



Nutritional Composition and Apparent Metabolizable Energy Value of Black Soldier Fly Larvae (*Hermetia illucens* L.) Full-Fat Meal for Broiler Chickens[#]

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ARTICLE INFO

ABSTRACT

[#]Presented at 5th International Research Conference (TRInCo, 2022), 9-11th November 2022, Trincomalee Campus Eastern University, Sri Lanka pp. 20.

Research Article

Received : 06-02-2023

Accepted : 08-10-2023

Keywords:

Apparent metabolizable energy
Broiler chickens
Black soldier fly larvae
Waste
Full fat meal

Black soldier fly larvae (BSFL; *Hermetia illucens* L.) is a proven high-cost protein source replacer and could be grown in a range of bio-degradable waste materials where hardly incorporated into broiler diets locally. The present study was aimed to assess the nutritional composition of BSFL, and apparent metabolizable energy (AME) value of BSFL meal provided to broiler chickens. BSFL full-fat meal produced from kitchen waste as a substrate were examined for their proximate composition, minerals and fatty acid profile. Eighty, 21-d old unsexed Cobb-500 broiler chickens (BW±SD: 665.8 ±14.3 g) were assigned randomly into 16 battery cages (04 replicates, five birds/replicate). A maize-soybean meal-based diet was used as the basal diet, which was partially substituted by pre-analyzed BSFL meal at the rates of 5%, 10%, and 15% to produce three test diets. Birds were fed in a completely randomized design for 7-d with a 4-d adaptation period. Excreta were collected for three days from day 25 to 28. The results envisaged that the crude protein (CP) and ether extract (EE) contents of the kitchen waste were 12.3%, and 10.5%, respectively. BSFL meal when analyzed had 34.4% CP and 47.3% EE. The fatty acid (FA) profile of the kitchen waste was more or less similar to that of BSFL's meal. The estimated AME of the BSFL full-fat meal fed for broilers was estimated to be 15.7 MJ/kg. The BSFL full-fat meal can be utilized sustainably in feed formulation and has a high potential to replace costlier feed ingredients.

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Introduction

Producing food for increasing population is quite challenging in modern agriculture. According to the FAO (2009), current human population is expected to reach 9 billion in 2050. As a consequence, it has been estimated that the world's food production should increase by 70%. Concomitantly, protein supplementation through livestock products should be doubled than the current output (Schivone et al., 2017). The broiler production is in front of fulfilling the protein gap efficiently and economically due to its faster growth rate and for excellent feed conversion ratio (Khusro et al., 2012). The broiler profit chain is marginalized, and the cost of production is mainly governed by the feed cost which accounts 70% of the total cost of production (Teguia and Beynen, 2005). The broiler performance which based on nutrient utilization is determined mainly by the respective diet's metabolizable energy to crude protein (CP) ratio (Zaman et al., 2020; Ravindran, 2013). Among the different feed ingredients

that are used in boiler feed, the CP sources are the most cost demanded ingredients and are supplied by plant sources and animal by-products. However, some plant sources remain as staple foods in certain developing countries (Khusro et al., 2012).

The crop yield is known to be affected by population growth, climate change, and global warming scenarios (Dar and Laxmipathi Gowda, 2013). Additionally, the plant protein sources are also challenged with an imbalanced amino acid profile, lower CP content, and the presence of antinutritional factors (Bandara, 2018). Fish meal (FM) has been identified as one of the widely used animal-originated dietary CP sources, averaging 60 – 72 % of CP level (Cho and Kim, 2011). Rising global poultry feed requirement has been fortified to produce 7 to 7.75 million tonnes in 2022 (Lem et al., 2014). Fish meal is currently considered a scarce ingredient in many regions of the world; hence its price is inelastic. Numerous

environmental consequences have also created marine overexploitation (Bandara, 2018). Therefore, the poultry feed industry is always encouraged to explore alternative CP sources for sustainability.

