Complete replacement of soybean meal with defatted black soldier fly larvae meal in Shaver White hens feeding program (28–43 wks of age): impact on egg production, egg quality, organ weight, and apparent retention of components[1]

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ABSTRACT Effects of total replacement of soybean meal (**SBM**) with defatted black soldier fly larvae meal (**BSFLM**) on egg production and quality, organ weight, and apparent retention (**AR**) of components were investigated in Shaver White hens from 28 to 43 wk of age. A total of 108 birds, (6 birds/cage) were assigned to three diets (6 replicates/diet). Diets were control corn–SBM diet and two additional diets made with the addition of either 10 or 15% BSFLM. Diets met or exceeded breeder specifications, contained TiO_2 as an indigestible marker, and were prepared in pellet form. Birds had free access to feed and water throughout the experiment. Hen-day egg production (**HDEP**) was monitored daily. Feed intake (**FI**) and body weight (**BW**) were monitored in 4-wk intervals. All eggs laid on the sixth day of wks 31, 35, 39, and 43 were used for egg weight (**EW**), Haugh units (**HU**), yolk color (**YC**), shell breaking strength (**SBS**), and shell thickness (**ST**). Excreta samples were collected for 3 consecutive days on wk 33 for AR and two birds/cage were necropsied at the end. There were no ($P > 0.05$) diet effects on HDEP, FI, and HU. Inclusion of BSFLM linearly decreased ($P < 0.05$) egg mass and feed conversion ratio (**FCR**) and quadratically increased ($P < 0.05$) BW. There was no ($P > 0.05$) interaction between diet and sampling time point on egg quality parameters. Inclusion of BSFLM increased SBF and YC linearly ($P < 0.05$) and ST quadratically ($P = 0.028$). Inclusion of BSFLM quadratically ($P \leq 0.01$) reduced empty ceca weight and increased liver weight and had no effect ($P > 0.05$) on gizzard, small intestine, and pancreas weights. Feeding BSFLM linearly ($P = 0.001$) and quadratically ($P = 0.007$) increased apparent metabolizable energy (**AME**). Data showed that defatted BSFLM resulted in deeper orange yolks and improved eggshell quality; however, unfavorable FCR linked to lighter eggs as well as heavier birds and liver warrants further investigations.

Key words: apparent retention of components, defatted black soldier fly larvae meal, egg production and quality, FCR, organ weight

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INTRODUCTION

The projected increase in human population and disposable income, especially in developing countries, will stimulate increased production of animal protein in the next 3 decades (FAO, 2011). This demand for animal-derived protein will require huge resources; feed being most affected because of diminishing land for cultivation of traditional feedstuffs (FAO, 2011). Poultry production is one of the cheapest and easiest means of meeting anticipated demand in animal protein because they grow fast and have favorable feed conversion efficiency (FAO, 2011). Eggs in particular are low-cost high-quality protein food and contain essential vitamins and minerals (Miranda et al., 2015). Feed cost accounts for over 65% of variable cost of producing poultry products, and energy and amino acids (**AA**) account for more than 90% of this cost (Kiarie et al., 2013). As such feed price has a huge impact on egg production costs. Protein-rich feed ingredients used in poultry feed in Canada and most of the world are mainly plant based. Severe shortages of feed ingredients due to unfavorable climatic conditions, land unavailability, and overexploitation of marine sources could lead to

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profound consequences on feed and food production, and the situation will be further aggravated by food–feed–fuel competition (Woyengo et al., 2014). This has prompted the feed industry to seek for sustainable alternative protein sources for poultry feed formulation.

Insects have been shown to be good alternative ingredients for animal feed formulation, especially poultry as they form part of natural chicken diet (Bovera et al., 2016). Insects are rich in AA, fatty acids, and micronutrients (Makkar et al., 2014; Bovera et al., 2016) and have been shown to grow rapidly on a variety of waste organic matters (Bava et al., 2019). Black soldier fly (Li et al., 2011; Borgogno et al., 2017), common housefly (Čičková et al., 2015), and yellow mealworm (Barker et al., 1998) are the insects with potential for large-scale production. Black soldier fly larvae have high growth rate and are excellent converters of organic waste to larvae and produce meal (**BSFLM**) with consistent AA profiles when grown on diverse substrates (Diener et al., 2009; Nguyen et al., 2015; Spranghers et al., 2017).

