The effects of partially replacing animal protein sources with full fat black soldier fly larvae meal (*Hermetia illucens*) in nursery diets on growth performance, gut morphology, and immune response of pigs

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ABSTRACT: One hundred and forty-four newly weaned pigs (6.74 ± 0.23 kg initial BW; 21 d of age) were used to determine the effect of partially replacing animal protein sources with black solider fly larvae meal (BSFLM) in nursery diets on growth performance, gut morphology, and immune response. After weaning, pigs were placed in 24 pens (six pigs per pen) and pens were randomly assigned to one of four dietary treatments (study d 0; $n = 6$), which were fed over three phases (phases I, II, and III were fed for 7, 14, and 21 d, respectively). Two nursery diets were formulated with 25% (LowFF) and 50% (HighFF) of the animal protein sources replaced by full fat BSFLM. Conventional nursery diets including animal protein sources without (CON−) and with antibiotics (220 mg Aureomycin per kg of complete feed; CON+) served as controls. On d 8, two pigs per pen were sacrificed to collect organ weights and for intestinal histomorphological measurements. On d 9 and d 23, two pigs per pen were vaccinated with the novel antigen ovalbumin (OVA). Blood samples were collected on d 9, d 23, and d 38 to assess concentrations of plasma haptoglobin and OVA-specific immunoglobulins G (IgG) and IgG1. On d 38, the same two pigs per pen underwent a dermal hypersensitivity test and skin-fold thickness was measured at 0, 6, 24, and 48 h postintradermal injection with OVA. Pigs fed the CON- had greater ADFI and lower G:F in phase II vs. those fed CON+ and HighFF diets ($P < 0.05$ and $P < 0.05$); intermediate ADFI was observed for pigs fed the LowFF diet. Overall in the nursery period, ADG (496 ± 13 g), ADFI (743 ± 23 g), G:F, and final BW (27.61 ± 0.66 kg) were not different among dietary treatments. There were no differences in organ weights, jejunal or ileal villus heights, or crypt depths among dietary treatments. There were no differences in OVA-specific IgG, IgG1, or plasma haptoglobin among dietary treatments at any of the blood sampling times. Although not different, pigs fed the LowFF, HighFF, and CON+ diets had respectively 2.0×, 1.7×, and 1.4× greater dermal hypersensitivity response to OVA versus those fed CON−. Both inclusion levels of BSFLM in nursery diets supported growth performance, gut morphology, and indices of immune function not different from the CON+, which suggest that full fat BSFLM can replace at least 50% of animal protein sources in nursery diets of pigs without any deleterious effects on pig growth.

Key words: black soldier fly larvae meal, growth performance, immune response, nursery pig

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INTRODUCTION

Nursery pig diets often contain highly digestible sources of animal proteins to promote increased absorption of nutrients and minimize the postweaning growth lag that is caused, in part, by exposure to food allergens and pathogens, an immature gastrointestinal tract, and reduced feed intake (Lallès et al., 2004). Recent studies in pigs (Crosbie et al., 2020) and poultry (Mwaniki and Kiarie, 2019) have demonstrated that the amino acid digestibility coefficients of BSFLM are high (i.e., 88% standardized ileal digestibility of Lys for growing pigs and 84% apparent ileal digestibility of Lys for broilers, respectively) and comparable to protein sources that are commonly used in nursery pig diets. While black soldier fly larvae meal (*Hermetia illucens*; BSFLM) is still cost-prohibitive compared to other commonly fed animal proteins, such as blood and fish meals, its proposed functional benefits may make it useful for inclusion in nursery diets, as these diets are typically fed for shorter durations and when feed intake is low (Biasato et al., 2019).

Black soldier fly larvae meal contains medium chain fatty acids (MCFA), particularly lauric acid, which makes up approximately 70% of the total saturated fatty acid content in BSFLM (depending on rearing substrate; Spranghers et al., 2016; Surendra et al., 2016; Barragan-Fonseca et al., 2017). Lauric acid, and its metabolites, have antimicrobial and anti-inflammatory properties in the small intestine (Devi and Kim, 2014; Spranghers et al., 2016). Additionally, the exoskeleton of black soldier fly larvae contains chitin, which can act as a prebiotic to support a balanced and diverse population of beneficial gut microbes and has immunostimulatory properties (Borrelli et al., 2017). Together, these functional attributes of BSFLM could reduce the colonization of pathogenic bacteria in the gut and lead to increased nutrient absorption and subsequent growth performance, making it an attractive feed ingredient for use in the postweaning period (Fioramonti et al., 2003; Heo et al., 2013).

The favorable amino acid profile and digestibility and functional attributes of BSFLM make it a possible protein source for inclusion in pig diets during instances of reduced digestive capacity and low feed intake (Crosbie et al., 2020). Therefore, the objective of the current study was to determine the effect of partially replacing animal protein sources with BSFLM in nursery pig diets on growth performance, gut morphology, and immune response.