The potential of insects in the livestock feed sector has been documented in past research, evidencing a positive influence on broiler production (Józefiak et al., 2016; Khan et al., 2018; Onsongo et al., 2018). This application establishes a sustainable approach, ensuring positive environmental impacts; less energy, land area requirement, and less environmental footprint for production (Makkar and Ankers, 2014). The black soldier fly larvae (BSFL, *Hermetia illucens* L.) is a promising insect for industrial poultry feed production. Its larvae thrive on a diverse range of waste materials that are unsuitable for human consumption. The larvae utilize very dense populations of organic wastes as varied as manures (Sheppard et al., 1994; Liu et al., 2008), rice straw (Zheng et al., 2012), food waste (Schiavone et al., 2017), distillers' grains (Webster et al., 2016), fecal sludge (Lalander et al., 2013; Banks et al., 2014), and animal offal and kitchen waste (Schiavone et al., 2017). Other insect larvae such as crickets and mealworms have lower feed conversion percentages. Black soldier fly larvae can convert these substrates into high-quality protein with a dry matter content ranging from 38 to 46%. Black soldier fly larvae's amino acid (AA) profile is high in methionine and lysine (9.05 and 22.3 g/kg DM, respectively) (Oonincx et al., 2015). It has been compared to or outperformed soybean meal which is the most popular and commonly used plant originated protein ingredient for poultry (Veldkamp et al., 2012).

Organic waste is common in low-to-middle-income countries, whereas paper, metals, and glasses are common in high-income countries. Recycling, incineration, waste-to-energy conversion, composting, and landfilling are the most well-known waste disposal processes available worldwide (Nanda and Berruti, 2021). Global municipal solid waste generation is predicted to be 2 billion tonnes, with an average of 0.74 kg/cap/day of garbage generation, with 33% of the waste remaining uncollected by municipalities (Kaza et al., 2018). Sri Lanka creates 7410 tonnes of municipal waste every day, where it has a higher fraction of organic materials with high moisture content. As a result, municipal solid waste is a recyclable and a cost-effective resource with great potential for addressing complex environmental concerns (Basnayake, 2019). The BSFL is a non-pest with a more extended larval period that allows for higher bio-accumulation through a wide variety of organic degradation. Because of the low level of technological readiness and economic feasibility of manufacturing, the BSFL's organic bio-conversion can be positively incorporated into the livestock feed industry (Čičková et al., 2015).

Scott and Boldaji (1997) have experienced in utilizing acid insoluble ash (AIA) as an inert marker in broiler digestibility experiments, where they found it as more suitable than synthetic chromium oxide. Nonetheless, data on BSFL digestibility and apparent metabolizable energy (AME) of BSFL for broilers is highly limited at the moment, limiting the formulation of sufficient BSFL-based broiler diets. Therefore, this study aimed to assess the nutritional composition and AME of BSFL full fat meal provided to broiler chickens.

Materials and Methods

The study was conducted at the farm premises of the Faculty of Agricultural Sciences, the Sabaragamuwa University of Sri Lanka according to the guidelines of the current ethical review committee (Reference no. ERC/A/01/2022/01 dated 15/09/2022) of the Sabaragamuwa University of Sri Lanka.

Preparation of the BSFL meal

Kitchen waste containing food and refuses were used as the common waste substrate to grow the BSFL (Ellewidana et al., 2020). The pre-pupae stage of BSFL were harvested, cleaned, and were stored under -20°C until further processing. At the collection point, the larvae weighed around 0.21 g ± 0.02 and 22.1 mm ± 4.7 in average length. The frozen larvae were thawed to room temperature and forced air dried for 20 hrs under 60°C in a convection oven (Model: BOC-V640F, Biobase Bioindustry (Shandong) Co; Ltd, China) (Schiavone et al., 2017). The dried larvae were ground into a fine powder using a kitchen grinder and were stored under 4 °C until mixed into diets.

Experimental Diets

A maize-soybean meal-based diet was used as the basal diet (NRC, 1994). The basal diet was partially replaced by pre-analyzed BSFL meal at the rates of 5%, 10% and 15% to produce three test diets.