There is limited research on the use of BSFLM as replacement of conventional feedstuffs in laying hen diets and subsequent effects on egg production and quality. Results from study done by Secci et al. (2018) showed that total replacement of soybean meal (**SBM**) with BSFLM in diets fed to Lohmann Brown Classic laying hens had no effect on egg quality traits and improved yolk color (**YC**). A study by Maurer et al. (2016) found no effect on egg production, feed intake (**FI**), and feed conversion ratio (**FCR**) when defatted BSFLM replaced SBM in diets for 64- to 74-wk-old Lohmann Select Leghorn classic hens. Total replacement of fish meal with maggot meal significantly decreased egg production in 50-wk-old layers (Isa Brown and Black Nera breeds), and the effect was different between the 2 strains (Agunbiade et al., 2007). Other studies showed that chitin present in insects had a positive influence on gastrointestinal physiology and metabolism of the Lohmann Brown Classic laying hens (Borgogno et al., 2017; Marono et al., 2017). We previously reported the effect of defatted BSFLM on egg production and quality in Shaver White pullets (19–27 wks) (Mwaniki et al., 2018). The partial replacement (41%) of SBM with defatted BSFLM improved YC, shell breaking strength (**SBS**), and shell thickness (**ST**). However, egg weight (**EW**), hen-day egg production (**HDEP**), and egg mass (**EM**) were reduced. Moreover, FCR was poor, linked to increased FI and lower EM, and birds fed BSFLM were heavier.

Hens have different nutrient requirements depending on strain and age among other factors (Leeson and Summers, 2005). Young pullets, particularly white leghorns, have high nutrient requirements at onset of lay to meet body growth requirements as well as egg production, yet they have much less capacity for feed consumption (Khanal et al., 2019). However, brown hens eat more as compared with their white counterparts (Leeson and Summers, 2005; Khanal et al., 2019). At the onset of lay, the bird is not only adjusting to her new environment, but she must consume enough energy and nutrients for her body weight development and to reach peak egg production (Leeson and Summers, 2005). Thus it is imperative to increase their FI from the end of the rearing period toward the peak of production in a short time (Leeson and Summers, 2005). In this context and given the unpredictable production performance responses, we reported in our previous study (Mwaniki et al., 2018), the intent of the present study was to investigate the impact of total replacement of SBM with BSFLM in postpeak period. Therefore, the objective was to investigate effects of total replacement of SBM with defatted BSFLM on egg production, egg quality, visceral organ weight, and apparent retention of components in Shaver White hens from 28–43 wks of age.

MATERIALS AND METHODS

The use of animals was approved by the University of Guelph Animal Ethics Committee and complied with the Canadian Code of Practice for the Care and Use of Animals for Scientific Purposes (CCAC, 2009).

Diets, Birds, and Housing

Defatted BSFLM (approximately 6% crude fat as fed) was procured from a commercial manufacturer and vendor (Enterra Feed Corp., Vancouver, BC, Canada), and its chemical composition was reported previously (Mwaniki et al., 2018). Coefficients for AA digestibility and apparent metabolizable energy (**AME**) values for BSFLM were from published literature (De Marco et al., 2015; Schiavone et al., 2017) and the other feedstuffs from Evonik Aminodat 5.0 (https://animal-nutrition.evonik.com/product/animal-nutrition/aminodat). A standard corn–soybean meal diet was formulated to meet specification for 28-week-old White Shaver pullets according to Shaver White commercial management guidelines (Table 1) (Danzeisen et al., 2011). The present study was a continuation of a previous study where the same birds were fed 0, 5, and 7.5% BSFLM inclusion levels to wk 27 of age (Mwaniki et al., 2018). For the present study, the birds were transitioned to diets containing 0, 10, and 15% BSFLM inclusion levels (Table 1). For the 15% level, BSFLM totally replaced SBM as the main source of AA in the diet. The diets were iso-caloric and iso-nitrogenous and contained TiO_2 as digestibility marker (Table 1). One hundred and eight, 28-wk-old Shaver White Leghorns previously placed in cages (6 birds per cage) based on wk 19 BW were transitioned to the present study, with each diet having 6 replicates. Cage dimensions were 66.0 cm × 62.2 cm × 49.5/46.4 cm (front/rear) for depth × width × height, respectively and were housed in an environmentally controlled room (20°C) at the Arkell Poultry Research Station, University of Guelph. The birds received 14 h of incandescent light (15 lux, 06:00 to 19:00 h) and 10 h of dark per day.