MATERIALS AND METHODS

Animal care and use protocols were approved by the University of Guelph Animal Care and Use Committee (AUP #4044). Pigs were cared for in accordance with the Canadian Council on Animal Care guidelines (CCAC, 2009). The study was conducted at the University of Guelph Arkell Swine Research Station (Guelph, ON, Canada).

Animals and Experimental Diets

One hundred and forty-four newly weaned pigs (Yorkshire × Landrace × Duroc; 6.74 ± 0.23 kg initial BW; 21 d of age) were selected and housed in an environmentally-controlled (26 °C) room in pens that measured 148 × 107 cm with fully slatted, plastic-coated, expanded metal floors. The experiment was conducted using a randomized complete block design (n = 6 over two blocks). In each block, pigs were divided into 12 pens at weaning (six pigs per pen, three castrated males, and three females; balanced for BW within the pen and assigning littermates to different pens) and pens were randomly assigned to one of the four dietary treatments, which were fed over three phases. Feed and water, through a low-pressure drinking nipple, were provided ad libitum. Phases I, II, and III were fed for 7, 14, and 21 d, respectively, and individual pig BW and per-pen feed disappearance were recorded weekly to determine ADG, ADFI, and G:F in each phase.

One full fat (FF; Oreka Solutions, Markham, ON, Canada) BSFLM source was used and was ground using a 0.6 mm screen size (Crosbie et al., 2020). Two control diets were generated (CON− with no antibiotics; CON+ with 220 mg Aureomycin/kg of complete feed; Table 1), which contained highly digestible animal proteins (i.e., dried whey, fish meal, spray dried blood meal, and blood plasma) and crystalline amino acids, to mimic commercial nursery diets. For the BSFLM-containing diets, 25% (LowFF) and 50% (HighFF) of the animal protein sources were replaced by FF BSFLM. Diets were formulated to meet or exceed the estimate nutrient requirements of nursery pigs (NRC, 2012). Phase I diets were provided as a crumble, and phases II and III were pelleted. Subsamples of all diets were collected on a weekly basis. Each diet composite sample was analyzed for dry matter (AOAC, 2005; method 930.15), crude protein (AOAC, 2005; method 968.06), calcium, phosphorus, potassium, and magnesium using inductively coupled plasma

Table 1. Ingredient and calculated nutrient composition of experimental diets (as-fed basis)[a]

Item	CON–/+			LowFF			HighFF		
	Phase I	Phase II	Phase III	Phase I	Phase II	Phase III	Phase I	Phase II	Phase III
Ingredient, %									
Corn	16.30	37.90	50.07	19.02	37.90	50.06	19.78	38.29	49.03
Soybean meal, dehulled	13.00	16.00	22.00	11.00	16.50	22.00	10.00	16.00	23.00
Barley	25.00	25.00	20.00	25.00	25.00	20.00	25.00	25.00	20.00
Oat groats	10.00	–	–	10.00	–	–	10.00	–	–
Whey, dried	20.00	8.00	–	15.00	6.00	–	10.00	4.00	–
Fish meal	5.00	3.00	–	3.75	2.25	–	2.50	1.50	–
Blood meal, spray dried	–	2.00	2.00	–	1.50	1.50	–	1.00	1.00
Blood plasma[b]	4.50	2.00	–	3.38	1.50	–	2.25	1.00	–
Black solider fly larvae meal, full fat[c]	–	–	–	7.38	3.75	0.50	14.76	7.50	1.00
Animal vegetable fat	3.50	3.00	2.50	2.50	2.50	2.50	2.50	2.50	2.50
L-Lysine·HCl	0.41	0.37	0.38	0.52	0.42	0.43	0.60	0.51	0.44
DL-Methionine	0.16	0.16	0.14	0.26	0.20	0.15	0.34	0.25	0.16
L-Threonine	0.13	0.13	0.14	0.17	0.14	0.15	0.20	0.18	0.15
L-Tryptophan	–	0.01	0.01	0.03	0.03	0.01	0.07	0.05	0.02
Limestone	0.50	0.58	0.86	0.50	0.46	0.80	0.50	0.37	0.80
Monocalcium phosphate	0.80	1.05	1.00	0.80	1.05	1.00	0.80	1.05	1.00
Salt	0.10	0.20	0.30	0.10	0.20	0.30	0.10	0.20	0.30
Vitamin and mineral premix[d]	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Total	100.00	100.00	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated nutrient composition[e]									
ME, kcal/kg	3470	3395	3351	3508	3415	3358	3586	3457	3361
CP, %	21.34	20.31	19.23	21.61	20.64	19.05	22.15	20.59	19.23
Total Lys, %	1.63	1.49	1.34	1.62	1.49	1.35	1.62	1.49	1.35
SID Lys, %[f]	1.46	1.34	1.21	1.46	1.34	1.21	1.46	1.34	1.22
SID Thr, %[f]	0.86	0.79	0.72	0.86	0.79	0.72	0.86	0.79	0.72
SID Met, %[f]	0.46	0.44	0.40	0.56	0.49	0.41	0.65	0.54	0.41
SID Met + Cys, %[f]	0.80	0.74	0.67	0.80	0.74	0.67	0.80	0.74	0.67
SID Trp, %[f]	0.25	0.23	0.21	0.24	0.23	0.21	0.24	0.23	0.21
SID Val, %[f]	0.90	0.89	0.81	0.89	0.88	0.78	0.90	0.85	0.77
Total calcium,w %	0.85	0.76	0.69	0.91	0.75	0.68	0.97	0.75	0.70
Total phosphorus, %	0.83	0.75	0.63	0.77	0.73	0.63	0.72	0.70	0.64
STTD phosphorus, %[g]	0.61	0.52	0.39	0.53	0.49	0.39	0.45	0.45	0.35