Table 1. Ingredients and the calculated composition of the basal diet (g/kg as fed)

Ingredient	g/kg
Maize	485.8
Soybean Meal (44% CP)	224.7
Rice Polish	150.0
Fish Meal-Anchovy	62.6
Coconut Oil	50.0
Limestone Powder	13.0
Di-Calcium Phosphate	4.5
Common Salt	2.5
Vitamin Premix ¹	2.5
Mineral Premix ¹	2.5
L-Lysine HCL	0.84
DL-Methionine	0.56
Coccidiostat	0.50
Calculated composition	
Metabolizable Energy (MJ/kg)	13.4
Dry Matter, %	90.1
Crude Protein, %	20.0
Ether Extract, %	8.99
Crude Fiber, %	3.32
DL-Methionine, %	0.44
L-Lysine HCL	1.20
Calcium, %	0.91
Total Phosphorus, %	0.72
Non Phytate Phosphorus, %	0.36

¹Vitamin and mineral premixes (IU/mg per kilogram): Vitamin A 15,000,000; Vitamin D 3,800,000; Vitamin E 30,000; Vitamin K 2,500; Vitamin A 15,000,000; Vitamin B 1 2,500; Vitamin B 2 6,000; Calcium Pantothenate 12,000; Vitamin B 6 5,000; Vitamin B 12 24; Niacin 40,000; Folic acid 1,200; Biotin 180; Choline Chloride 2,000; Iron 40,000; Copper 10,000; Zinc 60,000; Manganese 80,000; Iodine 1,000; Cobalt 200; Selenium 150.

Chemical Analysis

The kitchen waste substrate samples where the larvae were reared were analyzed for dry matter (DM), Ash, CP, EE, gross energy (GE), calcium (Ca), total phosphorus (TP) contents and for its fatty acid (FA) profile. Black soldier fly larvae meal was analyzed in triplicates for its proximate composition, GE, Ca, TP, and FA profile (AOAC, 2005). For the determination of AME, the GE (AOAC, 2005) and AIA (Vogtmann et al., 1975) of test diets and excreta samples were analyzed in triplicates.

Estimation of AME of BSFL full-fat meal for broiler chickens

A total of hundred and fifty (150), vaccinated, unsexed, day-old broiler chicks (Cobb 500) were purchased from a commercial hatchery in Sri Lanka. They were raised in floor pens and were fed a commercial diet (12.5 MJ/kg and 21% CP) till day-21. On day 21, birds were individually weighed (665.8 ±14.3 g), and eighty (80) birds having uniform weights were randomly distributed into 16 battery cages (60 cm × 60 cm x 60 cm) (four replicates, five birds per cage). From day 21 to day 28, birds were fed with four test diets. The ingredient composition of the basal diet and its calculated composition are indicated in Table 1. The feed intakes throughout the experimental period were recorded. The collection trays were introduced on day 25 and lasted after three consecutive days. Daily excreta collected from each cage after removing feathers and feed residues were weighed, labeled, and were stored at -20°C. Daily collections were pooled within a cage and were forced air-dried (Model: BOC-V640F, Biobase Biodustry (Shandong) Co; Ltd, China) until constant weights were obtained and were ground into a fine powder.

Calculations

The AME (MJ/kg diet) value of each diet in each inclusion level was calculated using Equation 1 as described by Scott and Boldaji (1997).

$$AME = GE_{diet} - [GE_{excreta} \frac{Marker_{diet}}{Marker_{excreta}}] \quad (1)$$

GE = gross energy, MJ/ kg (diet, excreta)
 Marker = concentration of AIA

AME value of BSFL meal at each inclusion level was calculated using Equation 2 (Wu et al., 2020).

$$AME_d = AME_{bd} + (AME_{bd} - AME_{BSFL}) \times P_{BSFL} \quad (2)$$

Where; AME_d= AME diet, AME_{bd} = AME basal diet, AME_{BSFL} = AME Black Soldier Fly Larvae, P_{BSFL}= BSFL Proportion %.

The AME of BSFL meal was finally calculated by obtaining the mean value from obtained three AME values of BSFL meal.

Results and Discussion

Nutrient composition of kitchen waste substrate and full fat BSFL meal

The nutrient composition of rearing substrate and harvested BSFL meal are presented in Table 2. Nutrient composition of BSFL varies with the quality of the growing substrate.

Analytical data on nutritional composition of BSFL rearing substrates are highly limited. Kierończyk et al. (2020) and Shumo et al. (2019) documented the nutritional composition of BSFL reared on a similar growing substrate but resulted in different nutrient compositions (Table 3).

