Veterinary Integrative Sciences 2026; 24(1): e2026018-1-12. DOI: 10.12982/VIS.2026.018

Veterinary Integrative Sciences
ISSN; 2629-9968 (online)

Research article

Utilization of Silkworm (*Bombyx mori*) pupae meal and Javanese bird grasshopper (*Valanga nigricornis*) meal as alternative protein sources to replace meat and bone meal in ruminant feed: An *in vitro* study

Sri Ayu Agung Purnami[1], Muhammad Ridla[1,2,*], Nahrowi[1,2], Ki Ageng Sarwono[3], Sri Utami[3], Sih Kahono[3], Roni Ridwan[3], Rusli Fidriyanto[3], Ainissya Fitri[3], Rohmatussolihat[3] and Dudi Firmansyah[4]

[1] Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia
[2] Center for Tropical Animal Studies (CENTRAS), IPB University, Bogor 16680, Indonesia
[3] Research Center for Applied Zoology, National Research and Innovation Agency (BRIN), Cibinong, Bogor 16911, Indonesia
[4] Animal Technology and Management, Vocational School, IPB University, Bogor 16151, Indonesia

Abstract

This study aimed to evaluate the effects of replacing meat and bone meal (MBM) with insect-based protein sources, Silkworm pupae (*Bombyx mori*) meal (SPM), and Javanese bird grasshopper (*Valanga nigricornis*) meal (FGM) on rumen degradability, fermentation characteristics, total gas production, and methane emissions *in vitro*. Six experimental diets, each with five replicates, were tested, namely P1 (100% Napier grass), P2 (80% Napier grass + 20% MBM), P3 (80% Napier grass + 20% full-fat SPM (FSPM)), P4 (80% Napier grass + 20% defatted SPM (DSPM)), P5 (80% Napier grass + 20% full-fat FGM (FFGM)), and P6 (80% Napier grass + 20% defatted FGM (DFGM)). The result showed that the total gas and methane production were significantly lowered by insect-based protein meal and MBM ($P < 0.05$). *In vitro* dry matter degradability (IVDMD) and *in vitro* organic matter degradability (IVOMD) of insect-based protein meal were not significantly different from MBM and control diet (IVDMD: $P = 0.87$; IVOMD: $P = 0.966$). Insect-based protein meal and MBM reduced total volatile fatty acid (VFA) and propionate proportion while also increasing NH_3-N and acetate:propionate ratio, as evidenced by $P < 0.05$. These results showed that insect-based proteins, particularly DSPM and FGM, had a similar digestibility to MBM, reduced methane emissions, and could effectively replace MBM in ruminant diets.

Keywords: Feed efficiency, Fermentation dynamics, Methane reduction, Protein digestibility, Sustainable livestock nutrition.

***Corresponding author**: Muhammad Ridla, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia. E-mail: hmridla@apps.ipb.ac.id.

Article history; *received manuscript: 8 February 2025,*
revised manuscript: 26 March 2025,
accepted manuscript: 17 May 2025,
published online: 26 May 2025,
Academic editor; *Saowaluck Yammuen-art*

INTRODUCTION

The primary protein sources for livestock feed formulations in Indonesia are animal- and plant-based ingredients, including fish, blood meal, meat and bone meal (MBM), palm kernel, soybean, and legumes. MBM is a widely used source of animal crude protein in feed formulations, containing 45–55%. This meal also provides essential amino acids, such as lysine, methionine, and cysteine, and energy and minerals, particularly calcium and phosphorus (Yanuartono et al., 2020). However, the use of MBM in ruminant feed is prohibited due to the association with *Bovine Spongiform Encephalopathy* (BSE), commonly called mad cow disease. The concerns about disease transmission have led to the European Union ban on the use of MBM as animal feed in 2001 (Hlasny, 2024). In Indonesia, regulations governing MBM usage include the Ministry of Agriculture Regulation Number 471/Kpts/OT.210/V/2002, which explicitly bans the inclusion of ruminant-derived ingredients in ruminant feed.