[a] Dietary treatments: conventional nursery diet that contained multiple sources of proteins, including plant and animal proteins without (CON−) or with 220 mg of Aureomycin per kg of complete feed (CON+), conventional nursery diet with 25% (LowFF) or 50% (HighFF) of animal proteins replaced with full fat black soldier fly larvae meal (BSFLM). Diets were fed for 7, 14, and 21 d in phases I, II, and III, respectively.

[b] AP920; manufactured by APC Nutrition Inc. (Ames, IA).

[c] Full fat BSFLM obtained from Oreka Solutions (Markham, ON, Canada).

[d] Provided, per kilogram of diet, 12,000 IU vitamin A as retinyl acetate, 1,200 IU vitamin D3 as cholecalciferol, 48 IU vitamin E as DL-α-tocopherol acetate, 3 mg vitamin K as menadione, 18 mg pantothenic acid, 6 mg riboflavin, 600 mg choline, 2.4 mg folic acid, 30 mg niacin, 18 mg thiamine, 1.8 mg pyridoxine, 0.03 mg vitamin B12, 0.24 mg biotin, 1,200 mg Ca from $CaCO_3$, 18 mg Cu from $CuSO_4 \cdot 5H_2O$, 120 mg Fe from $FeSO_4$, 24 mg Mn from MnSO4, 126 mg Zn from $ZnSO_4$, 0.36 mg Se from Na_2SeO_3, and 0.6 mg I from KI (DSM Nutritional Products Canada Inc., Ayr, ON, Canada).

[e] Calculated on the basis of the NRC (2012) ingredient values with BSFLM ingredient values from Crosbie et al. (2020).

[f] Standardized ileal digestible.

[g] Standardized total tract digestible.

mass spectrophotometry (AOAC, 2005; method 985.01), and sodium using inductively coupled plasma-optical emission spectrometry (AOAC, 2005; method 2011.14) at Agrifood Laboratories (Guelph, ON, Canada).

Assessment of Gut Morphology and Immune Response

On d 8 postweaning, 12 pigs per treatment (one castrated male and one female per pen with BW similar to the pen average) were euthanized with 3 mL of Euthasol (Virbac, TX). Immediately thereafter, the entire gastrointestinal tract was excised, and gut and organ weights were measured. Five centimeters of the jejunum (approximately 1.5 m distal to the ligament of Trietz) and ileum (approximately 0.5 m proximal to the ileocecal junction) were removed, rinsed with saline, and placed in 10% formalin until further analysis. Ileal and jejunal segments were prepared for histology analysis according to the procedures of Carleton et al. (1980). Measurements of villi height and crypt depth were collected from a minimum of the five longest villi in each intestinal section using a Leica DMR fluorescence microscope (Leica Microsystems Inc., Wetzlar, Germany) and Openlab Computer Imaging System (Perkin Elmer, Waltham, MA).

On d 9 and 23 postweaning, an additional two pigs per pen (one castrated male and one female) were vaccinated with 1 mL of ovalbumin (OVA) with Quil A as the adjuvant (0.5 mg OVA plus 0.5 mg Quil A/mL of physiological saline; Sigma-Aldrich Co., St Louis, MO). On d 38, pigs were injected intradermally with 0.1 mL of OVA (0.1 mg/mL) into the inner thigh of the hind leg to assess the dermal hypersensitivity response (DHR). Physiological saline (0.1 mL; Sigma-Aldrich Co., St Louis, MO) was also intradermally injected in each thigh at least 5 cm from the OVA test site to act as a control. Skin-fold thickness was measured at the injection sites at 0 h (prior to intradermal injection), 6, 24, and 48 h postinjection as an indicator of the dermal immune response. All skin-fold thickness measurements were taken using callipers (Model RH15 9LB, Creative Health Products Inc., Ann Arbor, MI) and performed by the same individual to reduce subjective bias. The change in skin-fold thickness (mm) over time relative to the saline-injection measurement was used to determine the DHR (Eq. 1).

