Comparative protein quality in black soldier fly larvae meal vs. soybean meal and fish meal using classical protein efficiency ratio (PER) chick growth assay model

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ABSTRACT Black soldier fly larvae meal (**BSFLM**) is a relatively new ingredient that has attracted attention for application in human, farm, and companion animal nutrition. However, there is limited data on protein quality (**PQ**) of BSFLM. This study compared PQ in BSFLM with soybean meal (**SBM**) and fishmeal (**FM**) using the classical protein efficiency ratio (**PER**) chick growth assay model. A total of 240-male day-old Ross × Ross broiler chicks were allocated to 6 test diets for 10 d test. The corn starch-dextrose based diets were: 5 test diets containing 10% crude protein (**CP**) from casein (as a standard assay diet), SBM, FM, or BSFLM without or with (BSFLM+) additional essential amino acids (EAA) to match the levels of the SBM diet on digestible basis. A sixth diet was nitrogen-free (**NFD**), to enable calculation of net protein ratio (**NPR**). The birds had ad libitum access to feed and water, body weight (**BW**) and feed intake (**FI**) were recorded at the beginning and end of the experimental feeding period. Body weight gain (**BWG**), gain to feed ratio (**G:F**), crude protein intake (**CPI**), PER, and NPR were calculated. The analyzed CP (as fed) was 10.1, 12.8, 9.5, 11.7, and 14.9% for casein, SBM, FM, BSFLM, and BSFLM+ diets, respectively. Birds fed BSFLM+ had greater BWG ($P < 0.01$) than birds fed other diets. Feed intake was greater ($P < 0.01$) for birds fed BSFLM+ and FM diets than birds fed other diets. Birds fed BSFLM+ ate ($P < 0.01$) more CP than birds fed other diets. Diets had no ($P > 0.05$) effects on PER and NPR. In terms of ranking, the PER was 2.44, 2.38, 2.34, 2.28, and 2.10 g BWG/g CPI for BSFLM+, SBM, FM, casein, and BSLM, respectively. Corresponding values for NPR were 2.69, 2.78, 2.70, 2.74, and 2.48 g BWG/g CPI, respectively. In conclusion, the protein quality of BSFLM without or with additional EAA was comparable with FM and SBM.

Key words: black soldier fly larvae, protein quality, PER chick growth assay

2023 Poultry Science 102:102255
https://doi.org/10.1016/j.psj.2022.102255

INTRODUCTION

Edible insects have elicited interest for application in human, farm, and companion nutrition (Bosch and Swanson, 2021; Lange and Nakamura, 2021). Insects are rich in amino acids, fatty acids, and micronutrients, and have been shown to grow rapidly on a variety of waste organic matters (Bava et al., 2019). As such insect farming has been suggested to offer a sustainable food/feed ingredients supply in the context of limited natural resources, climate change pressure, supply chain disruptions and food-feed-biofuel competition among others (Muscat et al., 2020). Black soldier fly larvae (**BSFLM**) is one of the insect protein that has been approved and commercialized in many jurisdictions (Spranghers et al., 2017). Protein quality (**PQ**) is a key criterion for evaluating bioavailability of amino acids in ingredients (Mansilla et al., 2020). Standardized ileal digestibility (**SID**) of amino acids is the gold standard method for evaluation PQ in poultry feedstuffs (NRC, 1994; Adedokun et al., 2015) and values for BSFLM in poultry have been reported in numerous reports, for example (Schiavone et al., 2017; Mwaniki and Kiarie, 2019; Matin et al., 2021). Although not without its shortcomings, protein efficiency ratio (**PER**) chick growth assay model is valuable in evaluating PQ of feedstuffs for human and companion animals (Mansilla et al., 2020). Moreover, the PER method allows evaluation of single ingredients that would not be permitted in dog or cat tests (Donadelli et al., 2019). The objective of this study was to use PER chick assay to compare protein utilization in BSFLM

Received June 12, 2022.
Accepted October 11, 2022.

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in comparison with conventional protein sources (soybean meal and fishmeal).

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the University of Guelph Animal Care Committee and birds were cared for in accordance with the Canadian Council on Animal Care guidelines (**CCAC** 2009).

Protein Sources and Feed Preparation

Soybean meal and fish meal samples were procured from local commodity markets (Floradale Feed Mill Limited, Floradale, ON, Canada). Casein was from Sigma-Aldrich (Oakville, ON, Canada) and BSFLM was from Enterra Corporation (Maple Ridge, BC, Canada). A nitrogen free diet (**NFD**) of corn starch-dextrose diet was formulated to allow estimation of net protein ratio (**NPR**). The NFD diet was formulated to meet or exceed AMEn and all the nutrient specifications (Aviagen, Ross 708) with exception of crude protein and amino acids. Four experimental diets were formulated by adding respective protein source to NFD to supply 10% CP in replacement of the 2:1 cornstarch to dextrose mix. Additional test diet (BSFLM+) was formulated by adding synthetic amino acids: L-Arginine, L-Lysine HCL, L-Isoleucine, DL-Methionine, L-Threonine, and L-Tryptophan to the level of SBM diet on standardized ileal digestible basis. The BSFLM+ diet was tested to confirm previous studies that indicated supplementation of synthetic amino acids ameliorated growth depressing effects of BSFLM in poultry (Neumann et al., 2018a,b,c; Velten et al., 2018). Six mash diets were prepared and tested: casein, soybean meal, fishmeal, BSFLM, and BSFLM+, along with NFD. Samples of protein ingredients and diets were collected and placed in plastic pound bags for subsequent analysis.