Experimental Procedures

The birds were allowed free access to feed and water to wk 43 of age. Egg production on a cage basis was monitored daily. Feed intake and BW were monitored in 4-wk intervals. Egg weight and quality characteristics were assessed on individual eggs collected on the sixth day of weeks 31, 35, 39, and 43. Fresh excreta samples were collected on week 33 for three consecutive days. Briefly, excreta collection boards were placed under each cage every morning and sample collection done at the end of the day. Feathers and feed particles were removed, and samples put in sealable labeled polythene bags and frozen at −20°C for later analyses. At the end of wk 43, two birds per cage were randomly selected, weighed, and sacrificed via cervical dislocation. The liver, pancreas and empty gizzard, small intestine, and ceca were weighed.

Laboratory Analyses

Egg quality measurements. Egg weight, height of albumin (Haugh units, **HU**), and YC were determined by egg Analyzer (ORKA Food Technology Ltd, Ramat HaSharon, Israel). The systems detect, calculate, and report values for YC (1–15 colors scale based on DSM/Roche Yolk Color Fan), HU, and EW (g). Prior to measurements, the unit was calibrated as per manufacturer recommendations. The eggshell thickness was measured using a high-resolution nondestructive device that measures ST without breaking using precision ultrasound (ESTG-1, ORKA Food Technology Ltd, Ramat HaSharon, Israel). Briefly, a gel is applied on the egg followed by placement of the egg on cradle to read ST in mm. Shell breaking strength (kgf) was measured by Force Reader (ORKA Food Technology Ltd, Ramat HaSharon, Israel); the unit measures accurately the breaking point of the egg shell by applying mechanical force on vertically placed egg on the cradle.

Chemical analyses. Excreta samples were pooled for each cage and oven-dried at 60°C. Samples of diets and excreta were finely ground in a coffee grinder (CBG5 Smart Grind, Applica Consumer Products Inc., Shelton, CT) and thoroughly mixed for analysis. Diets and excreta samples were analyzed for DM, CP, gross energy, crude fat, and neutral detergent fiber (**NDF**). Dry matter determination was carried out according to standard procedures method 930.15 (AOAC, 2005). Nitrogen was determined by the combustion method 968.06 (AOAC, 2005) using a CNS-2000 carbon, N, and sulfur analyzer (Leco Corporation, St. Joseph, MI). The CP values were derived by multiplying the assayed N values by a factor of 6.25. Gross energy was determined using a bomb calorimeter (IKA Calorimeter System C 5000; IKA Works, Wilmington, NC). The NDF contents were determined according to Van Soest et al. (1991) using Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Crude fat content was determined using ANKOM XT 20 Extractor (Ankom Technology, Fairport, NY). Diet samples for AA analysis were prepared by acid hydrolysis according to AOAC (2005, method 982.30), and as modified by Mills et al. (1989). Briefly, about 100 mg of each sample was digested in 4 mL of 6 N HCl for 24 h at 110°C, followed by neutralization with 4 mL of 25% (weight/volume) NaOH and cooled to room temperature. The mixture was then equalized to 50 mL volume with sodium citrate buffer (pH 2.2) and analyzed using an AA analyzer (Sykam, Germany). Samples for analysis of sulfur-containing AA (methionine and cystine) were subjected to performic acid oxidation prior to acid hydrolysis. Tryptophan was not determined. Minerals (Ca, P, K, Mg, and Na), ethanol soluble carbohydrates, and starch were analyzed in a commercial laboratory (SGS Canada Inc, Guelph, ON, Canada). Titanium content in the diet and excreta was measured on a UV spectrophotometer following the method of Myers et al. (2004). The diets and excreta samples were wet acid digested with nitric and perchloric acid mixture (AOAC International, 2005; method 990.08) and concentrations of Ca and P in the supernatant read on an inductively coupled plasma mass spectrometer (Varian Inc, Palo Alto, CA) at the School of Environmental Sciences, University of Guelph.