$$\text{DHR (mm)} = (\text{SFT}_{\text{time x}} - \text{SFT}_{\text{time 0}}) - (\text{Saline}_{\text{time x}} - \text{Saline}_{\text{time 0}}) \quad (1)$$

where $\text{SFT}_{\text{time } x}$ is the skin-fold thickness in mm at the OVA injection site at h 0, 6, 24, or 48, $\text{SFT}_{\text{time 0}}$ is the skin-fold thickness in mm at the OVA injection site at h 0, $\text{Saline}_{\text{time } x}$ is the skin-fold thickness in mm at the saline injection site at h 0, 6, 24, or 48, and $\text{Saline}_{\text{time 0}}$ is the skin-fold thickness in mm at the saline injection site at h 0.

Blood samples (6 mL) from the vaccinated pigs were collected via orbital-sinus puncture into plasma vacutainer tubes containing an anticoagulant (EDTA; BD Vacutainer, BD, Franklin Lakes, NJ, USA) to assess the antigen-specific immune response to vaccination on d 9 (basal levels), d 23 (primary response), and at 0 h on d 38 (secondary response) postweaning for determination of plasma OVA-specific immunoglobulins G (IgG), OVA-specific IgG1, and plasma haptoglobin (Hp). Blood samples were stored on ice and then centrifuged for 20 min at 3,000 × *g* and 4 °C. The resulting plasma was aliquoted into microcentrifuge tubes, and subsequently stored at −20 °C further until analysis.

The OVA-specific IgG response was quantified using an indirect ELISA method as described by Begley et al. (2008) and the OVA-specific IgG1 response was quantified using an indirect ELISA method as described by Lee et al. (2019). For both OVA-specific IgG and IgG1, high-affinity binding 96-well microtiter plates (Corning, Acton, MA) were used; the basal (plasma pooled from all pigs on d 9), quality control, and plasma samples were tested in triplicate, and the optical density was measured at 405 nm using a Wallac 1420 Victor3 Multilabel counter (Perkin Elmer, Waltham, MA). The optical densities for individual plates were adjusted using a correction factor (Eq. 2). The average intra- and inter-assay variations were 3.4% and 4.3% for OVA-specific IgG and 4.4% and 2.1% for OVA-specific IgG1, respectively.

$$\text{Correction factor} = \frac{\text{Overall mean of reference samples from all plates}}{\text{Actual mean of individual plate basal sample}} \quad (2)$$

Plasma Hp concentrations were determined by the clinical pathology service of the Animal Health Laboratory, University of Guelph, using a Roche Cobas c501 biochemistry analyzer (Roche Diagnostics, Indianapolis, IN).

Statistical Analysis

Statistical analyses of growth performance, organ and gut weight measurements, and histology were conducted using the GLIMMIX procedure of

Table 2. Analyzed nutrient composition of experimental diets (%, as-fed basis)[a]

Item	CON−			CON+			LowFF			HighFF		
	Phase I	Phase II	Phase III	Phase I	Phase II	Phase III	Phase I	Phase II	Phase III	Phase I	Phase II	Phase III
Dry matter	87.6	88.8	88.6	87.6	88.8	88.7	88.2	88.8	88.9	87.4	88.2	89.2
Crude protein	19.9	19.5	19.6	20.0	19.9	19.4	21.5	20.8	17.9	22.4	19.5	18.7
Calcium	0.82	0.74	0.61	0.89	0.78	0.63	0.88	0.69	0.63	0.97	0.74	0.57
Phosphorus	0.78	0.72	0.55	0.81	0.74	0.58	0.73	0.68	0.58	0.70	0.66	0.58
Sodium	0.28	0.22	0.15	0.32	0.23	0.16	0.27	0.18	0.13	0.22	0.17	0.14
Potassium	0.86	0.76	0.76	0.92	0.77	0.73	0.84	0.80	0.74	0.77	0.75	0.79
Magnesium	0.13	0.14	0.16	0.13	0.14	0.15	0.14	0.15	0.15	0.15	0.15	0.16

[a] Dietary treatments: conventional nursery diet that contained multiple sources of proteins, including plant and animal proteins without (CON−) or with 220 mg of Aureomycin per kg of complete feed (CON+), conventional nursery diet with 25% (LowFF) or 50% (HighFF) of animal proteins replaced with full fat black soldier fly larvae meal. Diets were fed for 7, 14, and 21 d in phases I, II, and III, respectively.

