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Substitution of Soybean Meal with Defatted Black Soldier Fly Larvae Meal in Starter Concentrate on Growth Performance, Rumen Fermentation, Apparent Nutrient Digestibility, and Blood Hemato-Biochemical Profile of Growing Lambs

Research Article

D.M. Fassah[1], F. Hanifah[1], K. Kiswantoro[1], S.P. Damayanti[1], Y.M. Alfia[1], L. Khotijah[1], D. Diapari[1] and D.A. Astuti[1*]

[1] Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, 16680, Bogor, Indonesia

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*Correspondence E-mail: dewiaa@apps.ipb.ac.id

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ABSTRACT

This study aimed to assess the effect of partially substituting soybean meal (SBM) with defatted Black soldier fly larvae meal (DBSFL) on growth performance, rumen fermentation characteristics, apparent nutrient digestibility, and blood hemato-biochemical profile of growing lambs. A total of 10 Javanese thin-tail growing lambs (BW 13.95±3.07 kg) were equally assigned to two dietary treatments: P1= starter concentrate with 22% SBM (control), and P2= starter concentrate with 11% SBM and 10% DBSFL. The trial lasted for 56 days, including 14 days of adaptation. The results revealed that substituting 10% of SBM with DBSFL did not affect (P>0.05) nutrient intake and growth performance of growing lambs. The inclusion of 10% DBSFL had no adverse effects (P>0.05) on rumen fermentation characteristics, feed apparent digestibility, and blood hemato-biochemical profiles of growing lambs. In conclusion, DBSFL at 10% inclusion level can effectively substitute SBM in the starter concentrate, ensuring comparable health and productivity in growing lambs.

KEY WORDS blood haemato-biochemical profile, defatted black soldier fly larvae meal, digestibility, growing lamb, performance, rumen fermentation.

INTRODUCTION

Protein is a dominant nutrient required to support animal growth, especially in post-weaning ruminants. The most common protein source for ruminants is plant-based, such as legumes, oilseed meals, grain and by-products, and non-protein nitrogen. Soybean meal (SBM) has traditionally been recognized as a crucial protein source in young ruminant diets, primarily due to its excellent digestibility and comprehensive amino acid profile. However, the demand for soybeans in the global market and its reliance on imports has increased costs, making it less economically viable for many livestock producers (McFarlane and O'Connor, 2014). This economic issue, alongside the concerns regarding the environmental sustainability of soybean production. Therefore, an alternative, more cost-effective protein source for young ruminant feed is required.

One potential alternative is insect-derived protein, which has gained traction recently as a sustainable and nutritionally valuable feed ingredient. The larvae of black soldier fly (BSF; *Hermetia illucens*) have been recognized as an exceptionally promising insect-derived protein source, offering high crude protein content ranging from 40%-50%, crude fat content 29%-32%, and rich amino acid composition comparable to SBM (Bosch *et al.* 2019; Mikhailova *et al.* 2022). The defatted black soldier fly larvae meal (DBSFL), a byproduct obtained after the extraction of fat or oil from the larvae, is particularly notable for its sustainability and nutrient profile. The DBSFL meal comprises roughly 47.70%-51.65% crude protein, exhibiting high essential amino acids, including methionine, lysine, and threonine, which are vital for supporting animal growth and health (Edah and Owolabi, 2023; Saputra and Lee, 2023). Furthermore, BSF larvae can be produced on organic waste, contributing to their sustainability profile and making them more environmentally friendly than traditional feed ingredients (Nekrasov *et al.* 2022; Amin *et al.* 2024).

