{c}	17.09	<0.001	<0.001	0.116
Feed intake, g/bird	1,218^{a}	1,208^{a}	1,128^{a}	1,124^{ab}	1,106^{ab}	1,009^{b}	45.18	<0.001	<0.001	0.685
FCR	1.651^{bc}	1.877^{ab}	1.462^{c}	1.714^{abc}	1.741^{abc}	1.963^{a}	0.11	<0.001	0.008	0.031
Finisher, d 25–49										
Final BW, g/bird	3,257^{a}	2,996^{b}	3,041^{b}	3,067^{b}	2,748^{c}	2,449^{d}	41.85	<0.001	<0.001	0.056
BW gain, g/bird	2,275^{a}	2,082^{b}	2,105^{b}	2,137^{ab}	1,921^{c}	1,771^{c}	37.96	<0.001	<0.001	0.317
Feed intake, g/bird	3,801^{a}	3,625^{abc}	3,680^{ab}	3,720^{ab}	3,520^{bc}	3,377^{c}	59.65	<0.001	<0.001	0.302
FCR	1.671^{b}	1.743^{b}	1.746^{b}	1.741^{b}	1.831^{a}	1.909^{a}	0.02	<0.001	<0.001	0.600
Overall, d 0–49										
BW gain, g/bird	3,211^{a}	2,950^{b}	2,995^{b}	3,022^{b}	2,703^{c}	2,403^{d}	41.84	<0.001	<0.001	0.055
Feed intake, g/bird	5,258^{a}	5,076^{ab}	5,056^{ab}	5,082^{ab}	4,859^{bc}	4,566^{c}	110.6	<0.001	<0.001	0.336
FCR	1.668^{d}	1.746^{c}	1.711^{cd}	1.714^{cd}	1.815^{b}	1.910^{a}	0.02	<0.001	<0.001	0.042
Mortality, %	3.6^{b}	1.2^{b}	6.0^{b}	1.8^{b}	3.6^{b}	18.5^{a}	1.96	<0.001	<0.001	0.009

[a-d]Within a row LSMeans with different superscripts differs, $P < 0.05$.
[1]Coccidiostat (Monteban 100, 70 mg Narasin/kg, Elanco Canada Ltd, Guelph, ON) and antibiotic (BMD 110 G, 55 mg Bacitracin Methylene Disalicylate/kg, Zoetis Canada Inc., Kirkland, QC).
[2]Contrasts in LSMeans tested among diets 0, 12.5, 25, 50, and 100
[3]BSFLM: black soldier fly meal.

BWG during the finisher phase also decreased linearly as birds fed diets 0 and 12.5 had greater BWG than birds fed diets 50 and 100 ($P < 0.01$), while birds fed diet 25 were intermediate. Likewise, BWG for birds fed diet 0 + AGP was the greatest compared to all other diet treatments ($P < 0.01$). The FI decreased linearly where birds fed diets 12.5 and 25 had greater FI than birds fed diet 100 ($P < 0.01$). Birds fed diet 0 + AGP had greater BW than birds fed diets 50 and 100 ($P < 0.01$). The FCR increased linearly where birds fed diets 0, 12.5, and 25 had lower FCR than birds fed diets 50 and 100 ($P < 0.01$). There was no difference between the 0+AGP diet and diets 0, 12.5, and 25.

Overall (d 0–49), the BWG and FI decreased linearly as birds fed diets 0, 12.5, and 25 had greater BWG than birds fed diets 50 and 100 ($P < 0.01$). Birds fed all other diet treatments had lower BWG than birds fed diet 0 + AGP ($P < 0.01$). The overall FCR decreased linearly and quadratically where birds fed diets 0, 12.5, and 50 had lower FCR than birds fed diets 50 and 100 ($P < 0.01$), with birds fed 12.5 and 25 intermediate to birds fed diets 0 and 0 + AGP, given that birds fed diet 0 + AGP had the lowest result. Overall, mortality increased linearly and quadratically where birds fed diet 100 had greater mortality compared to all other diets and birds fed diets 0 and 25 had the lowest mortalities ($P < 0.01$).

Table 10. Effects of replacing soybean meal (SBM) with increasing proportions of black soldier fly larvae meal (BSFLM) on organ weights (g/kg BW) and breast muscle attributes in broiler chickens.