Due to these restrictions, alternative protein sources were required to replace MBM in feed formulations. Insect is a potential alternative protein source to MBM because of the high protein content (Siemianowska et al., 2013; Makkar et al., 2014). Increasing concerns about climate change and food security have driven the development of insect production industries and the use of feed ingredients in developed and developing countries. Some promising alternatives include Silkworm pupae and Javanese bird grasshopper. The use of these insects as a feedstuff may have several benefits, such as a relatively cheap protein source and a sustainable waste and pest utilization method.

Silkworm pupae (*Bombyx mori*) are a by-product of the silk production industry, primarily cultivated for silk, with pupae being a significant by-product (Estetika and Endrawati, 2018). The pupae constitute approximately 86.84% of the total cocoon weight (Rahmasari et al., 2014) and exhibit substantial nutritional potential. Herliatika et al. (2021) reported that Silkworm pupae meal (SPM) contains 58.28% and 28.93% crude protein and fat, respectively. SPM has been used as feed for monogastric animals (e.g., poultry, pigs, and fish) and ruminants (Rashmi et al., 2018; Rashmi et al., 2022; Gao et al., 2024). It is a rich protein source that can complement ruminant feed due to the low degradability and favourable amino acid profile (Ji et al., 2015). However, the application in ruminant feed is limited by high-fat content, necessitating extraction before the inclusion in large quantities (Sheikh et al., 2018).

Another promising alternative is the Javanese bird grasshopper (*Valanga nigricornis*). This species is classified as a pest in rubber and palm oil plantations, and the population outbreak is mostly during the dry season. Javanese bird grasshopper has a nutrient profile of 72.5% protein and 9.45% fat (Paulin and Purwanto, 2020). The high protein content makes the Javanese bird grasshopper a suitable candidate for replacing MBM in animal feed.

Despite this growing interest, limited data exist on the use and effects of insects as alternative feed ingredients for ruminants. Consequently, more extensive scientific studies are required to elevate the topic to policymakers' agendas to aid the development of regulatory frameworks for licensing insects as ruminant feed ingredients (Toral et al., 2022). Insects have been subject to a variety of regulations, including outright prohibitions and vague or non-specific legislation that governs the usage as feed and food for ruminants (Lähteenmäki-Uutela et al., 2021).

The aim of using Silkworm pupae and Javanese bird grasshopper as alternative protein sources in ruminant feed is to mitigate reliance on MBM. A comprehensive evaluation of these feed ingredients is essential, including analyses of the nutrient composition and effects on *in vitro* rumen fermentation parameters, such as pH, volatile fatty acid (VFA), ammonia nitrogen (NH_3-N), *in vitro* dry matter degradability (IVDMD), *in vitro* organic matter degradability (IVOMD), gas production, and methane emissions.

MATERIALS AND METHODS

Sample preparation

Full-fat and defatted meals of Silkworm pupae and Javanese bird grasshopper were used in this study. Silkworm pupae were collected from the Center for Standardization of Sustainable Forest Management Instruments, Indonesia's Ministry of Environment and Forestry. Similarly, the Javanese bird grasshopper was collected from Meru Betiri National Park in East Java, Indonesia. The grasshopper was identified by the Research Center of Applied Zoology, National Research and Innovation Agency. Samples were dried at 60°C for 48 hours and ground into SPM and Javanese bird grasshopper meal (FGM). Fat removal was achieved through Soxhlet extraction to produce defatted samples according to the method of Jayanegara et al. (2020). This study used six experimental treatments, each with five replicates. The control treatment consisted of dried and ground Napier grass (*Pennisetum purpureum*). The other treatments included P1 = 100% Napier grass, P2 = 80% Napier grass + 20% meat and bone meal (MBM), P3 = 80% Napier grass + 20% full-fat SPM (FSPM), P4 = 80% Napier grass + 20% defatted SPM (DSPM), P5 = 80% Napier grass + 20% full-fat FGM (FFGM), and P6 = 80% Napier grass + 20% defatted FGM (DFGM).