SAS (SAS Inst. Inc., Cary, NC) where the pen was considered the experimental unit, diet was the fixed effect, and block and pen within treatment were considered random effects. Statistical analyses for plasma Hp, plasma OVA-specific IgG, and DHR were conducted using the GLIMMIX procedure of SAS with a pen as the experimental unit, dietary treatment, and time, and the interaction between dietary treatment and time as fixed effects, and using the repeated measures option. Block and pen within a dietary treatment were considered random effects. Villus height-to-crypt depth ratio and plasma Hp were log-transformed prior to statistical analysis. When appropriate, initial BW at d 0 was used as a covariate. Differences among individual means were assessed using the Tukey−Kramer posthoc test. Linear contrasts were constructed to compare the responses of pigs fed increasing inclusion levels of BSFLM in the CON-, LowFF, and HighFF diets, however, no significance was detected so these contrasts were not presented. A probability of less than 0.05 was considered significant, whereas $0.05 \leq P \leq 0.10$ was considered a tendency, and $P > 0.10$ was considered not significant.

RESULTS

Two pigs (one each from CON- and HighFF) were removed from the study due to lameness and death unrelated to dietary treatments. One pen fed the LowFF diet was removed from the study due to water drinker malfunction in the first week after weaning. All other pigs remained healthy throughout the study.

The chemical analyses of nursery diets were comparable to calculated values, except for the calcium content for phase III of the HighFF diet, which was 19% lower than expected and likely reflected a systematic error during calcium analysis (Tables 1 and 2).

Growth Performance

On d 7, the BW of pigs fed the LowFF diet were greater than pigs fed the HighFF diet ($P < 0.05$) and intermediate BW were observed for pigs fed the CON− and CON+ diets (Table 3). The BW of pigs on d 21 and 42 (end of study) were not different among dietary treatments and there were no differences in ADG among pigs fed dietary treatments in any phase. Pigs fed the CON− diet had greater ADFI in phase II vs. pigs fed the CON+ and HighFF diets ($P < 0.05$); ADFI was intermediate for pigs fed the LowFF diet. The ADFI

Table 3. Effect of black soldier fly larvae meal inclusion in nursery diets on growth performance of pigs after weaning

Item	Dietary treatment[a]				SEM[b]	*P*-value
	CON+	CON−	LowFF	HighFF		
No.	6	6	5	6		
BW, kg						
d 0	6.46	6.68	7.30	6.50	0.25	0.094
d 7	7.53[a,b]	7.49[a,b]	7.69[a]	7.01[b]	0.17	0.018
d 21	13.28	13.56	13.69	12.94	0.39	0.345
d 42	27.88	27.34	27.93	27.30	0.66	0.754
ADG, g						
Phase I	78	100	96	50	24	0.154
Phase II	400	431	412	442	21	0.437
Phase III	685	652	696	678	25	0.600
Overall	493	493	514	483	13	0.442
ADFI, g						
Phase I	167	164	164	138	14	0.384
Phase II	467[b]	543[a]	516[a,b]	447[b]	20	0.003
Phase III	1123	1093	1162	1077	36	0.355
Overall	743	737	780	712	23	0.238
G:F						
Phase I	0.47	0.62	0.57	0.31	0.10	0.103
Phase II	0.86[a]	0.79[b]	0.85[a]	0.90[a]	0.04	0.018
Phase III	0.61	0.59	0.60	0.63	0.01	0.156
Overall	0.67	0.66	0.66	0.68	0.01	0.434

[a] Dietary treatments: conventional nursery diet that contained multiple sources of proteins, including plant and animal proteins without (CON−) or with 220 mg of Aureomycin per kg of complete feed (CON+), conventional nursery diet with 25% (LowFF) or 50% (HighFF) of animal proteins replaced with full fat black soldier fly larvae meal. Diets were fed for 7, 14, and 21 d in phases I, II, and III, respectively.

[b] Maximum value of standard error of the means.

[a,b] Values with different letters within the same row differ ($P < 0.05$).

did not differ among pigs fed dietary treatments in phases I and III or over the entire experimental period. Pigs fed the CON− diet had lower G:F in phase II compared to pigs fed the CON+, LowFF, and HighFF diets ($P < 0.05$), but G:F did not differ among pigs fed dietary treatments in phases I and III or over the entire experimental period.

Gut Morphology

There were no differences for stomach, small and large intestine, and empty and full gut relative weights 8 d after weaning, though the relative liver weight tended to be influenced by the main effect of dietary treatment ($P = 0.093$; Table 4). There were also no differences in ileal and jejunal morphology (Table 5) among dietary treatments.