DBSFL has become a feasible protein source. Studies on poultry and aquaculture species indicate that DBSFL can effectively substitute conventional protein sources such as meat bone meal and fish meal (Eide *et al.* 2024; Nahrowi *et al.* 2024). However, the substitution of SBM with DBSFL in the starter concentrates of growing lambs has not been extensively studied. Previous studies have shown that insect-derived protein meals, such as BSF larvae meal, positively impact *in vitro* fermentation and digestibility in goat and dairy cows (Kahraman *et al.* 2023; Lu *et al.* 2024). Jayanegara *et al.* (2017a) reported that utilization of 20%-40% BSF larvae meal reduces rumen fermentability *in vitro*, but it also decreases methane emission. However, 9% utilization of BSF larvae meal from tea waste and sago pulp did not negatively affect rumen fermentation *in vitro* (Fassah *et al.* 2022). Including BSF larvae meals in the milk replacer of Ettawa crossbreed goats has demonstrated improvements in growth and physiological health (Astuti and Komalasari, 2020; Sepriadi *et al.* 2022). The utilization of 15% and 30% BSF meal in creep feed diet for growing Etawah crossbred goats showed better performance (Astuti and Wiryawan, 2022). However, the specific substitution of SBM with DBSFL in lamb starter concentrates, especially concerning its effects on growth performance, apparent nutrient digestibility, and blood hemato-biochemical parameters remains unknown.

By filling the knowledge gap, our study evaluates the implications of partially substituting SBM with DBSFL in the starter concentrate of growing lambs. We hypothesize that this substitution will result in comparable growth performance, rumen fermentation, apparent nutrient digestibility, and blood hemato-biochemical parameters. This study provides novel perspectives regarding the efficacy and feasibility of DBSFL as an eco-friendly, sustainable, cost-effective protein source for growing lambs.

MATERIALS AND METHODS

This study was conducted at the Meat and Draught Animal Nutrition Laboratory at the Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Indonesia. This study's conditions and handling practices are approved by the IPB University Animal Care and Use Committee (ACUC) no. 217-2021 IPB.

Animals, diets, and management

A total of 10 Javanese thin-tail growing lambs aged 5 months (BW 13.95±3.07 kg) were randomly allocated into one of two experimental diets, e.g., P1= starter concentrate containing 22% SBM, and P2= starter concentrate containing 11% SBM and 10% DBSFL. The diet consisted of starter concentrate and forage (*Pennisetum purpureum*) with a ratio of 70:30. Diets were formulated to fulfill the lamb's nutrient requirements (NRC, 2007) and prepared in pelletized form using a pellet machine. The defatted black soldier larvae meal was obtained from PT. Biocycle Indo (Kampar, Riau, Indonesia). The chemical composition of concentrate diets and forage are shown in Table 1. The amino acid contents of DBSFL and SBM are available in Table 2.

Growth performance and apparent nutrient digestibility

The total experiment duration is 56 days, including 14 days of adaptation. Lambs were housed in individual cages equipped with feeders and drinkers. Lambs were fed 4% of their body weight and were afforded free access to clean water. The amount of feed offered and refused was recorded daily for each lamb to calculate daily feed intake. Feed and refused feed samples were collected weekly and stored at 20 ^{o}C until analysis. All lambs were weighed biweekly prior to morning feeding. Average daily gain (ADG) was determined by dividing the cumulative weight gain by the total number of feeding days. Feed efficiency was evaluated by calculating ADG by dry matter intake.

During the final seven days (week six) of the experimental period, the total fecal output from each lamb was collected to assess apparent nutrient digestibility. Fresh feces were collected immediately after defecation to prevent urine contamination.

The daily fecal output from each lamb was weighed, and a 10% subsample was taken. Afterward, the fecal samples were thawed and combined over seven days for each lamb, with a 200 g subsample collected for chemical analysis. Feed, refused feed, and fecal samples were dried at 65 ^{o}C for 48 hours and ground for chemical analysis in accordance with AOAC (2019). The total digestible nutrient was calculated following Wardeh (1981).

Rumen fermentation characteristics

Rumen fluid was collected using a stomach tube (Preston, 1985) on day 56 after 2 hours of morning feeding. Ruminal pH was measured immediately using a portable pH meter (Hanna HI 98191, Cluj-Napoca, Romania). A 0.5 mL sample was put into glycerol media for total bacteria analysis, and 1 mL was put into the *Trypan Blue Formalin Saline* (TBFS) solution for protozoa analysis.