Sample analysis

The parameters included nutrient composition (crude fiber, crude protein, ash, organic matter, dry matter, crude fat, and moisture content) and *in vitro* rumen fermentation, such as IVDMD, IVOMD, gas production, methane production, pH, NH_3-N, and total VFA. Nutrient composition was analyzed using proximate analysis AOAC (2005). Furthermore, the *in vitro* analysis of rumen fermentation was conducted following the description by Theodorou et al. (1994) and Putra et al. (2023). The National Research and Innovation Agency (BRIN) Animal Ethics Committee accepted the animal handling protocol (Approval number 08/KE.02/SK/10/2022). Rumen fluid was collected from four fistulated male Ongole crossbred cattle, aged 3-4 years, with body weights ranging from approximately 275 to 349 kg. The animals were fed a diet consisting of 70% fresh Napier grass and 30% concentrate containing approximately 14% crude protein.

For each treatment, 0.5 g of sample was placed in a 100 mL vial, and 50 mL of a rumen fluid–McDougall's buffer mixture (1:2, v/v) was added. The mixture was saturated with CO_2 for 30 seconds, sealed with a rubber stopper, and incubated at 39°C for 48 hours. After incubation, the residual substrate was collected for IVDMD and IVOMD measurements. The supernatant was used for pH, NH_3-N, and VFA analyses.

Gas production was measured at 48 hours using a 50 mL syringe. Simultaneously, methane production was determined using a Bedetech GT 8220 gas detector. pH was measured with a digital pH meter, while NH_3-N concentration was quantified using a UV-VIS spectrophotometer, as described by Sagala et al. (2024). VFA concentrations were measured using a GC-MS-QP2010 SE (Shimadzu, Japan) according to the method of Sarwono et al. (2022).

Statistical analysis

The collected data were subjected to a factorial analysis of variance (ANOVA) with a randomized complete block design, including six treatments and five replications. An effect was considered significant at the probability threshold of $P < 0.05$. After accepting the result at a probability level of $P < 0.05$, a Duncan test was performed to assess the differences among the treatments. Statistical analysis was conducted using IBM SPSS Statistics version 25.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Nutrient content of feedstuff

The feedstuffs (Table 1) exhibited substantial variation in their nutrient composition. Napier grass (Elephant Grass) is characterized by a high crude fiber content (23.51% DM) and elevated non-fiber carbohydrate levels (52.01% DM), confirming its role as a primary source of roughage and energy. However, its crude protein (12.70% DM) and fat (1.27% DM) concentrations are relatively low. MBM possesses a high crude protein content (35.91% DM) with a low crude fiber level (4.46% DM). The high crude ash content (22.85% DM) of MBM shows its mineral-rich profile, which is a critical consideration when formulating ruminant diets.

Table 1 Nutrient content of feedstuff

Nutrient content	Feedstuff					
	Grass	MBM	FSPM	DSPM	FFGM	DFGM
Dry matter, %	21.56	91.44	89.17	82.75	93.88	94.84
Organic matter, % DM	89.49	77.15	94.61	93.51	95.62	95.24
Crude ash, % DM	10.51	22.85	5.39	6.49	4.38	4.76
Crude protein, % DM	12.70	35.91	48.46	62.90	58.83	65.37
Crude fat, % DM	1.27	12.11	13.22	2.63	9.39	2.34
Crude fiber, % DM	23.51	4.46	0.13	0.27	8.39	6.83
Nitrogen – Free Extract, % DM	52.01	24.67	22.8	27.71	19.01	20.70

Note: MBM = Meat and bone meal; FSPM = Full-fat silkworm pupae meal; DSPM = Defatted silkworm pupae meal; FFGM = Full-fat Javanese bird grasshopper meal; DFGM = Defatted Javanese bird grasshopper meal