	SBM replacement with BSFLM, %								Responses to BSFLM[3]	
Item	0 + AGP[1]	0	12.5	25	50	100	SEM	*P*-value	Linear	Quadratic
24 d old										
Gizzard	25.1^{ab}	28.8^{a}	23.7^{b}	23.3^{b}	25.4^{ab}	26.8^{ab}	0.90	0.001	0.644	0.002
Small intestine	29.6^{b}	31.9^{ab}	33.2^{a}	31.5^{ab}	31.2^{ab}	34.5^{a}	0.84	0.005	0.063	0.053
Pancreas	3.0^{c}	3.2^{bc}	3.2^{bc}	3.3^{bc}	3.5^{ab}	3.8^{a}	0.12	<0.001	<0.001	0.902
Liver	18.5^{c}	21.1^{bc}	22.8^{ab}	20.8^{bc}	21.1^{bc}	25.4^{a}	1.59	0.001	0.005	0.058
Spleen	0.8	0.9	0.9	0.8	0.8	0.8	0.05	0.879	0.320	0.505
Bursa	2.6	2.7	2.5	2.6	2.8	2.9	0.19	0.676	0.163	0.605
49 d old										
Gizzard	16.8^{b}	19.7^{ab}	20.1^{ab}	17.9^{ab}	20.6^{a}	19.7^{ab}	0.81	0.014	0.696	0.998
Small intestine	16.7^{b}	19.9^{a}	18.9^{ab}	19.2^{ab}	19.5^{a}	20.8^{a}	0.90	0.002	0.110	0.193
Pancreas	2.0^{b}	2.2^{ab}	2.1^{ab}	2.2^{ab}	2.2^{ab}	2.4^{a}	0.13	0.015	0.032	0.405
Liver	20.5^{b}	22.7^{ab}	22.1^{b}	23.0^{ab}	24.1^{ab}	26.2^{a}	1.31	0.001	0.001	0.709
Spleen	1.1	1.0	1.1	1.1	1.0	1.2	0.07	0.502	0.101	0.392
Bursa	1.7	1.4	1.7	1.5	1.7	1.9	0.14	0.230	0.043	0.921

[a-c]Within a row LSMeans with different superscripts differs, $P < 0.05$.
[1]Coccidiostat (Monteban 100, 70 mg Narasin/kg, Elanco Canada Ltd, Guelph, ON) and antibiotic (BMD 110 G, 55 mg Bacitracin Methylene Disalicylate/kg, Zoetis Canada Inc., Kirkland, QC).
[2]Contrasts in LS Means tested among diets 0, 12.5, 25, 50, and 100.

Relative Organ Weights

At the end of the grower phase (d 24), gizzard weight quadratically increased among the BSFLM diets, where birds fed diets 12.5 and 25 had the lowest gizzard weights ($P < 0.01$) (Table 10). Small intestine weight increased where birds fed the 0 + AGP diet had the lowest weight, birds fed the 12.5 and 100 diets had the highest weights, and all other diets were intermediate ($P < 0.01$). The pancreas weight increased linearly with increasing dietary BSFLM inclusion, where birds fed diet 100 had the greatest pancreas weight, and birds fed diets 0, 12.5, 25, and 50 were intermediate to birds fed diet 0 + AGP and diet 100 ($P < 0.01$). Birds fed diet 0 + AGP had a lower pancreas weight than birds fed diets 50 and 100. The liver weight increased linearly with increasing dietary BSFLM inclusion where birds fed diets 0, 25, and 50 had lower liver weights than birds fed diet 100, yet birds fed diet 12.5 were intermediate ($P < 0.01$). Birds fed diet 0 + AGP had the lowest liver weight ($P < 0.01$). The spleen and bursa were not influenced by dietary treatment.