Table 1 Ingredient and chemical composition of concentrate diets and forage

Items	Concentrate[1] P1	Concentrate[1] P2	*Pennisetum purpureum*
Ingredients (%)			
Ground corn	44.50	43.00	-
Pollard	24.00	27.00	-
Tapioca meal	1.50	1.50	-
Palm oil	3.50	3.00	-
Molasses	1.00	1.00	-
Soybean meal	22.00	11.00	-
Defatted BSF[2] larvae meal	0.00	10.00	-
$CaCO_3$	2.00	2.00	-
Dicalcium phosphate	0.50	0.50	-
Salt	0.50	0.50	-
Premix	0.50	0.50	-
Total	100.00	100.00	-
Chemical composition (%)			
Dry matter (DM)	92.56	93.29	16.50
Ash	5.96	5.93	8.59
Crude protein (CP)	21.93	21.92	12.58
Ether extract (EE)	5.38	5.61	1.92
Crude fiber (CF)	2.86	4.03	28.83
Nitrogen-free extract (NFE)	63.87	62.50	48.08
Total digestible nutrient (TDN)	90.86[3]	89.90[3]	67.47[4]

[1] P1= starter concentrate containing 22% soybean meal and P2= starter concentrate containing 11% soybean meal + 10% defatted black soldier fly larvae meal.
[2] BSF: black soldier fly.
[3] TDN of concentrate= -37.3039 + (1.3048×% CP) + (1.3630×% NFE) + (2.1302×% EE) + (0.3618×% CF) (Wardeh, 1981),
[4] TDN of forage= 1.6899 + (1.3844×% CP) + (0.7526×% NFE) - (0.8279×% EE) + (0.3673×% CF) (Wardeh, 1981).

Table 2 Comparison of amino acids contained in defatted black soldier fly larvae meal and soybean meal

Amino acid	Composition (%) DBSFL	Composition (%) Soybean meal[1]
L-Histidin	2.01	1.30
L-Glisin	4.07	2.03
L-Arginin	2.94	3.39
L-Tirosin	3.36	1.53
L-Methionine	0.45	0.59
L-Valin	3.43	1.69
L-Phenylalanine	2.47	2.10
L-Isoleusin	2.31	1.67
L-Leusin	3.71	3.36
L-Lysine	3.45	2.91

[1] Upadhaya *et al.* (2016), soybean meal containing dry matter: 86.48%, crude protein: 44.00%, ether extract: 1.10%.
DBSFL defatted black soldier fly larvae meal. Laboratory analysis (2022) and DBSFL containing dry matter: 94.65%, crude protein: 68%, ether extract: 6.74%.

The bacterial and protozoan populations were quantified according to the method described by Ogimoto and Imai (1981).

The remaining rumen fluid was filtered through 4 layers of cheesecloth to measure the total and individual proportions of VFA and NH_3. Samples for NH_3 and total VFA were given ± 2 drops of $HgCl_2$ solution to kill microbes and stop fermentation and two drops of H_2SO_4 to reduce the pH value and stabilize the sample.

The total VFA concentration was determined using the steam distillation method according to Despal *et al.* (2022), while the individual VFA molar proportion was determined using gas chromatography (GC Bruker S/N BR1303 M 705 with the Scion 436 GC-MS model; Billerica, MA, USA), and NH_3 concentration was analyzed using Conway's micro-diffusion method according to Jayanegara *et al.* (2016). Methane gas concentration was estimated based on the proportion of individual VFA (Moss *et al.* 2000).