Nutrient content of experimental diets

The experimental diets (Table 2) showed significant differences in nutrient composition depending on the protein source incorporated. Diet P1, consisting of 100% Napier grass, had the lowest crude protein concentration (12.70% DM), as well as the highest crude fiber (23.51% DM) and non-fiber carbohydrate content (52.01% DM), reflecting the fibrous nature and primary role as an energy source. When 20% of Napier grass was substituted with alternative protein sources (diets P2-P6), there was a significant increase in crude protein levels, ranging from 17.34% to 23.23% DM. There was also a concomitant reduction in crude fiber (18.97-22.50% DM) and non-fiber carbohydrates (41.71-46.54% DM). Diet P6 (80% Napier grass + 20% DFGM) achieved the highest crude protein concentration (23.23% DM) and the lowest ash content (8.45% DM), showing an optimal nutrient profile. Meanwhile, diet P3 (80% Napier grass + 20% FSPM) recorded the highest crude fat content (5.66% DM). The diets incorporating defatted insect-based meals (P4 and P6) showed enhanced protein content with reduced fiber levels, thereby optimizing the nutrient balance and potentially enhancing the degradability of the feed.

Table 2 Nutrient content of experimental diets

Nutrient content (% DM)	Experimental diets					
	P1	P2	P3	P4	P5	P6
Dry matter (%)	21.56	77.46	75.65	70.51	80.18	79.41
Organic matter, % DM	89.49	79.61	93.58	92.70	94.39	94.04
Crude ash, % DM	10.51	20.38	6.41	7.29	5.60	5.91
Crude protein, % DM	12.70	31.26	41.30	52.86	49.60	54.83
Crude fat, % DM	1.27	9.94	10.83	2.35	7.76	2.12
Crude fiber, % DM	23.51	8.27	4.80	4.91	11.41	10.16
Nitrogen – Free Extract, % DM	52.01	30.13	36.64	32.57	25.61	26.96

Note: The nutritional content was calculated from the feedstuff nutritional content result. P1 = 100% Napier grass; P2 = 80% Napier grass + 20% MBM; P3 = 80% Napier grass + 20% FSPM; P4 = 80% Napier grass + 20% DSPM; P5 = 80% Napier grass + 20% FFGM; P6 = 80% Napier grass + 20% DFGM

Total gas production and methane emission

In vitro gas production assays in Table 3 showed that the inclusion of insect protein meals led to a significant reduction in total gas production by approximately 9-11% compared to the control diet (P1). The values increased from 84.13 mL in P1 to a range of approximately 73-77 mL ($P < 0.01$). The addition of insect protein meals significantly reduced methane production in absolute terms (a reduction of 22-34% in methane volume) and as a proportion of the total gas produced (a reduction of 20-31%) relative to the control ($P < 0.05$). These results suggested that the supplementation of insect protein could serve as an effective strategy to mitigate enteric methane emissions, which is of considerable importance in reducing the environmental footprint of ruminant livestock.

Table 3 Total gas production and methane emission of the experimental diets

Parameter	Experimental diets						
	P1	P2	P3	P4	P5	P6	P-value
Total gas production, ml	84.13 ± 2.27^{b}	74.88 ± 3.57^{a}	74.40 ± 3.68^{a}	76.63 ± 3.82^{a}	73.70 ± 3.23^{a}	74.30 ± 4.70^{a}	0.021
Methane, ml	3.55 ± 0.68^{b}	2.64 ± 0.41^{a}	2.31 ± 0.39^{a}	2.75 ± 0.55^{a}	2.43 ± 0.54^{a}	2.59 ± 0.56^{a}	0.046
Methane, % total gas	4.58 ± 0.63^{b}	3.50 ± 0.41^{a}	3.15 ± 0.39^{a}	3.57 ± 0.58^{a}	3.29 ± 0.61^{a}	3.66 ± 0.80^{a}	0.044

Note: Different superscripts in the same row are significantly different at $P < 0.05$; P1 = 100% Napier grass; P2 = 80% Napier grass + 20% MBM; P3 = 80% Napier grass + 20% FSPM; P4 = 80% Napier grass + 20% DSPM; P5 = 80% Napier grass + 20% FFGM; P6 = 80% Napier grass + 20% DFGM.