At the end of the finisher phase (d 49), birds fed diet 0 + AGP had lower gizzard weight than birds fed diet 50, while all other diets were intermediate ($P < 0.05$), and lower small intestine weight than birds fed diets 0, 50, and 100, with intermediate values observed for diets 12.5 and 25 ($P < 0.01$). Pancreas weight increased linearly among the BSFLM diets, where birds fed diet 100 had the highest pancreas weight and all other diets were intermediate to birds fed diets 0 + AGP and 100 ($P < 0.05$). Liver weight also increased linearly among the BSFLM diets, where birds fed diet 12.5 had a lower liver weight than birds fed diet 100, while all other diets were intermediate ($P < 0.01$). Birds fed diet 0 + AGP only had a lower liver weight than birds fed diet 100 ($P < 0.01$). Spleen weight did not change in response to BSFLM inclusion; however, bursa weight increased linearly with inclusion of BSFLM ($P < 0.05$).

DISCUSSION

The objective of the study was to examine the influence of partial to complete replacement of SBM and BSFLM in a broiler feeding program on growth performance. The diets were formulated based on book value specifications for ingredients. Key indispensable amino acids (Lys, Met, Met + Cys) were formulated to meet the requirements, and most other amino acid concentrations met or exceeded specifications. However, diet analyses revealed several amino acids that were rather low. For example, total Arg displayed consistent deficiencies among the diets relative to digestible Arg specifications of 1.40, 1.27, and 1.17% for starter, grower, and finisher, respectively (Aviagen, 2014). Additionally, Thr, Val, and Ile also displayed deficiencies in some diets. The high mortalities in diet 100 was due to the increased need for euthanasia, due to mainly lameness in the form of leg problems, and non-uniform growth leading to more runts. Runts may be the result of a poor gut microbiome (Metzer Farms, 2018) and must be euthanized to avoid welfare issues when the feeders and waterers are adjusted according to bird growth.

A study conducted by Dabbou et al. (2018) reported quadratic responses in BW to increasing BSFLM level throughout the starter, grower, and finisher phases, with the highest BW observed for the 10% inclusion diet, similarly to the quadratic decrease observed in the starter phase in the present study. However, Dabbou et al. (2018) reported most statistically significant responses in the later phases (grower and finisher) as opposed to the starter phase in the present study. Dabbou et al. (2018) saw otherwise no change in BW, BWG, FI, or FCR for diets including 5% and 10% BSFLM and reduced BW and FCR in the diet containing 15% inclusion of BSFLM compared to the control diet (0% BSFLM). It is thought that BSFLM and oil possess prebiotic and antimicrobial properties from the chitin and medium-chain fatty acid contents (Dörper et al., 2020); however, previous studies used partially defatted BSFLM without additional BSFLO (Dabbou et al., 2018), indicating that even relatively low amounts of chitin and MCFA can positively impact growth performance to achieve BW not different from birds fed AGP diets. Furthermore, Dabbou et al. (2018) reported an increase in FI only during the starter phase, compared to the present study, where we observed a decrease in FI with increasing BSFLM inclusion through all 3 phases. The FI depression in the present study may help explain the unsustained benefits of BSFLM in later phases, where Dabbou et al. (2018) observed maintained FI and thus sustained levels of functional compounds such as chitin and MCFA. Likewise, de Souza Vilela et al. (2021) fed broilers up to 20% inclusion of full-fat BSFLM and observed no change in growth performance in the starter phase, and a linear increase in BW, BWG, and FCR in the grower and finisher phases with no change in FI. Additionally, Cullere et al. (2016) and Onsongo et al. (2018) reported no change in growth performance parameters, including FI, when feeding broilers below 55.5% replacement of SBM with BSFLM or BSF pupae meal. However, any additional benefits were not reported despite these studies utilizing diets with similar amounts of crude fat compared to the present study. The crude fat content is considered the source of these benefits, given the fatty acid profile (MCFA). Cullere et al. (2016) and Onsongo et al. (2018) used defatted BSF products, and supplemented with either SBO or corn oil, rather than BSFLO and therefore would not have the functional benefits from high MCFA as BSFLO does. Diets 0, and 25 had the lowest mortalities. Additionally, several relative organ weights increased with increasing BSFLM inclusion levels including the gizzard, small intestine, and liver. Given this evidence, further research should be conducted on the use of BSFLM and oil as a supplement ($<\sim 25\%$) to replace antibiotics and coccidiostats in AGP diets.