The effect of different kitchen waste substrates on the nutrient composition of BSFL is summarized in Table 4. The nutrient composition of BSFL was in agreement with the values obtained by previously researchers. The highest EE level was reported from the current experiment. The CP content reported in the present study can suppress some commonly used feed ingredient such as sunflower meal (Willis, 2003). The body composition of BSFL is known to influence by the quality and the quantity of food consumed (Nguyen et al., 2015). Therefore, it is suggested that the composition of the rearing medium should be well balanced (Kierończyk et al., 2020). Moreover, the nutrient percentage of BSFL is also influenced by the larval growth stage. Considering the CP percentage of BSFL, Rachmawati et al. (2010) reported a decline in their growth stage (5 days old: 61%; 15 days old: 44% and 20 days old: 42% larvae).

Variations in nutritional composition of BSFL in diverse waste substrates other than kitchen waste have been well documented. The larvae fed on cattle manure (Newton et al.,1977; Li et al.,2011), swine manure (Newton et al., 2005; St-Hilaire et al., 2007; Li et al., 2011; Manzano-Agugliaro et al.,2012), chicken manure (Sheppard et al., 1994; Li et al., 2011), chicken feed (Bosch et al., 2014; Nguyen et al., 2015; Oonincx et al., 2015), palm kernel meal (Rachmawati et al., 2010), liver (Nguyen et al., 2015), fish (Nguyen et al., 2015) and Barry (2004) had varying CP (40 – 62.7%) and EE (6 – 49%) percentages.

Table 2. Analyzed nutrient composition of the kitchen waste substrate and the black soldier fly larvae meal on dry matter basis

Nutrient	Kitchen Waste Substrate (% DM)	BSFL Meal (% DM)
Crude Protein	12.3	34.4
Ether Extract	10.5	47.3
Crude fiber	-	5.97
Dry matter	92.0	92.7
Calcium	0.42	0.46
Total Phosphorus	0.50	1.20
Crude Ash	12.9	9.57
Gross Energy (MJ/kg)	17.0	17.8

Table 3. Comparison of some published data on nutrient composition of different substrates used for Black soldier fly larvae on dry matter basis

Reference	CP	EE	CF	DM	C	TP	CA	Substrate
The present experiment	12.3	10.5	-	92.0	0.42	0.50	12.9	KW
Kierończyk et al. (2020)	13.0	1.30	8.73	-	-	-	5.75	KW
Shumo et al. (2019)	20.0±0.5	7.2±0.3	-	92.7±0.1	-	-	7.20±0.3	KW
Spranghers et al. (2016)	8.60	2.10	33.6	-	0.683	0.293	10.8	Vegetable waste
Spranghers et al. (2016)	15.7	13.9	4.1	-	0.141	0.237	4.50	Restaurant waste

CP: Crude protein (%); EE: Ether extract (%); CF: Crude Fiber (%); DM: Dry Matter (%); C: Calcium (%); TP: Total Phosphorus (%); CA: Crude Ash (%); KW: Kitchen waste (Mixture of Wheat bran, Carrots, Cabbage, and Potatoes)

Table 4. Comparison of some published data on nutrient composition of Black soldier fly larvae utilized different substrates on dry matter basis

Reference	CP	EE	CF	DM	C	TP	A	Substrate
The present experiment	34.4	47.3	5.97	92.7-	0.46	1.20	9.57	KW
Kierończyk et al. (2020)	45.4	14.0	9.83	-	-	-	8.16	KW
Rawski et al. (2020)	35.0	29.8	7.90	-	-	-	5.30	FVF
Jansen (2018)	36.1	42.9	8.10	93.3	4.29	0.72	11.9	KW
Shumo et al. (2019)	33.0±1.0	34.3±0.4	-	87.7±1.0	1.93±0.42	2.0±0.58	9.6±1.6	KW
Spranghers et al. (2016)	39.9	37.1	-	-	2.87	0.404	9.6	Vegetable waste
Spranghers et al. (2016)	43.1	38.6	-	-	0.123	0.408	2.7	Restaurant waste

CP: Crude protein (%); EE: Ether extract (%); CF: Crude Fiber (%); DM: Dry Matter (%); C: Calcium (%); TP: Total Phosphorus (%); A: Ash (%); KW: Kitchen waste (Mixture of Wheat bran, Carrots, Cabbage, and Potatoes); FVF: Fresh vegetable and fruit mix