Table 1. Composition of experimental diets, *as fed.*

	Black fly soldier larvae meal, %		
Item	0	10	15
Corn	45.3	50.6	51.1
Wheat	20.0	20.0	20.0
Soybean meal 46%	18.8	5.24	-
Black fly soldier larvae meal	-	10.0	15.0
Soy oil	1.48	-	-
Limestone fine	7.14	6.99	6.91
Limestone course	2.86	2.79	2.76
Monocalcium phosphate	2.11	2.08	2.04
Vitamin and trace element premix[1]	1.00	1.00	1.00
Salt	0.30	0.31	0.32
DL-Methionine	0.25	0.27	0.28
L-Lysine HCL	0.20	0.18	0.13
L-Threonine	0.09	0.11	0.11
L-Tryptophan	-	0.03	0.05
Sodium bicarbonate	0.13	0.07	0.01
Titanium dioxide	0.30	0.30	0.30
Calculated provisions			
AME, kcal/kg	2,750	2,803	2,847
Crude protein, %	15.00	15.00	15.48
Linoleic acid, %	1.95	1.29	1.27
SID Lys, %	0.75	0.75	0.75
SID Met, %.	0.45	0.52	0.55
SID Met+Cys, %	0.67	0.67	0.67
SID Try, %	0.17	0.15	0.15
SID Thr, %	0.52	0.52	0.52
Ca, %	4.30	4.30	4.30
Available P, %	0.45	0.45	0.45
Na, %	0.18	0.18	0.18
Cl, %	0.25	0.25	0.25

[1]Vitamin mineral premix provided per kilogram of premix: vitamin A, 880,000 IU; vitamin D3, 330,000 IU; vitamin E, 4,000 IU; vitamin B12, 1,200 mcg; biotin, 22,000 mcg; menadione, 330 mg; thiamine, 400 mg; riboflavin, 800 mg; pantothenic acid, 1500 mg; pyridoxine, 300 mg; niacin, 5,000 mg; folic acid, 100 mg; choline, 60,000 mg; iron, 6,000 mg; and copper, 1,000 mg.

Table 2. Analyzed chemical composition of experimental diets, as fed basis.

Item	Black fly soldier larvae meal, % 0	10	15
Dry matter, %	89.2	89.8	90.5
Gross energy, kcal/kg	3,411	3,335	3,457
Crude protein, %	15.1	14.7	14.8
Crude fat, %	3.36	2.65	2.81
Starch, %	39.6	43.3	43.4
Ethanol soluble carbohydrates, %	3.64	1.79	1.43
NDF, %	6.90	9.00	9.68
Ca, %	4.36	4.22	4.56
P, %	0.72	0.74	0.74
K, %	0.67	0.57	0.52
Mg, %	0.18	0.18	0.19
Na, %	0.20	0.20	0.20
Indispensable amino acids, %			
Arg	0.94	0.78	0.71
His	0.45	0.70	0.84
Ile	0.59	0.59	0.63
Leu	1.23	1.24	1.29
Lys	0.92	0.84	0.79
Met	0.67	0.65	0.67
Met+Cys	0.92	0.84	0.81
Phe	0.92	0.91	0.77
Thr	0.73	0.72	0.76
Val	0.78	0.78	0.79
Dispensable amino acids, %			
Ala	0.75	0.88	0.87
Asp	1.47	1.29	1.01
Glu	3.08	2.69	2.34
Gly	0.62	0.66	0.71
Pro	1.07	1.05	1.02
Ser	0.83	0.75	0.66
Tyr	0.51	0.57	0.56

Calculations and Statistical Analyses

Hen-day egg production (%) was calculated in 4-week periods (wks 28–31, 32–35, 36–49, and 40–43). Average EW (g/bird/d), EM (g/bird/d), FI (g/bird/d), and FCR (FI/EM) were calculated as described by Mwaniki et al. (2018). Organ weights (liver, pancreas, gizzard, small intestine, and ceca) were standardized by individual bird BW. Apparent retention (**AR**) of dietary components was calculated as described by Mwaniki and Kiarie (2018).