Blood haemato-biochemical profile

Blood was taken for each lamb at day 56 after 2 hours of morning feeding. Blood samples were obtained aseptically from the jugular vein of the animals using a sterile disposable syringe. The blood samples were immediately preserved on vacuum tubes preloaded with EDTA, gently mixed, and then centrifuged at 2500 × g for 15 minutes. Plasma was removed and used for blood hematology and metabolite profiles. Blood hematology, including erythrocytes, leukocytes, hemoglobin, hematocrit, and leukocyte differentiation was quantitatively analyzed according to Sastradipraja *et al.* (1989). Blood metabolite profiles, including glucose, cholesterol, total protein, and albumin, were assessed using commercial kits (cat. no: 112191, 101592, 157092, and 156092) from Greiner GMBH (Bahlingen, Germany) following company instructions.

Statistical analysis

All data were analyzed using the independent sample T-test performed in SPSS 20.0 (SPSS, 2011). A significance was defined at $P < 0.05$, while a trend was observed for $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Growth performance

Substitution of 50% SBM with DBSFL did not affect (P>0.05) total dry matter intake as well as the nutrient intake (including intakes of crude protein, ether extract, crude fiber, and NFE), average daily gain, final body weight, and feed efficiency of growing lambs (Table 3).

Rumen fermentation characteristics and apparent nutrient digestibility

Substitution of 50% SBM with DBSFL had no effect (P>0.05) on rumen pH, total bacteria, and total protozoa compared to control (Table 4). The substitution of 50% SBM with DBSFL tended to increase (P=0.07) butyrate proportion up to 20.4% compared to control (Table 4). However, the substitution did not affect (P>0.05) NH_3 concentration, total VFA, proportion of acetate, propionate, iso-butyrate, iso-valerate, C_2:C_3 ratio, VFA/NH_3 ratio, and concentration of CH_4 of growing lambs (Table 4). Furthermore, substituting 50% SBM with DBSFL did not affect (P>0.05) the apparent nutrient digestibility of growing lambs (Table 5).

Blood haemato-biochemical profiles

Substitution of 50% SBM with DBSFL had no effect (P>0.05) on the number of erythrocytes, hemoglobin, hematocrit, leukocytes, as well as leukocytes differentiation (lymphocytes, neutrophils, monocytes, eosinophils, and basophils) (Table 6). The substitution of 50% SBM with DBSFL did not change (P>0.05) blood metabolite profiles, including glucose, cholesterol, total protein, and albumin (Table 6).

DBSFL, as a substitute protein source for SBM in lamb diets, has garnered significant interest in ruminant nutrition. In the present study, 10% DBSFL to substitute SBM in starter concentrate did not impair the nutrient intakes of growing lambs. This suggests that DBSFL provides a nutritionally adequate protein source that supports normal feed intake and nutrient utilization. A possible explanation is that both diets were formulated in isonitrogenous and isoenergetic, providing lambs with equivalent amounts of protein and energy regardless of the protein source. Previous studies demonstrated that varying levels of BSF larvae meal in isonitrogenous and isoenergetic diets do not significantly change nutrient intake in poultry, ruminants, and aquatic animals (Dalle Zotte *et al.* 2019; Harlystiarini *et al.* 2020; Mulianda *et al.* 2020; Zhao *et al.* 2023; Silva *et al.* 2024). Furthermore, in line with Wei *et al.* (2024), the inclusion of DBSFL may improve the palatability of the diet, as it contains a high amount of essential amino acids (Table 2), which contribute to enhanced flavor and feed intake stimulation. Additionally, DBSFL contains lauric acid, a medium-chain fatty acid that has been reported to exert antimicrobial properties (Fortuoso *et al.* 2019), potentially modulate microbial populations, stabilize the fermentation process, and promote nutrient utilization efficiency. Therefore, our study supports that 10% DBSFL in starter concentrate provides similar nutritional benefits to SBM, thereby supporting comparable nutrient intake and ensuring an adequate nutrient supply.

The results of this study revealed no notable differences in nutrient intakes, resulting in comparable growth performance and feed efficiency in growing lambs when SBM was substituted with DBSFL at a 10% inclusion rate in starter concentrate. This indicates that DBSFL provides a balanced amino acid composition necessary for maintenance, metabolic function, and growth. DBSFL exhibits a high protein content (37%-63%) with a superior amino acid profile compared to SBM, which is crucial for promoting optimal animal growth (Barragan-Fonseca *et al.* 2017; Dabbou *et al.* 2018).