The crude fiber (CF) content of BSFL reported in the present study is comparatively lower (5.97%) than the values reported by Jansen (2018) (8.10%), and Newton et al. (2005) (7%) who used pig manure as the rearing substrate. Diets high in fiber are favorable in feed application as they influence the mucosal lining of the intestine (Montagne et al., 2003). Moreover, fiber has been found to favor the ensuing effects on the intestinal mucus barrier of chickens (Sumbule et al., 2021). According to Mwaniki et al. (2018), high feed intake by birds fed insect-based diets may be related to increased fiber content at different BSFL inclusion levels. Fibers aid in increased ceca fermentation in birds, resulting more excellent nutrient absorption and development (Bovera et al., 2016). Also, CF content could have an influence on protein digestibility (El-Wahab et al., 2021). Therefore, growing economic insect-based feeds might be more beneficial if fiber percentages were mixed in various feeds. It is practiced that feeding fiber to rations at low levels has a positive effect, but levels more than 3% in a ration have been proven to impact voluntary feed intake significantly and nutrient digestibility, resulting poor bird performance (Tejeda and Kim, 2021).

Additional to major nutrients, Ca and TP are known to ensure physiological functions of birds; egg shell formation, and maintenance of bones (Jansen, 2018). Calcium and TP deficiency can cause bone loss, stunted growth, and poor posture (Shumo et al., 2019). The present study reported comparatively lower levels of Ca (0.42%) and TP (1.2%) than the values reported by other researchers (Table 4) (Newton et al., 2005; Spranghers et al., 2016). Influence of the rearing substrate on the mineral composition of BSFL is well documented. Moreover, the outer layer of the skin of BSFL secretes calcium carbonate (CaCO₃), thus affecting the mineral concentration (Shumo et al., 2019). The ash content of BSFL revealed in the present study (9.57%) is in a similar range to that of previous studies (Table 4). However, it has been revealed that the crude ash content may influence the fat digestibility variably (El-Wahab et al., 2021).

The analyzed lipid profile of the kitchen waste or the rearing substrate and the BSFL full fat meal are presented in Table 5. The results indicated that the FA profile of the substrate is more or less similar to that of full fat BSFL meal. It is suggested that the FA profile of BSFL is reflected by the FA profile of the substrate (Makkar and Ankers, 2014; Spranghers et al., 2016). According to the results, lauric acid, palmitic acid, myristic acid, oleic acid, linoleic acid, and stearic acid were dominant in BSFL meal. The FA concentrations are in line with the previous studies where BSFL were grown in similar and or different rearing substrates (Sealey et al., 2011; Spranghers et al., 2016; Barragan-Fonseca, 2018). Saturated fatty acids (SFA) are composed of palmitic and myristic acids, which enhance the low-density lipoproteins (LDL) by suppressing the LDL receptors' expression (Sacks and Willett, 1991). Unsaturated fatty acids are more favorable in poultry diets and are abundant than SFAs in BSFL meals. More importantly, a higher lauric acid content (53.04%) of BSFL meal reported in the present experiment is more or less similar to those reported by Spranghers et al. (2016) (60.8% in vegetable waste; 57.5% in restaurant waste) and St-Hilaire et al. (2007) (49.37% in swine manure). Lauric acid is a natural antimicrobial substance, which can suppress lipid-enveloped viruses, many pathogenic bacteria, and protozoa (Spranghers et al., 2016). Therefore, BSFL meal-enriched diets are hygienic and are possibly abolish the usage of synthetic antibiotics in poultry diet formulae.

Apparent metabolizable energy (AME) of BSFL meal

To the best of author's knowledge, the research conducted to estimate AME of BSFL are highly limited. The AME of BSFL at 5%, 10% and 15% inclusion levels in the present study were estimated to be -0.49, 25.8 and 15.7 MJ/kg, respectively (Table 6). However, negative AME values of BSFL meal were not been reported previously. Based on the past avian feeding experiments, only very few studies have published negative AME values for feed ingredients (Table 7). According to Sibbald (1982), the inclusion level of the test material is crucial for estimating the AME value of a test ingredient.