The data were analyzed using general linear models (**GLM**) procedures (SAS Inst. Inc., Cary, NC) with the cage as experimental unit. Data on egg production, egg quality, and body weight were subjected to a 2-way ANOVA according to the following model; $Y_{ijk} = \mu + D_i + W_j + DWij + e_{ijk}$, where Y is one observation, μ is general mean, D is the diet effect (i = 0, 10, or 15% BSFLM), W is the 4-week period effect (j = 28–31, 32–35, 36–49, and 40–43), DW is the diet × week interaction, and e is the error term. Data on organ weight and AR of components were subjected to a 1-way ANOVA according to the following model; $Y_{ij} = \mu + D_i + e_{ij}$, where Y is one observation, μ is general mean, D is the diet effect (i = 0, 10, or 15% BSFLM), and e is the error term. Contrast coefficients from unequally spaced BSFLM were generated using the interactive matrix language procedure of SAS. Tukey test in SAS was used for further separation of diet and time effects where applicable. An α level of $P \leq 0.05$ was used as the criterion for statistical significance.

RESULTS AND DISCUSSION

The analyzed chemical composition of the diets is shown in Table 2. The analyzed gross energy concentration in 10% BSFLM diet was 2% (−76 kcal/kg) lower than control; however, the value for 15% BSFLM was 3% (+96 kcal/kg) higher than control. This difference in gross energy concentration could not be explained by trends in the concentration of energy-yielding components (CP, starch, crude fat, and NDF).

There was no ($P > 0.05$) diet effect on HDEP (Table 3), suggesting that the noted differences in the analyzed concentration of gross energy in the diets had no impact on egg production (Leeson and Summers, 2005). However, EW decreased quadratically ($P =$

Table 3. Effects of replacing soybean meal with black soldier fly larvae meal (BSFLM) in diets fed to Shaver White layers (28–43 wk of age) on egg production, egg mass, feed intake, FCR, and body weight.

BSFLM inclusion, %	Hen-day egg production, %	Egg weight, g/bird/D	Egg mass, g/D	Feed intake, g/bird/D	FCR	Body weight, kg
0	96.7	57.8[a]	55.9	106.9	1.913	1.548[b]
10	94.6	56.9[b]	53.9	106.0	1.976	1.586[a]
15	95.5	56.5[b]	54.0	109.0	2.022	1.556[a]
SEM	0.96	0.31	0.69	1.67	0.04	0.01
Period, week						
28 to 31	96.6[b]	54.9[c]	53.1[b]	94.6[d]	1.791[b]	1.506[d]
32 to 35	96.8[b]	56.9[b]	55.1[ab]	105.3[c]	1.913[b]	1.536[c]
36 to 39	92.9[b]	57.9[a,b]	53.8[b]	110.8[b]	2.064[a]	1.591[b]
40 to 43	96.2[a]	58.6[a]	56.3[a]	118.6[a]	2.114[a]	1.621[a]
SEM	1.11	0.36	0.80	1.93	0.04	0.01
Probabilities						
BSFLM	0.312	0.010	0.065	0.442	0.130	0.042
Week	0.050	<0.001	0.028	<0.001	<0.001	<0.001
BSFLM x week	0.906	0.933	0.963	0.453	0.746	0.995
Response to BSFLM inclusion						
Linear	0.270	0.884	0.030	0.498	0.045	0.686
Quadratic	0.291	0.003	0.377	0.280	0.849	0.049

[a-d]Means assigned different letters within a factor of analysis (BSFLM, period) are significantly different, $P < 0.05$.

Table 4. Effects of replacing soybean meal with black soldier fly larvae meal (BSFLM) in diets fed to Shaver White layers (28-43 week of age) on egg quality characteristics.[1]

BSFLM inclusion, %,	Yolk color	Haugh units	Shell breaking strength, kgf	Shell thickness, mm
0	4.27[c]	65.3	4.69	0.436
10	4.85[b]	66.5	4.78	0.434
15	5.03[a]	66.3	4.87	0.438
SEM	0.046	1.325	0.068	0.002
Week of age				
31	5.04[a]	66.5	5.12[a]	0.433[c]
35	4.82[b]	67.4	4.58[c]	0.438[a,b]
39	4.29[c]	64.7	4.54[c]	0.432[b,c]
43	4.72[b]	65.4	4.87[b]	0.441[a]
SEM	0.05	1.53	0.08	0.002
Probabilities				
BSFLM	<0.001	0.596	<0.176	0.273
Week	<0.001	0.785	<0.001	0.034
BSFLM × week	0.561	0.328	0.782	0.684
Response to BSFLM inclusion				
Linear	<0.001	0.506	<0.001	0.863
Quadratic	0.993	0.266	0.129	0.028

[a-c]Means assigned different letters within a factor of analysis (BSFLM, week) are significantly different, $P < 0.05$.