Immune Response to OVA Vaccination

Neither dietary treatment, time, or the interaction between dietary treatment and time after weaning influenced plasma Hp (Table 6). As expected, plasma OVA-specific IgG and OVA-specific IgG1 concentrations were greater on d 38 (secondary response) than on d 23 (primary response; $P < 0.001$). However, neither dietary treatment, nor the interaction between dietary treatment and time after weaning, influenced plasma OVA-specific IgG, or OVA-specific IgG1 concentrations. The DHR to OVA challenge was not influenced by either dietary treatment, or the interaction between dietary treatment and time, however, time had a significant effect on DHR ($P < 0.001$). Though not significant, as indicated by the mean separation with the Tukey adjustment, pigs fed the LowFF, HighFF, and CON+ diets had 2.0×, 1.7×, and 1.4× greater DHR to OVA versus those fed CON− 6 h after the intradermal OVA injection (Table 6).

DISCUSSION

The current study demonstrated that nursery diets supplemented with FF BSFLM supported growth performance, gut morphological parameters 8 d after weaning, and immune response comparable to conventional nursery diets with or without in-feed antibiotics included at

Table 4. Effect of black soldier fly larvae meal inclusion in nursery diets on relative organ (g/kg of live weight) of pigs 8 d postweaning

	Dietary treatment[a]					
Item, g/kg live weight	CON+	CON−	LowFF	HighFF	SEM[b]	*P*-value
No.[c]	6	6	5	6		
Liver	23.7	24.6	25.7	26.8	1.54	0.093
Stomach	7.3	7.6	7.3	8.4	0.52	0.274
Small intestine	47.9	46.4	48.1	46.6	2.77	0.924
Large intestine	16.2	17.3	17.2	18.4	0.88	0.274
Empty gut	71.3	71.3	72.7	73.4	3.11	0.895

[a]Dietary treatments: conventional nursery diet that contained multiple sources of proteins, including plant and animal proteins without (CON−) or with 220 mg of Aureomycin per kg of complete feed (CON+), conventional nursery diet with 25% (LowFF) or 50% (HighFF) of animal proteins replaced with full fat black soldier fly larvae meal. Diets were fed for 7 d in phase I.

[b] Maximum value of standard error of the means.

[c] Each observation represents the mean of two animals (one castrated male and one female) per pen.

Table 5. Effect of black soldier fly larvae meal inclusion in nursery diets on ileal and jejunal morphology of pigs 8 d postweaning

	Dietary treatment[a]					
Item	CON+	CON−	LowFF	HighFF	SEM[b]	*P*-value
No.[c]	6	6	5	6		
Jejunum						
Villi height, μm	463	483	464	467	46	0.971
Crypt depth, μm	254	288	307	276	24	0.171
Villus:crypt ratio	1.80	1.65	1.52	1.68	0.17	0.742
Ileum						
Villi height, μm	498	442	446	454	34	0.545
Crypt depth, μm	278	258	287	274	19	0.444
Villus:crypt ratio	1.81	1.70	1.53	1.66	0.11	0.422

[a]Dietary treatments: conventional nursery diet that contained multiple sources of proteins, including plant and animal proteins without (CON−) or with 220 mg of Aureomycin per kg of complete feed (CON+), conventional nursery diet with 25% (LowFF) or 50% (HighFF) of animal proteins replaced with full fat black soldier fly larvae meal. Diets were fed for 7 d in phase I.

[b] Maximum value of standard error of the means.

[c] Each observation represents the mean of two animals (one castrated male and one female) per pen.

growth-promoting levels. The G:F of pigs fed the CON+, LowFF, and HighFF diets in phase II were approximately 9% greater than that of pigs fed the CON− diet, which was largely driven by differences in ADFI. The relatively lower feed intake for pigs fed the HighFF diet could be due to palatability issues with high inclusion levels of BSFLM in this phase (7.5%), but this inclusion level was less than for phase I where no differences in ADFI were observed. Though, it is noted that the variability in feed intake during phase I could have precluded the detection of differences among treatment groups. In addition, pigs fed the CON+ diet also had lower ADFI in phase II, which was contrary to the typical response to in-feed antibiotic inclusion where ADFI is increased compared to pigs fed diets with no in-feed antibiotics (Williams et al., 2018). Regardless, over the entire nursery period, there were no differences in ADG, ADFI, and G:F ratios among dietary treatments, indicating that pigs fed diets containing BSFLM performed just as well as those that received conventional nursery diets, with and without in-feed antibiotics as growth promoters. Therefore, at least under the current experimental conditions, BSFLM could be used to replace either 25% or 50% of the highly digestible animal protein sources in nursery diets, and up to an inclusion level of approximately 15% during the first week after weaning.