Our current study found that DBSFL exhibited nearly double the amounts of key amino acids, such as histidine, glycine, tyrosine, valine, isoleucine, and lysine compared to SBM (Table 2), which plays a critical role in protein synthesis, tissue repair, and immune function. Another factor is that DBSFL has demonstrated more excellent resistance to rumen protein degradation relative to SBM, which may enhance protein availability in post-ruminal digestion (Ido *et al.* 2021; Mulianda *et al.* 2020).

Table 3 Effect of substitution soybean meal with defatted black soldier fly larvae meal in starter concentrate on nutrient intake, performance, and efficiency of growing lambs

Parameters	Concentrate[1]		SEM	P-value
	P1	P2		
Dry matter intake (g/day)	660.48	647.70	31.52	0.78
Crude protein intake (g/day)	132.15	129.67	4.48	0.80
Ether extract intake (g/day)	30.83	31.48	1.10	0.79
Crude fiber intake (g/day)	54.19	58.82	1.81	0.22
nitrogen-free extract intake (g/day)	400.38	385.80	14.01	0.61
Average daily gain (g/day)	190.19	175.81	7.16	0.34
Final body weight (kg)	21.96	21.67	1.01	0.90
Feed efficiency (%)	28.95	27.20	0.83	0.58

P1= starter concentrate containing 22% soybean meal and P2= starter concentrate containing 11% soybean meal + 10% defatted black soldier fly larvae meal.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effect of substitution soybean meal with defatted black soldier fly larvae meal in starter concentrate on rumen fermentation characteristics of growing lambs

Parameters	Concentrate		SEM	P-value
	P1	P2		
pH	6.79	6.87	0.11	0.73
Total bacteria (log CFU/mL)	9.17	9.84	0.39	0.42
Total protozoa (log cell/mL)	5.96	6.34	0.22	0.41
NH_3 (mM)	10.30	10.51	1.20	0.94
Total VFA (mM)	117.00	116.88	3.89	0.99
VFA molar proportion (%)				
C_2	50.98	50.36	2.21	0.90
C_3	37.98	36.82	2.20	0.81
$_nC_4$	7.79	9.38	0.44	0.07
$_iC_4$	0.83	0.95	0.06	0.31
$_nC_5$	1.08	1.18	0.07	0.55
$_iC_5$	1.33	1.30	0.05	0.82
C_2/C_3 ratio	1.42	1.45	0.16	0.94
VFA/NH_3 ratio	12.89	12.18	1.38	0.81
CH_4 (mM/100 mM)	15.81	14.03	1.27	0.52

P1= starter concentrate containing 22% soybean meal and P2= starter concentrate containing 11% soybean meal + 10% defatted black soldier fly larvae meal.
NH_3: ammonia; VFA: volatile fatty acid; C_2: acetate; C_3: propionate; C_4: butyrate; C_5: valerate and CH_4: methane.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of substitution soybean meal with defatted black soldier fly larvae meal in starter concentrate on the digestibility of growing lambs

Parameters (%)	Concentrate		SEM	P-value
	P1	P2		
Dry matter	76.43	74.97	0.52	0.18
Crude protein	78.27	75.94	0.95	0.24
Crude fat	92.66	91.12	0.58	0.20
Crude fiber	47.82	52.22	1.89	0.27
Nitrogen-free extract	81.13	80.84	0.41	0.75
Total digestible nutrient	58.99	58.86	1.94	0.98

P1= starter concentrate containing 22% soybean meal and P2= starter concentrate containing 11% soybean meal + 10% defatted black soldier fly larvae meal.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effect of substitution soybean meal with defatted black soldier fly larvae meal in starter concentrate on blood haemato-biochemical profile of growing lambs