Animals and Experimental Design

A total of 240, day-old male broiler chicks were equally distributed by body weight (**BW**) to 48 floor pens and fed a commercial starter diet until d 14 to allow for acclimation. Access to feed and nipple drinkers were provided ad libitum throughout the duration of the trial period. The commercial broiler starter feed (Floradale Feed Mill Ltd., Floradale, ON, Canada) had 20% crude protein and was prepared as fine crumble. The temperatures and lighting schedules started at 32°C with 23 h, respectively and gradually decreased to 21°C with 16 h, respectively. On d 14, pen BW were recorded, and 6 diets were distributed in a randomized block (room) design to 8 replicates pens per diet. The BW, feed intake (**FI**), and mortality were monitored for calculation of BW gain (**BWG**) and gain-to feed-ratio (**G:F**).

Laboratory Analyses

Protein ingredients (casein, SBM, FM, and BSFLM) and diet samples were finely ground and submitted to a commercial lab (SGS Canada, Guelph) for dry matter, crude protein, crude fat, starch, and minerals analyses according to AOAC (2005).

Calculation and Statistical Analyses

The crude protein intake (CPI, g/bird), PER, and NPR were calculated according to the following equations (Cramer et al., 2007; Donadelli et al., 2019):

$$\mathrm{CPI} = \mathrm{FI} \times \text{Analyzed CP of diet (\%DM)} \tag{1}$$

$$\mathrm{PER} = \mathrm{BWG} / \mathrm{CPI} \tag{2}$$

$$\mathrm{NPR} = (\mathrm{BWG} - \mathrm{GN-free}) / \mathrm{CPI} \tag{3}$$

where GN-free is the BWG of birds fed NFD.

Statistical analysis was performed using GLIMMIX procedures of SAS with diet as fixed and room (block) as random factor. Pen was considered the experimental unit. Tukey test was used to separate LSmeans and $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The analyzed composition of ingredients and diets is shown in Table 1. The concentrations of CP in casein, SBM (hulled solvent extracted), fishmeal and BSFLM (partially defatted) were comparable with literature

Table 1. Analyzed composition of test ingredients and experimental diets, as fed (%).[1]

	Ingredients				Experimental diets					
Item	Casein	SBM	Fishmeal	BSFLM	NFD	Casein	SBM	Fishmeal	BSFLM	BSFLM+
Dry matter	90.7	89.8	92.4	95.8	86.6	87.0	87.5	88.0	89.1	89.3
Crude protein	88.0	47.4	56.0	54.9	0.85	10.1	12.80	9.49	11.7	14.9
Crude fat	0.12	1.72	8.60	13.3	5.82	4.49	7.24	3.41	7.11	7.26
Starch	0.05	1.05	0.74	7.79	71.7	63.9	51.7	62.6	59.6	56.5
Calcium	0.01	0.20	7.38	1.01	0.84	0.96	1.08	1.59	0.93	1.08
Phosphorous	0.24	0.64	3.97	0.87	0.41	0.43	0.66	0.76	0.60	0.69
Sodium	0.07	0.05	0.70	0.17	0.20	0.21	0.20	0.26	0.18	0.18
Potassium	0.04	2.23	0.66	1.35	0.73	0.75	0.97	0.83	0.83	0.86
Magnesium	0.00	0.28	0.19	0.37	0.04	0.04	0.09	0.06	0.09	0.10

[1]Abbreviations: BSFLM, black soldier fly larvae meal; BSFLM+, BSFLM supplemented with amino acids to match soybean meal diet; NFD, nitrogen free diet; SBM, soybean meal.

Table 2. Growth performance, crude protein intake (CPI), protein efficiency ratio (PER), net protein ratio (NPR), and gain-to-feed ratio (G:F) of broiler chicken fed various protein ingredients from day 14 to day 21 of age (n = 8).