Current literature indicates that the overall linear decrease in performance observed throughout the study with high inclusion levels of BSFLM could be due to greater fiber contents or limited available amino acid supply. Murawska et al. (2021) observed a negative linear relationship in BW, BWG, FI, and FCR in broilers fed 50%, 75%, and 100% replacement of SBM with BSFLM, and suggested the decreased performance mainly results from the reduction in FI. It is suggested that reduced FI could be caused by impaired protein utilization linked to increased dietary chitin content when BSFLM is included at higher levels in the diet. Chitin is representative of the fiber in BSFLM, and as seen in Murawska et al. (2021) and the present study, the NDF content of the diets increases as the level of BSFLM inclusion increases. High fiber content or NDF, has been seen to increase anterior digestive tract weight (gizzard), reduce digesta transit time, and subsequently reduce nutrient digestibility (Krás et al., 2013; Sanchez et al., 2021).

Therefore, given the effects seen on not only reduced FI, but also increased FCR in the present study, it is thought that the decreased BW and BWG is not only due to reduced FI, but also a reduction in nutrient digestibility resulting from high crude fiber, and could have further adverse effects on other areas of the body such as feather development and organ function. According to the current literature, the gizzard, primarily functioning as the site for grinding and breaking down feed particles, is expected to increase in size and mass as larger particles and more structural and fibrous material are added to the diet (Kiarie and Mills, 2019). An increase in gizzard weight was seen in diets containing high levels of BSFLM (50 and 100), which also contained the highest concentration of chitin, and consequently NDF, suggesting that the high fiber content in diets 50 and 100 caused the increase in gizzard weight. In addition to reduced nutrient digestibility via increased NDF, reduced nutrient absorption in the small intestine could also have contributed to poor performance. In the present study, the small intestinal weight increased with increasing levels of BSFLM inclusion; however, on d 24 and 49, diet 0 + AGP had significantly lighter small intestine compared to all other diets. Earlier research has shown that AGP inclusion causes intestinal wall thinning (Coates et al., 1955). Thinned intestinal walls allow nutrients to pass more easily, increasing overall nutrient absorption and hence feed utilization (Madge, 1969). The thinned intestinal wall likely led to the decrease in intestinal weight in the 0 +AGP fed birds. On the other hand, research has shown that the protein source in poultry diets, notably vegetable versus animal sources, can influence the microbiome in the gut through increased microbial diversity (Bean-Hodgins and Kiarie, 2021). Animal proteins contain high amounts of crude protein and can cause the fermentation of excess crude protein in the intestinal tract, which feeds harmful bacteria such as *C. perfringens* (Drew et al., 2004; Wilkie et al., 2005; Bean-Hodgins and Kiarie, 2021). Indeed, much of the protein in insect meal has been demonstrated to be bound in chitin and concentration of chitin had negative correlation with protein digestibility (Marono et al., 2015). Therefore, it is possible that BSFLM can trigger increased bacterial populations in the gut, causing the gut wall to thicken via increased mucosa or muscularis production, resulting in a greater overall weight.