Parameters	Concentrate		SEM	P-value	Normal value
	P1	P2			
Erythrocytes (10^6/mm^3)	11.14	11.06	0.32	0.91	9-15[S]
Hemoglobin (g%)	13.24	13.08	0.18	0.68	9-15[S]
Hematocrit (%)	30.80	31.20	0.86	0.83	29-45[s]
Leukocytes (10^3/mm^3)	6.07	7.46	0.45	0.13	4-12[s]
Lymphocytes (%)	56.68	57.08	1.03	0.86	50-75[s]
Neutrophils (%)	31.63	31.01	0.88	0.74	17-50[s]
Monocytes (%)	3.05	3.10	0.39	0.95	0-6[s]
Eosinophils (%)	7.11	6.96	0.33	0.86	0-8[s]
Basophils (%)	1.52	1.85	0.27	0.57	0.5-3[s]
Glucose (mg/dL)	82.35	87.65	2.00	0.20	45-135[d]
Cholesterol (mg/dL)	54.80	66.97	3.72	0.24	52-76[k]
Total protein (g/dL)	6.00	5.74	0.18	0.52	6.00-7.90[k]
Albumin (g/dL)	3.16	3.22	0.06	0.66	2.40-4.00[Si]

P1= starter concentrate containing 22% soybean meal and P2= starter concentrate containing 11% soybean meal + 10% defatted black soldier fly larvae meal.
[S]: Smith and Mangkoewidjojo (1988); [d]: Desco *et al.* (1989); [k]: Kaneko *et al.* (2008) and [Si]: Sidki and Hirst (1998).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

This is particularly important in highly productive ruminants, which require increased levels of rumen undegradable protein (RUP) to fulfill the amino acid demands during the post-ruminal absorption (Sharif *et al.* 2019). The higher rumen undegradable protein fraction of DBSFL suggests a greater proportion of protein bypassing rumen microbial degradation, leading to increased direct absorption of essential amino acids in the small intestine, which is beneficial for growth performance. Despite the differences in amino acid composition and degradation properties, the overall weight gains of lambs remained similar between treatments. This aligns with Obeidat (2023), who proposed that the weight gain of lambs is determined mainly by the nutrient intake regardless of specific dietary composition. This highlights the idea that a sufficient dietary nutrient can sustain optimal growth and feed efficiency. Thus, the inclusion of 10% DBSFL in starter concentrate not only meets the nutrient requirement of growing lambs but also helps reduce the reliance on SBM, supporting sustainability of ruminant productivity.

Bacteria and protozoa play pivotal roles in rumen fermentation, facilitating the breakdown of feed nutrients through a complex microbial ecosystem. In this study, using 10% DBSFL in starter concentrate did not alter the total bacteria and protozoa population, suggesting that dietary change did not disrupt the rumen microbial balance. The balance of the microbial population significantly contributes to VFA production and microbial synthesis, which is crucial for energy supply and metabolic function in ruminants. Our result aligns with Ahmed *et al.* (2021), confirming that incorporating BSF larvae had no significant impact on the rumen microbial population. In addition, a symbiotic relationship between bacteria and protozoa is crucial for maintaining rumen homeostasis. Protozoa derive energy and nutrients by consuming bacteria, while they regulate bacteria populations by grazing (Perez *et al.* 2024). The unchanged microbial populations with DBSFL inclusion suggest that it does not contain inhibitory compounds that could interfere with microbial activity. In this study, the bacteria population was maintained in 9-10 log CFU/mL (McDonald *et al.* 2022), indicating favorable rumen conditions to support efficient rumen fermentation.