Table 5. Analyzed lipid profile of kitchen waste substrate and Black soldier fly larvae meal (g/ 100g of fat)

Fatty acid	Kitchen Waste Substrate	BSFL Full Fat Meal
Caproic acid (C 6:0)	0.07	ND
Caprylic acid (C 8:0)	1.90	ND
Capric acid (C 10:0)	2.80	0.94
Lauric acid (C 12:0)	33.4	53.0
Tridecanoic acid (C 13.0)	ND	0.02
Myristic acid (C 14:0)	14.7	12.6
Palmitic acid (C16:0)	16.4	13.1
Palmitoleic acid (C 16:1 c)	0.51	2.29
Heptadecanoic acid (C17:0)	0.07	0.05
Cis-10- Heptadecanoic acid (C17: 1c)	0.05	0.03
Stearic acid (C 18:0)	3.48	1.76
Elaidic acid (C18: 1 9t)	0.10	ND
Oleic acid (C18 :11 9 C)	15.8-	10.9
C 18:2 (9C,12t)	0.05	ND
C 18:2 (9t, 12c)	0.04	ND
Linoleic acid (C 18:2 n6c)	8.49	4.42
C 18:3 (9t,12t,15c + 9t, 12c, 15t)	0.22	0.13
Cis-11-Eicosenoic acid (C 20:1)	1.06	ND
Linoleic acid (c 18: 3n3)	0.23	0.52
Heneicosanoic acid (C21:0)	ND	ND
Cis-11, 14-eicosadienoic acid (C 20:2)	ND	ND
Behenic acid (C22:0)	ND	ND
Cis-8,11,14- Eicosatrienoic acid (C20:3n6)	0.12	0.06
Erucic acid (C22:1n9)	0.04	ND
Cis-11,14,17- Eicosatrienoic acid (C20:3n3)	0.46	0.03
Lignoceric acid (C 21:0)	ND	0.02

BSFL, Black soldier fly larvae; ND, Not detected.

Table 6. Apparent metabolizable energy of full fat Black soldier fly larvae meal at different substitution levels

Criteria	Basal diet	Diet BSFL 5%	Diet BSFL 10%	Diet BSFL 15%
Average AME (MJ/kg) ¹	8.95	8.62	10.32	9.83
SEM AME diet	0.30	0.99	1.13	0.99
AME _{BSFL} (MJ/kg)	-	-0.49	25.82	15.66
Dry Matter %	82.92	84.35	83.96	85.37

¹Each value represents the mean of four replicates. AME, Apparent metabolizable energy.

Table 7. Negative apparent metabolizable energy values for feed ingredients reported in the past research studies

Reference	Feed ingredient	Inclusion level (%)	¹ AME value	Method	Bird type
Potter et al. (1960)	Alpha cellulose	0, 20, 33.3	(-0.189) ± 0.062 cal/g	Multiple regression method	White Plymouth Rock chicken
	Aquatic liverwort (Ricciocarpus natans)	30.7	(-0.06) ± 0.05 kcal /g	Marker method	Ducks
Sugden (1973)	Dock fruits (Rumex maritimus)	39.8	(-0.37) ± 0.07 kcal /g	Marker method	Ducks
	Pondweed foliage (Potamogeton Richardsonii)	40.2	(-0.45) ± 0.05 kcal /g	Marker method	Ducks
Petersen et al. (1976)	Barley hulls	25	(-0.29±0.18) kcal /g	Marker method	4-week-old male broiler chicks
	Barley hulls	25	(-0.22±0.13) kcal /g	Marker method	4-week-old female broiler chicks
Ortiz et al. (2001)	Linseed	16	-2.96 MJ/kg	Total collection method	28-d old broilers
	Linseed	24	-0.42 MJ/kg	Total collection method	29-d old broilers

¹AME, Apparent metabolizable energy.

Table 8. Average feed intake of birds during the experimental period (g/bird)

Criteria	Basal diet	Diet BSFL 5%	Diet BSFL 10%	Diet BSFL 15%
Average feed intake (g/ bird) ± SEM	134.5±2.95	121.1±1.46	130.7±3.61	136.6±6.74
P-value	0.09			

SEM, Standard error of mean; BSFL, Black soldier fly larvae.