0.003) with BSFLM inclusion, and as a result there was a significant linear decrease in EM (P = 0.03); the EM for the control, 10% BSFLM, and 15% BSFLM was 55.9, 53.9, and 54.0 g/bird/D, respectively. This could be due to low analyzed values of Met + Cys and Lys in 10 and 15% BSFLM diets (Table 2).The supply of adequate protein (**AA**) is critical for egg size in laying hens, and energy content must also be put into account to avoid utilization of protein to meet energy requirements (Leeson and Summers, 2005; Robinson and Kiarie, 2019). Moreover, linoleic acid is well known to affect egg size (March and MacMillan, 1990; Leeson and Summers, 2005). Although the intent was to design isocaloric diets, based on calculated provisions, 10 and 15% BSFLM diets had 35% less linoleic acid relative to the control. This could have partly contributed to the decrease in egg size with BSFLM inclusion. It also appears that BSFLM somewhat had a negative impact on AA utilization. Diets were formulated with equal AA concentration, but the smaller eggs in BSFLM fed diets suggested AA may have been limiting. Moderate and variable retention of protein and energy in broilers fed BSFLM has been attributed to the negative effects of chitin (De Marco et al., 2015; Schiavone et al., 2017; Mwaniki and Kiarie, 2018). Broiler chickens fed chitin derived from crustacean shell waste (37.3% CP) digested approximately 50% of chitin protein (Marono et al., 2015). A study by Hossain and Blair (2007) demonstrated that *in vitro* CP digestibility was negatively correlated to the chitin content. Although chitin concentration was not measured in the current study, the increased concentration of NDF with addition of BSFLM suggested increased dietary concentration of chitin. Increase in EW with age can be explained by increased proportion of yolk at the expense of albumen as the bird ages (Whitehead et al., 1991).

Feeding BSFLM linearly increased (P = 0.045) FCR (FI/EM) attributed to reduced EM as FI was not influenced by the diet (P = 0.442). The observed FCR was 1.91, 1.98, and 2.02 for the control, 10% BSFLM, and 15% BSFLM, respectively. The FCR was also shown to increase in Lohmann Brown Classic laying hens fed diet containing BSFLM as a total replacement of SBM (Marono et al., 2017) and with partial replacement of SBM (Mwaniki et al., 2018). There was a significant increase in HDEP (P = 0.05), EW (P < 0.001), and EM (P = 0.028) with increase in bird age. There was a quadratic increase in hen body weight because of BSFLM (P =

Table 5. Effects of replacing soybean meal with black soldier fly larvae meal (BSFLM) in diets fed to Shaver layers (28-43 week of age) on organ weights.

	Organ weight, g/kg body weight[1]				
BSFLM inclusion, %	Gizzard	Small intestine	Ceca	Liver	Pancreas
0	8.81	13.8	3.13[a]	24.0[b]	2.27
10	8.55	13.5	2.55[b]	27.9[a]	1.99
15	8.82	13.2	2.47[c]	29.8[a]	1.96
SEM	0.37	0.58	0.09	1.09	0.11
Probabilities	0.853	0.739	0.0004	0.006	0.117
Response to BSFLM inclusion					
Linear	0.605	0.838	0.003	0.074	0.135
Quadratic	0.839	0.461	0.002	0.005	0.115

[a-c]Means assigned different letters within a column are significantly different, $P < 0.05$.
[1]Values are average of two birds sacrificed at the end of week 43 of age.

Table 6. Effects of replacing soybean meal with black soldier fly larvae meal (BSFLM) in diets fed to Shaver White layers (28–43 wk of age) on apparent retention (AR, %) of components and AME (kcal/kg).

BSFLM inclusion, %	Dry matter	Gross energy	Crude fat	NDF	Ca	P	AME as fed	AME DM
0	77.8	84.7	86.6	40.8[c]	36.1[a]	29.2	2,913[b]	3,208[b]
10	78.9	85.4	86.2	64.9[b]	28.6[b]	25.4	2,921[b]	3,216[b]
15	79.1	86.1	86.6	68.9[a]	41.7[a]	29.4	3,043[a]	3,329[a]
SEM	0.74	0.58	0.99	1.86	2.52	5.11	20.3	22.2
Probabilities	0.399	0.275	0.946	<0.001	0.008	0.826	0.001	0.002
Response to BSFLM inclusion								
Linear	0.191	0.124	0.909	<0.001	0.375	0.935	0.001	0.003
Quadratic	0.784	0.686	0.757	0.036	0.003	0.547	0.007	0.033

[a,b]Means assigned different letters within a column are significantly different, $P < 0.05$.