Unchanged in ruminal pH and NH_3 concentration provide evidence that the substitution of 10% DBSFL did not interfere with nitrogen metabolism or ammonia metabolism by rumen microbes. Rumen pH is typically maintained in the range of 5.5 – 6.9 due to the absorption of fatty acids and NH_3 (Choudhury *et al.* 2015). Rumen microbes rely on NH_3 as a nitrogen source for protein synthesis, which directly impacts on ruminant productivity (Rosmalia *et al.* 2022). In this study, the NH_3 concentration remained within the optimal range of 6 - 21 mM (McDonald *et al.* 2022), suggesting DBSFL protein degradation was well-regulated and minimized nitrogen losses to ammonia. Ammonia concentration is affected by the type of feed and the level of rumen protein digestibility (Mashudi *et al.* 2024). This aligns with previous findings that DBSFL protein is 18% less degraded in the rumen (47%) compared to SBM (65%), indicating potential improvement in nitrogen utilization efficiency (Fukuda *et al.* 2022). The greater resistance to rumen degradation ensures high rumen undegradable protein, allowing more amino acids absorbed in the small intestine to support highly productive ruminants (Sharif *et al.* 2019).

A 10% DBSFL to substitute SBM did not change the total concentration and proportions of rumen's VFAs, VFA-NH_3 ratio, and methane concentration. It likely reflects the similar composition of feed energy sources, including fiber, NFE, and fat, degraded into simple sugars and fermented into VFAs. However, there was a tendency for an increase in butyric acid (P=0.07) in lambs fed the DBSFL diet, which may be attributed to differences in crude fiber level. High crude fiber contributes to elevated ruminal butyric acid concentrations, which serve as the key energy source for epithelial cells and play a vital role in maintaining rumen health and epithelial proliferation (Miguel *et al.* 2019; Świerk *et al.* 2024).

The major medium-chain fatty acid (MCFA) in BSF, lauric acid may affect the rumen fermentation. Lauric acid is known to exhibit antimicrobial properties against methanogenic archaea and ciliates by modulating the fermentation profile (Ewald *et al.* 2020; Yanza *et al.* 2021). This property may explain why the methane production remained unchanged despite a shift in butyric acid concentration. Previously, Dohme *et al.* (2000) demonstrated that 53 g/kg DM of fat ingredients rich in MCFA (lauric acid) significantly increased butyric acid production while reducing methane emissions by selectively inhibiting certain rumen microbial populations. However, our study did not observe an alteration in methane production. In this study, the ratio of VFA/NH_3 ranges from 12.18 to 12.89, categorized as the normal range, which is necessary for optimal rumen microbial metabolism. The VFA/NH_3 ratio ranges from 9.75 to 14.55, which is associated with positive live weight gains in lambs (Prayuwidayati and Widodo, 2007). Our findings observe that 10% DBSFL substitute SBM in the starter concentrate did not observe any methane suppression, but a slight increase of butyrate suggests that DBSFL may contribute to energy production efficiency in the rumen.

Our study reported that 10% DBSFL to substitute SBM did not affect the apparent nutrient digestibility in growing lambs, indicating that DBSFL was efficiently digested and did not interfere with feed utilization. The BSF larvae meal contains 47%-65% of neutral detergent insoluble crude protein fraction (Jayanegara *et al.* 2017b), significantly higher than that of SBM, which ranges from 2.4%-12.1% depending on the processing method (Mjoun *et al.* 2010). This suggests that a more significant proportion of BSF protein is available for direct absorption in the small intestine, improving the availability of amino acids. The presence of chitin in DBSFL may have also contributed to higher RUP levels, which form nanofibers embedded in a matrix of proteins and minerals, making it less accessible for ruminal microbial degradation (Hilkias *et al.* 2017; Tan *et al.* 2021). While chitin sometimes reduces digestibility due to its complex structure, the stable apparent nutrient digestibility suggests that 10% DBSFL substitution to SBM in starter concentrate was appropriately balanced, allowing efficient overall feed digestibility and supporting the viability as an alternative protein source for ruminant production.

In this study, the blood haemato-biochemical profile of the growing lambs remained within the normal physiological ranges, indicating that substituting 10% SBM with DBSFL did not negatively affect metabolic function or immune response. The stability of erythrocyte and leukocyte counts suggests that DBSFL provides sufficient nutrients since their production is influenced by adequate essential nutrients such as amino acids, minerals (zinc, iron, selenium), and vitamins (Parveen *et al.* 2010; Smith *et al.* 2018). Erythropoiesis, the process of erythrocytes formation, is strongly influenced by dietary protein quality, and amino acid availability. Certain amino acids, including glycine and lysine, play a critical role in hemoglobin synthesis and erythropoiesis (Garcia-Santos *et al.* 2017; Ma *et al.* 2024).