Reduced nutrient absorption and digestibility would also limit the amino acids available to the bird. A study conducted by Velten et al. (2018) replaced 50% SBM with BSFLM in broiler diets containing standard amino acid supplementation and fortified amino acid supplementation (addition of Met, Lys, Thr, Arg, Val). Reduced growth performance was observed when the broilers received the standard amino acid supplementation, but no change in performance parameters was observed when receiving supplemental amino acids, indicating that the birds were not receiving sufficient amino acids in a standard diet with BSFLM inclusion. Similarly, it was recently demonstrated that the addition of indispensable synthetic amino acids in BSFLM diet to equal levels of amino acids in soybean meal diet improved protein utilization efficiency in BSFLM (Cheng et al., 2022). In the present study, the diets were analyzed, and the most notable effect on amino acid concentrations was the Arg deficiency. This deficiency increased as the level of BSFLM increased, corresponding with lower levels of arginine in BSFLM versus SBM diet. Furthermore, the Arg: Lys ratio was below the NRC recommendation of 0.9 to 1.18 (Balnave and Barke, 2002) in diets 25, 50, and 100 for the starter and finisher phases, and diet 100 for the grower phase. Arginine plays an essential role in broiler growth, feather development, and immunity, and can influence the effectiveness of all other amino acids in protein synthesis; however, is heavily influenced by Lys concentrations (Balnave and Barke, 2002). The literature reviewed by Balnave and Barke (2002) suggest that Lys and Arg do not compete for absorption in the small intestine, but instead compete for renal reabsorption. If the Arg: Lys ratio is imbalanced towards Lys, excess Lys will be reabsorbed, a process which is also influenced by potassium levels, and will result in the overproduction of arginase by the kidney. Arginase activity is closely correlated to reduced FI and BWG in broilers as it also breaks down Arg, causing more of a deficiency (Balnave and Barke, 2002). This indicates that the reduced performance seen in Velten et al. (2018) could be caused by amino acid imbalances resulting from the Arg: Lys ratio and could be rectified with amino acid supplementation, or as also suggested by Balnave and Barke (2002), and potassium supplementation. Given that an Arg deficiency can reduce the effectiveness of other amino acids for use for protein synthesis, a build up of unavailable amino acids would require an increase of catabolism of amino acids via the liver. Therefore, the liver would be working harder to break down the excess amino acids (He et al., 2021). The increased metabolic activity taking place in the liver would cause the increase in size and relative weight as seen in the present study. An alternative possibility for hepatic hypertrophy is if amino acids are unavailable for protein synthesis, this may suggest they could also be unavailable for other typical functions in the body, such as for example the lipotropic ability of methionine. Methionine works with choline as a methyl donor to export fats from the liver (Kalbande et al., 2009; Saeed et al., 2017). Although liver fat was not analyzed in the present study. It is understood that a methionine deficiency can play a part in the development of fatty liver syndrome, a common metabolic condition in chickens resulting in fat accumulation in the liver (Qureshi et al., 2004). The lack of bioavailable methionine could have led to the development of fatty liver syndrome in this case, causing the increase in liver weight in birds fed higher BSFLM levels. Another factor which could have played a part in the development of fatty liver syndrome is the increase in starch contents of the diets as the BSFLM inclusion level increased. The starch content increased due to the increase in corn in the diets. Corn increased because of reduced SBM as a way to balance the diets for energy. However, the change in energy source may have influenced the overall composition of the liver, leading to fatty liver syndrome.

Several other organs became heavier with increasing BSFLM inclusion. Pancreas weight increased linearly with the BSFLM on d 24 and 49. It is unclear as to why pancreas hypertrophy occurred; however, research has established that the presence of antinutritional factors such as trypsin inhibitors in untreated SBM will cause a corresponding increase in pancreas weight, as the pancreas attempts to compensate with trypsin production (Pacheco et al., 2014). The increasing presence of chitin in the BSFLM diets could also be considered an antinutritional factor; however, research indicates that poultry produce small amounts of chitinase enzymes capable of digesting chitin (Tabata et al., 2017). Perhaps increased chitinase production influenced feedback loop involving the pancreas, resulting in pancreas hypertrophy, or perhaps BSFLM contains other antinutritional factors yet to be identified, which have a similar impact on the pancreas as trypsin inhibitors. Additionally, little change was seen in the lymphoid organs, except for a linear increase in bursa weight on d 49, where the AGP + 0 diet was similar to diets 12.5 and 50, and diet 100 was heavier than all of the other diets. The bursa acts as an indicator of immune function in poultry, being the main site for B-cell differentiation. Studies have used the weight of the bursa as an indicator for the potential of antibiotic alternatives, suggesting that heavier bursa is indicative of healthier immune system (Guo et al., 2003). Likewise, in the present study, bursa weight increased with the level of BSFLM, suggesting that the BSFLM does promote a healthy immune system, and is comparable to AGP diets. Alternatively, increased bursa weight could also indicate an increased need for immune responses given the potentially changing bacterial population in the gut leading to increased intestinal weight.

In conclusion, the results from this study indicated that broilers would experience reduction in growth performance when fed higher levels of BSFLM (≥50% of SBM) through reduced BW and BWG, as well as reduced FI and poorer FCR. These results indicated that a major, or full replacement of SBM with BSFLM is not viable under the conditions present in this study. However, further research is warranted on BSFLM inclusion in lower amounts (≤25% of SBM), as a value-added ingredient, which could promote growth similar to an AGP diet. Further research is also warranted on a comprehensive study of the metabolic and physiological effects of BSFLM inclusion, including the effects of high levels of BSFLM (≥50%) on organs.

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DISCLOSURES

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

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