0.042) (Table 3). Body weight increased significantly with the age of birds ($P < 0.001$). Feeding BSFLM linearly ($P < 0.001$) increased YC intensity (Table 4) in line with previous observations (Mwaniki et al., 2018). This also agreed with a study by Secci et al. (2018) where eggs from birds fed BSFLM had a deeper orange color in comparison with their SBM counterparts. B-carotene and lutein are the main pigments responsible for an orange-yellow color of yolks (Leeson and Summers, 2005).

A linear ($P < 0.001$) and quadratic ($P = 0.028$) increase in SBF and ST, respectively, was observed with increasing BSFLM levels. The quadratic increase in ST was a result of 10% BSFLM inclusion level having bigger eggs hence less ST as compared to 15% BSFLM. Our previous study showed a linear increase in ST with inclusion of BSFLM (Mwaniki et al., 2018). It is plausible that increase in SBS with addition of BSFLM could have resulted from smaller eggs in birds fed BSFLM as compared to those fed SBM (Table 4). Similar observations were also made in our previous study, Mwaniki et al. (2018) where 5% BSFLM level had smaller eggs with highest SBS compared to 0 and 7.5% levels. This suggested that shell strength is related to egg size in agreement with previous indications that EM is allometrically related to eggshell structural properties (Ar et al., 1979). Improved calcium absorption and metabolism in BSFLM-fed birds could also have contributed to the stronger eggshells. For example, Marono et al. (2017) showed that feeding laying hens (wk 24–45) 17% BSFLM increased circulating serum Ca levels relative to the control (0% BSFLM) despite the 2 diets having similar Ca concentration.

Feeding BSFLM quadratically ($P = 0.005$) increased liver weight (Table 5). Inclusion of BSFLM reduced empty ceca weight linearly ($P = 0.003$) and quadratically ($P = 0.002$). This was unexpected because high fiber diets tend to increase sizes of ceca and small intestine weight (Kiarie et al., 2013). There was, however, no effect ($P > 0.05$) on pancreas, small intestine, and gizzard weight (Table 5). There was no diet effect on AR of DM ($P = 0.399$), GE ($P = 0.275$), and crude fat ($P = 0.946$) on replacement of SBM with BSFLM (Table 6). However, the AR of NDF increased linearly ($P < 0.0001$) and quadratically ($P = 0.036$) with inclusion of BSFLM. Black Soldier Fly contains fiber in the form of chitin, and birds possess the ability to break down chitin because of presence of endogenous chitinase in the proventricular mucosa (Hossain and Blair, 2007). This could explain the increased fiber utilization in diets containing BSFLM. Increased retention of NDF could also indicate increased fermentation of chitin. Laying birds require Ca for eggshell formation, bone formation, and normal metabolic functions (Leeson and Summers, 2005). Dietary sources are not usually enough to meet the bird's requirements and, as such, inorganic sources of Ca and P are added to diets (Akbari Moghaddam Kakhki et al., 2018; 2019). Feeding BSFLM had a quadratic ($P = 0.003$) response on AR of Ca, with 10% BSFLM showing lower AR of Ca relative to control or higher 15% BSFLM (Table 6). Based on observations on shell quality (SBS and ST), reduced AR of Ca in birds fed 10% BSFLM was unexpected and could point at inaccuracies in Ca analyses in the diet and/or excreta. Replacing SBM with BSFLM did not affect AR of P ($P > 0.05$). The AME increased linearly ($P = 0.001$) and quadratically ($P = 0.007$) with inclusion of BSFLM. Because AR of GE was not affected by the diets, effects on AME were due to higher GE in 15% BSFLM diet.

CONCLUSIONS

This study showed that defatted BSFLM can totally replace SBM without negatively affecting HDEP; however, poor FCR was observed because of lower EW. The deeper orange yolks suggested BSFLM had pigments that changed the YC. Further investigations are warranted to unravel nutritional and metabolic consequences of feeding hens BSFLM with respect to EW, shell quality, and visceral organs.

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