Our study demonstrated that DBSFL contains higher glycine (4.07%) and lysine (3.45%) contents than SBM (2.03% and 2.91%), yet no significant changes were observed in erythrocyte counts, hemoglobin concentration, and hematocrit levels. This suggests that even with a partial shift in protein sources, the dietary amino acid supply remains sufficient to maintain physiological functions. Moreover, the similar crude protein intake between treatments supports this, as the protein sufficiency supply is the key factor to maintain the hematological balance.

The absence of changes in leukocyte counts and its differentiation confirm that DBSFL does not compromise immune function, since any negative impact on immunity could lead to increased susceptibility to infections. The lack of inflammatory response also suggests that DBSFL did not introduce any anti-nutritional factors or immune stressors, making it a safe protein alternative in growing lamb diets. Similar findings in aquatic animals observed that BSF larvae meal inclusion had no adverse effect on immune parameters (Lanes *et al.* 2021; Moutinho *et al.* 2024). Moreover, previous study indicates a possible influence of the higher growth rate with strong immunity (Gholizadeh *et al.* 2024). These support the viability of DBSFL as a safe and sustainable alternative protein source to SBM.

Substituting SBM with DBSFL did not alter the blood metabolite levels of growing lambs, indicating the DBSFL may support the normal metabolic homeostasis. Blood glucose is the primary energy source for cellular function, and its levels are influenced by the interplay between energy and carbohydrate intake, temperature, and hormonal responses (Zieba *et al.* 2020; Ge *et al.* 2023; Sudarman *et al.* 2023). Our result suggest that DBSFL did not alter the carbohydrate metabolism or energy balance, confirming that both SBM and DBSFL provide similar energy availability for animal physiological functions.

Cholesterol, a precursor for steroid hormone synthesis (Hu *et al.* 2010) remained unaffected by DBSFL inclusion. This finding is relevant since dietary fat can influence cholesterol metabolism. Black soldier fly larvae naturally contain higher lipid levels, but the defatting process may lower the lipid content and minimize its impact on lipid metabolism. In addition, both diets contain of similar energy and fat levels that prevent any shifts in lipid absorption and cholesterol biosynthesis.

Total blood protein, including albumin and globulin, which is essential for various biological functions and serves as a biocatalyst in metabolic processes remained unchanged with 10% DBSFL inclusion on diet. This result suggests that DBSFL provides a comparable supply of digestible and metabolizable protein, maintaining sufficient protein levels for animal physiological functions. Albumin is crucial in transporting fatty acids, minerals, drugs, and hormones and maintaining blood osmotic pressure (Belinskaia *et al.* 2021; De Simone *et al.* 2021). Despite substituting 47% SBM with DBSFL, both diets were formulated in isonitrogenous form, resulting in equivalent levels of blood total protein and albumin. Previous research demonstrated that the diet protein level influences total blood protein and albumin concentrations (Antunović *et al.* 2011; Fasae *et al.* 2016). Moreover, amino acids such as isoleucine, lysine, and tryptophan, precursors for albumin synthesis, were present in similar concentrations in both SBM and DBSFL. Therefore, the absence of adverse effects of DBSFL on hemato-biochemical profile parameters confirms that DBSFL can replace a portion of SBM in starter concentrates without compromising any physiological health, making it a sustainable option for ruminant production.

CONCLUSION

The inclusion of 10% defatted BSF larvae meal in starter concentrate of growing lambs did not affect growth performance, rumen fermentation, apparent nutrient digestibility, and the blood hemato-biochemical profile, demonstrating its viability as a sustainable protein alternative. Utilizing defatted BSF larvae meal supports eco-friendly livestock production by promoting circular economy practices through insect-derived protein utilization.

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