Rumen degradability and fermentation profiles *in vitro*

In vitro assessments of rumen degradability in Table 4 showed that neither IVDMD nor IVOMD was significantly affected by the inclusion of insect protein meals when compared with the control diet (IVDMD: $P = 0.870$; IVOMD: $P = 0.966$). Despite the unchanged degradability, the ruminal fermentation profile was changed by the supplementation of the protein sources. The concentration of NH_3-N in the rumen fluid was significantly elevated in diets supplemented with alternative protein sources ($P < 0.001$). Specifically, DSPM (P4) induced the most pronounced increase in NH_3-N, exhibiting a 708% elevation relative to the control, while MBM (P2) induced a more moderate increase of 258%. The inclusion of insect protein from Silkworm pupae was associated with a significant reduction in total VFA concentration, as evident by $P < 0.05$. In this regard, FFGM and DFGM (P5 and P6) reduced total VFA content by 14% and 23%, respectively. Total VFA concentrations in rumen fluid from diets supplemented with MBM, FSPM, and DSPM did not differ significantly from the control. The proportions of acetate and butyrate remained unaffected by the addition of MBM or insect protein meals. The propionate proportion was significantly reduced ($P < 0.05$), with the lowest production observed in DSPM group. Consequently, the acetate-to-propionate ratio increased significantly following the supplementation of insect protein meals and MBM ($P = 0.021$), showing a shift in ruminal fermentation dynamics that may have implications for the energy metabolism of ruminants.

Table 4 *In vitro* rumen degradability and fermentation dynamics of experimental diets

Parameter	Experimental diets						
	P1	P2	P3	P4	P5	P6	P-value
IVDMD, %	65.64 ± 3.54	66.76 ± 7.92	66.87 ± 4.28	64.80 ± 9.05	67.84 ± 2.34	69.85 ± 8.68	0.078
IVOMD, %	71.23 ± 3.47	71.89 ± 7.40	71.88 ± 4.07	69.94 ± 9.39	72.78 ± 1.65	73.08 ± 4.46	0.087
pH	6.78 ± 0.05	6.78 ± 0.06	6.77 ± 0.06	6.79 ± 0.06	6.76 ± 0.06	6.82 ± 0.08	1.825
NH_3-N, mM	6.16 ± 5.19[a]	22.11 ± 6.39[b]	32.48 ± 14.09[c]	55.96 ± 8.88[e]	48.14 ± 2.91[d]	49.79 ± 10.53[d]	0.018
TSCFA, mM	101.56 ± 6.52[c]	88.23 ± 8.53[abc]	91.14 ± 7.23[bc]	97.97 ± 1.86[bc]	86.94 ± 8.05[ab]	77.38 ± 12.28[a]	0.022
Acetate (%)	70.25 ± 1.97	72.05 ± 0.99	71.40 ± 1.04	71.72 ± 0.39	71.43 ± 0.63	70.97 ± 1.90	1.041
Propionate (%)	20.87 ± 1.68[b]	19.08 ± 0.43[a]	18.41 ± 0.66[a]	18.02 ± 0.33[a]	18.88 ± 0.58[a]	18.93 ± 0.82[a]	0.038
Butyrate (%)	5.78 ± 0.27	5.69 ± 0.71	6.15 ± 0.27	5.81 ± 0.18	5.57 ± 0.72	5.87 ± 0.77	0.672
A:P ratio	3.39 ± 0.37[a]	3.78 ± 0.1[b]	3.88 ± 0.19[b]	3.98 ± 0.09[b]	3.79 ± 0.14[b]	3.76 ± 0.26[b]	0.042

Note: Different superscripts in the same row are significantly different at $P < 0.05$; P1 = 100% Napier grass; P2 = 80% Napier grass + 20% MBM; P3 = 80% Napier grass + 20% FSPM; P4 = 80% Napier grass + 20% DSPM; P5 = 80% Napier grass + 20% FFGM; P6 = 80% Napier grass + 20% DFGM; IVDMD = In *vitro* dry matter degradability. IVOMD = In *vitro* organic matter degradability. NH_3-N =Ammonia-N; TSCFA=Total short-chain fatty acid; A:P = Acetate:Propionate.