With respect to humoral immunity, OVA-specific IgG and IgG1 were detected in all pigs that were immunized with OVA, with a greater response observed on d 38 than on d 23, which indicated that the immunization protocol was efficacious; a similar protocol has been used effectively in pigs previously (Crawley et al., 2005; Huber et al., 2018). The inclusion of BSFLM to replace a portion (25% or 50%) of animal protein sources and the inclusion of

Table 6. Effect of black soldier fly larvae meal on plasma haptoglobin concentrations, plasma anti-ovalbumin (OVA) IgG and IgG1 response, and the dermal hypersensitivity response to OVA of pigs after weaning

	Dietary Treatment[a]					*P*-value[b]		
Item	CON+	CON−	LowFF	HighFF	SEM[c]	Diet	Time	Diet × time
No.[d]	12	12	10	12				
Plasma haptoglobin, g/L								
d 9	0.40	0.67	0.44	0.57	0.17	0.826	0.343	0.172
d 23	0.60	0.54	0.93	0.45	0.29			
anti-OVA IgG[e]								
d 23	0.15	0.17	0.20	0.21	0.12	0.833	<0.001	0.443
d 38	1.06	0.83	0.95	1.02	0.12			
Anti-OVA IgG1[e]								
d 23	0.03	0.07	0.14	0.06	0.19	0.960	<0.001	0.864
d 38	0.66	0.74	0.73	0.85	0.19			
Change in skinfold thickness, mm								
h 0	−0.01	−0.01	0.05	0.01	0.21	0.401	<0.001	0.325
h 6	1.19	0.74	1.40	1.26	0.21			
h 24	0.89	0.63	1.20	0.97	0.21			
h 48	0.78	0.62	0.51	0.67	0.21			

[a] Dietary treatments: conventional nursery diet that contained multiple sources of proteins, including plant and animal proteins without (CON−) or with 220 mg of Aureomycin per kg of complete feed (CON+) or conventional nursery diet with 25% (LowFF) or 50% (HighFF) of animal proteins replaced with full fat black soldier fly larvae meal. Diets were fed for 7, 14, and 21 d in phases I, II, and III, respectively.

[b] *P*-values show main effects of dietary treatment, time after weaning (or time after intradermal injection of OVA for change in skinfold thickness), and the interaction between dietary treatment and time after weaning (or time after intradermal injection of OVA for change in skinfold thickness).

[c] Maximum value of standard error of the means.

[d] Each mean represents observations on 12 pigs (10 pigs for LowFF).

[e] Corrected optical density.

antibiotics in a conventional nursery diet allowed pigs fed the LowFF, HighFF, and CON+ diets to mount a 2.0×, 1.7×, and 1.4 × greater DHR to the novel antigen OVA, respectively, than pigs fed the CON− diet. The greater DHR was particularly noted at h 6, when the response to OVA is typically observed to be the highest (Koepke et al., 2017). Moreover, pigs that received the CON− diet had the lowest plasma OVA-specific IgG by 38 d after weaning, which is an indicator of the secondary response to vaccination. Despite the lack of statistical significance when separating the means with the Tukey–Kramer posthoc test, these observations indicate a more robust immune response to vaccination and subsequent challenge against OVA for pigs that received the LowFF, HighFF, and CON+ diets versus those that received the CON− diet.

The antigen OVA preferentially stimulates the differentiation of T helper 2 (Th2) cells from naïve T helper cells in mice, however, this effect is not well-characterized in pigs (Williams et al., 2005; Wu et al., 2006). Evidence suggests that the chitin component of BSFLM, in the form of chitio-oligosaccharides made up of β1–4 bonds between the *N*-acetylglucosamine amino polysaccharide units, can promote both humoral and cell-mediated immune responses in mice and rats (Lee et al., 2008; Xing et al., 2017). Specifically, chitin can enhance Th2 cytokine production via stimulating the generation and activation of a variety of innate immune cells such as macrophages, eosinophils, and basophils, which amplifies the humoral immune response (Lee et al., 2008). Further, chitin can act as an adjuvant for the stimulation of the Th2 immune response to OVA in mice (Lee et al., 2008). Such a mechanism could explain why pigs fed the LowFF and HighFF BSFLM diets had a relatively greater DHR to OVA versus pigs the CON− diet. However, the inclusion levels of BSFLM in the LowFF and HighFF diets by phase III (i.e. when the secondary OVA-specific IgG response and DHR were measured) were relatively low (i.e., 0.5% and 1%, respectively), while during the early nursery period and the initial vaccination, the inclusion levels of BSFLM were relatively high (i.e., phase I; 7.4% and 14.8%, respectively). In addition, the chitin content of BSFLM can range between 1.6% and 7.3%, depending on rearing and processing methods (Sheppard et al., 1994; Diener et al., 2009). Others have demonstrated that a certain threshold

of chitin intake can improve the immune response in challenged animals, while doses above this threshold have no further benefit to the animal, or may negatively affect the immune response (Yousef et al., 2012). Moreover, the processing methods used on the BSFLM or during the fractionation of chitin can also influence the immunomodulatory activities of BSFLM or its fractions (Xing et al., 2017). In order to optimize the immune response of nursery pigs, the effects of timing, dosing, and processing of chitin or other functional compounds found in BSFLM remain to be elucidated and were beyond the scope of the current study.