Table 9. Apparent metabolizable energy values of Black soldier fly larvae meal reported in the past research studies

Reference	Feed ingredient	Inclusion level (%)	AME value	Method	Bird type
Uushona (2015)	BSFL meal dried at 100 °C	50	14.8 MJ/kg	Marker method	43-d old, Cobb 500 broilers
	BSFL meal dried at 65 °C	50	17.4 MJ/kg	Marker method	43-d old, Cobb 500 broilers
	Defatted BSFL meal dried at 65 °C	40	16.5 MJ/kg	Marker method	43-d old, Cobb 500 broilers
Schiavone et al. (2017)	Partially defatted BSFL meal	250 g/kg (w/w)	16.3 MJ/kg DM	Marker method	Ross 308 male broilers
	Highly defatted BSFL meal	250 g/kg (w/w)	14.9 MJ/kg DM	Marker method	Ross 308 male broilers
	Dry-rendered BSFL larvae	50%	16.7MJ/kg	Total collection method	Cobb-500 broilers
Cockcroft (2018)	Extruded BSFL larvae	50%	8.84MJ/kg	Total collection method	Cobb-500 broilers
	Full-fat BSFL larvae	50%	15.8MJ/kg	Total collection method	Cobb-500 broilers

BSFL, Black soldier fly larvae; AME, Apparent metabolizable energy.

Furthermore, the effect of the experimental error on the estimated AME value decreases as the level of test material inclusion increases (Sibbald and Slinger, 1963). Moreover, at low inclusion levels, the test material may not be evenly mixed or sufficiently incorporated into the diet therefore may result inaccurate AME estimation if birds do selective intakes (Petersen et al., 1976). Therefore, it could possibly magnify the errors. The feed intake of birds is one of the vital factors which has a positive relationship when determining AME of a particular test ingredient (Sibbald, 1975; Farrell et al., 1991; Yaghobfar & Boldaji, 2002). Furthermore, the value for AME is also determined by endogenous energy loss (EEL) per unit of feed intake. Theoretically, as food intake decreases, the bird will force to meet more of its protein and energy needs through tissue catabolism, increasing endogenous energy output. Fasted birds therefore may have higher fecal and urinary energy losses than those birds full fed due to increased catabolism of tissue protein to provide energy for body maintenance. Uric acid, which is excreted as urine, is also a byproduct of protein catabolism (Sibbald, 1985). Though statistically not significant, in the present experiment the birds fed 5% BSFL meal indicated comparatively a lower feed intake as compared to those fed 15% inclusion level (Table 8). This might have an influence for a higher EEL resulting a negative AME value at 5% BSFL inclusion level.

The values reported in the past BSFL metabolic experiments are summarized in Table 9. Those results are in close agreement with the AME value obtained for 15% BSFL inclusion level (15.7 MJ/kg). Hence it is reasonable and noteworthy to consider 15.7 MJ/kg value as the AME of the BSFL full-fat meal under the conducted experimental conditions. Considering the plant-based AME experiments on white lupin (*Lupinus albus*) three cultivars namely, Ultra, Kiev mutant and Promore have

resulted AME values of 8.05, 9.58 and 9.68 MJ/kg DM, respectively) (Nalle et al., 2012). Interestingly, the present study demonstrated comparatively a higher AME value (15.7 MJ/kg) than the AME of plant-based protein ingredients in an economical manner.

The present AME study was conducted using Cobb 500 unsexed broilers aged between 25 to 28-days post hatch. Determination of AME of a particular feed does not depend only on the energy-gaining feed ingredients but also on the bird's health status, age, breed, feeding pattern, method of feeding assay, physiological conditions, housing conditions and environmental conditions (Härtel, 1986; Wu et al., 2020). By considering the effect of gender on AME, Nalle et al. (2012) has been proposed that the gender influences on digestive capabilities, gut structure, function, and metabolic activity of gut microflora. Furthermore, the AME value of a feed ingredient is known to be higher in adult chickens than in growing broilers (Sibbald and Wolynetz, 1985). Therefore, further studies are warranted to find out the AME of BSFL in the birds of the same sex.

Conclusion

The present study concluded that the full-fat BSFL meal is enriched with major nutrients, which could be substituted with conventional protein rich feed ingredients. Apparent metabolizable energy of full-fat BSFL estimated in the present study is 15.7 MJ/kg.

Acknowledgment

Authors wish to acknowledge the financial assistance provided by RIC Grant 6 of Sabaragamuwa University, AHEAD Project, Ministry of Higher Education funded by the World Bank. The assistance of the technical staff,

Department of Livestock Production, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka, Sri Lanka is also acknowledged.

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