Item	Diet[1]							
	NFD	Casein	SBM	Fishmeal	BSFLM	BSFLM+	SEM	P-value
Initial body weight (BW)	245.7	253.3	243.6	240.0	233.2	240.9	5.63	0.241
BW gain (BWG), g/bird	−17.1[c]	88.7[b]	105.8[b]	111.5[b]	98.3[b]	171.3[a]	8.79	<0.01
Feed intake (FI), g/bird	268.7[c]	330.1[bc]	308.8[bc]	449.0[a]	353.3[b]	420.6a	15.6	<0.01
BWG to FI	−0.07[d]	0.26[bc]	0.35[ab]	0.25[c]	0.28[bc]	0.41[a]	0.02	<0.01
CPI, g/bird	-	38.4[c]	44.5[bc]	48.4[b]	46.4[bc]	70.2[a]	2.04	<0.01
PER, g BWG/g CPI	-	2.28	2.38	2.34	2.10	2.44	0.19	0.754
NPR, g BWG/g CPI	-	2.74	2.78	2.70	2.48	2.69	0.18	0.828

[1]Abbreviations: BSFLM, black soldier fly larvae meal; BSFLM+, BSFLM supplemented with amino acids to match soybean meal diet; NFD, nitrogen free diet; SBM, soybean meal.

[a-d]Within a row, means without a common superscript differ $P < 0.01$.

values (NRC, 2012; Mwaniki and Kiarie, 2019). For feed formulation, the crude protein values for casein and FM were from book values and those of SBM and BSFLM from previous experiments from our laboratory (Mwaniki and Kiarie, 2019; Leung and Kiarie, 2020). With exception of casein diet, there were discrepancies between formulated (~10% CP) vs. analyzed dietary CP. Specifically, SBM and BSFLM+ diets assayed higher CP likely associated with sampling/analytical inaccuracies. Moreover, additional amino acids in BSFLM+ diets contributed to CP that was not accounted for during formulation.

The BWG, FI, G: F, CPI, PER, and NPR data are presented in Table 2. In broiler chickens, provision of balanced and adequate amino acids is crucial for maintenance and growth (NRC, 1994). The broiler chick assay used in the present study facilitates determination of protein utilization efficiency for growth and ranking of protein ingredients (Donadelli et al., 2019; Mansilla et al., 2020). Birds fed NFD produced negative BWG confirming the necessity for protein and AA for maintenance (Donadelli et al., 2019). Birds fed casein, SBM, FM, and BSFLM had similar ($P > 0.05$) BWG. Addition of synthetic essential amino acids in BSFLM+ diet to the level of SBM resulted in superior ($P < 0.05$) BWG than for birds fed other diets. Birds fed FM and BSFLM+ diets consumed more ($P < 0.05$) feed than birds fed SBM, BSFLM and NFD. However, birds fed SBM and BSFLM diets exhibited similar ($P > 0.05$) feed intake. Birds fed BSFLM+ had a higher ($P < 0.05$) G:F compared to bird fed other diets with exception of SBM birds. These observations agreed with studies that demonstrated that extended fortification of amino acids in practical diets in which SBM was replaced with more than 50% BSFLM improved growth and protein utilization in broilers and pigs (Neumann et al., 2018a,b,c; Velten et al., 2018). Specifically, 50% replacement of SBM with BSFLM in a corn-wheat-SBM based diet plus basic addition of synthetic Met and Lys reduced growth, feed intake and protein utilization relative to control. However, increasing synthetic Met and Lys and adding other amino acids (Thr, Arg, and Val) restored these parameters to the level of control (Neumann et al., 2018a; Velten et al., 2018).

The PER and NPR values are dependent on BWG and the amount of consumed protein over the test period. Although birds fed BSFLM+ had higher BWG, this did not translate to superior ($P > 0.05$) PER and NPR indices relative to other proteins. This was partly linked to higher ($P < 0.05$) CPI in birds fed BSFLM+ relative to other birds. Nonetheless, in terms of ranking, the PER was 2.44, 2.38, 2.34, 2.28, and 2.10 for BSFLM+, SBM, FM, casein, and BSLM, respectively. Corresponding values for NPR were 2.69, 2.78, 2.70, 2.74, and 2.48 g BWG/g CPI, respectively. Although nonsignificant, PER and NPR values for BSFLM were inferior to BSFLM+ values; supporting previous observations that addition of amino acids in practical diets containing higher amounts of BSFLM improved net protein utilization in the broiler chickens (Neumann et al., 2018a,b).

The PER and NPR values are effective indicators of PQ for single ingredients, especially when determining the biological efficiency and economic value of new ingredients for multispecies application (Mansilla et al., 2020). A PER of an ingredient that is greater than the PER of casein is indicative of high-quality source (Mansilla et al., 2020). Based on PER and NPR observed in the present study, the protein quality in BSFLM was comparable to casein, SBM, FM, and BSFLM+. However, the data did indicate that fortifying BSFLM with select essential amino acids to the level SBM elevated growth performance to the level of SBM. Further investigations are warranted to characterize optimal amino acids balance in practical diets with BSFLM as the main protein ingredient.

ACKNOWLEDGMENTS

V. Cheng completed the experiment as part of her graduate course (ANSC*6900). The work was funded by the Ontario Agri-Food Innovation Alliance and Canada First Research Excellence Fund. Many thanks to the technical assistance and bird care of M. Mohammadi, C. Zhu, I. Wilson, R. Petersen, T. McElwain, D. Whey, and H. Naumann. Thank you for the moral support and the efforts of A. Gelinas, H. Facey, J. Gasarabwe, M. Kithama, and J. Lee.

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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