The Aureomycin used in this study is a broad-spectrum antibiotic that belongs to the tetracycline antibiotic family. Aureomycin elicits antimicrobial activity against Gram-positive and Gram-negative bacteria by inhibiting bacterial protein synthesis via preventing amino-acyl-tRNA attachment through binding the 30s subunit of bacterial ribosomes (Williams et al., 2005). Further, tetracyclines have been shown to reduce expression of the pro-inflammatory cytokines IFN-γ and TNF-β in humans; these molecules characterize the T helper 1 (Th1) immune response and could result in a reduced capability to mount a cell-mediated immune response, and perhaps favor a humoral immune response during instances of immune system stimulation (Krakauer and Buckley, 2003; Wu et al., 2006). Thus, it is possible the 1.4 × greater DHR to OVA for pigs fed the CON+ vs. CON− diet may be due to the addition of Aureomycin to the diet.

Dietary BSFLM inclusion did not influence intestinal morphology, though pigs used in the current study were from a high-health herd and not disease challenged, which may explain this lack of significance. Previous research has shown that the functional components of BSFLM, including chitin, can support intestinal morphological integrity via preventing attachment of pathogenic bacteria in the gut (e.g., *Escherichia coli*) and promoting the growth of beneficial gut bacteria (e.g., *Lactobacillus* and *Bifidobacterium*; Liaqat and Eltem, 2018). Furthermore, the MCFA in BSFLM, primarily made up of lauric acid, also have antimicrobial effects against some pathogenic bacteria, particularly *Clostridium perfringens* and many Gram-positive bacteria, while sparing beneficial *Lactobacilli* bacteria in the gut (Spranghers et al., 2017). This effect likely occurs via MCFA infiltrating the lipid membrane of the pathogenic bacterial cell, dissociating within the cell, and stimulating a reduction in cytosolic pH (Skrivanova et al., 2012). This causes bacteria to deplete cellular ATP in an effort to export excess protons and maintain neutral pH (Skrivanova et al., 2012). Therefore, it would be beneficial to determine whether the inclusion of BSFLM in nursery diets can mitigate common (e.g. *E. coli*) enteric bacterial challenges by either stimulating the gastrointestinal tract immune response or reducing the colonization of these pathogenic bacteria in the gut in instances where postweaning diarrhea is a challenge.

As previously mentioned, the current study was conducted at a high-health university research facility and pigs were not immune or disease-challenged as indicated by the plasma Hp concentrations. Haptoglobin is an acute-phase protein whose concentration increases during the innate acute-phase immune response due to acute bacterial or viral infections or stress related to transport or changes in feed administration patterns (Piñeiro et al., 2009). The plasma Hp concentrations at both sampling times in the current study were lower or within the reference range recommended by Piñeiro et al. (2009) and the lack of improvement in growth performance for pigs fed the CON+ versus the CON− diet provides further evidence that the pigs used in the current study were not as challenged as those from commercial farms. It is anticipated that the immune modulating and growth promoting benefits of BSFLM would have been more apparent if the pigs' immune systems had been compromised. Therefore, this study should be replicated in a commercial facility or performed with an immune challenge model to characterize any potential benefits of including BSFLM in the nursery diets of pigs.

In conclusion, the findings of the present study suggest that FF BSFLM can be supplemented into nursery diets at an inclusion level of up to 50% of the animal protein content and still support growth performance, gut morphology during the first week after weaning, and immune response not different from pigs fed conventional nursery diets containing Aureomycin at a growth-promoting level. Although, pigs were not disease or immune challenged in this study, this lack of difference may be due to the pigs already being near maximum levels of growth performance. Therefore, while this study indicates that BSFLM may be a viable alternative to animal proteins in nursery pig diets and even elicit some immune-modulating functional benefits, further research should be conducted to explore the extent of these effects on immune-challenged nursery pigs and/or pigs in commercial environments. Moreover, the cost-effectiveness of feeding

BSFLM as a protein supplement with functional benefits versus BSFLM fractions (e.g., chitin or MCFA) as immune-modulating additives remains to be determined.

ABBREVIATIONS

BSFLM, black soldier fly larvae meal; FF, full fat; Hp, haptoglobin; IgG, immunoglobulin G; IgG1, immunoglobulin G 1; MCFA, medium chain fatty acids; OVA, ovalbumin; Th1, T helper 1; Th2, T helper 2

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