DISCUSSION

Insect-origin meals, particularly those derived from Silkworm pupae and Javanese bird grasshoppers, have been characterized by elevated protein concentrations. Similarly, DSPM and DFGM exhibit crude protein values of 62.90% and 65.37% on a dry matter basis, respectively, as shown in Table 1. These values are significantly higher than those of full-fat counterparts, which is attributable to the reduction in non-protein fractions, such as lipids and fiber. The diminished presence of these non-protein components enhances the utility of defatted insect meals as concentrated protein supplements, particularly in high-protein diets. This observation is further corroborated by Herliatika et al. (2021), who reported nutrient profiles for SPM at 58.28% crude protein and 28.93% fat. In the case of FGM, the nutrient profile was at 33.11% crude protein and 18.7% fat. Consequently, defatted insect meals are becoming promising candidates for livestock feed formulations that aim to elevate dietary protein and also minimize excess fiber and lipid content.

SPM, a protein-rich animal-derived feed ingredient, typically exhibits a wide range of dry matter (DM) content, from 50% to 80% (Ullah et al., 2017), and crude protein content of 15% - 58%, depending on processing and preparation methodologies. In a more recent investigation, SPM was found to possess a DM content of 79.31% and an organic matter (OM) content of 83.79% (Sahib et al., 2023). When compared to conventional MBM, the elevated crude protein content observed in these insect meals showed the potential as an alternative source of animal protein for ruminants (Samal and Pattanaik, 2014).

As shown in Table 2, diets P4 and P6 exhibited crude protein concentrations of 22.74% and 23.23%, respectively, which were significantly higher than the 17.34% observed in P2 containing MBM. This increase in protein concentration is consistent with the results of Sahib et al. (2023), who documented incremental rises in crude protein content in controlled treatments when SPM was partially substituted into the diet. The data showed that the substitution strategies effectively elevate the protein profile of the diet, thereby enhancing its nutritional value.

The lipid content in SPM and FGM is considerably lower than MBM. According to Williams et al. (2020), the total fat content in ruminant diets should not exceed 6-7%, as excessive fat may compromise digestibility and adversely affect rumen fermentation. High-fat diets have been shown to impair the activity of fibrolytic bacteria, such as *Fibrobacter succinogenes,* and to reduce the protozoal populations critical for carbohydrate digestion (Rodríguez-Rodríguez et al., 2022). Therefore, defatted insect meals, such as DSPM and DFGM, with reduced lipid content and superior protein concentration, are particularly well-suited as protein sources for ruminants.

Total gas production *in vitro* was highest in P1 treatment, which consisted of roughage with the highest crude fiber content (23.51% DM, Table 2). The elevated gas production in P1 is due to the fermentation of fiber and other plant structural carbohydrates by ruminal fibrolytic bacteria, leading to the production of VFA and gases, such as CO_2 and H_2 (Moss et al., 2000). Consistent with previous studies, Ahmed and Nishida (2023), Renna et al. (2022), the incorporation of insect meals, as well as other alternative protein sources, such as cricket, black soldier fly, house fly, and mealworm, led to a reduction in total gas production.

In addition to lowering the gas production, the inclusion of insect protein meals and MBM led to a significant reduction in methane emissions relative to the roughage-only control. This reduction is related to the diminished availability of CO_2 and H_2, substrates necessary for methanogenesis through the hydrogenotrophic pathway in rumen methanogens. The results of this study are consistent with previous reports (Jayanegara et al., 2017a; Jayanegara et al., 2017b; Sarwono et al., 2019; Ridla and Nahrowi, 2025) that the supplementation of insect protein meals, or alternative protein sources, significantly decreases methane production *in vitro*. The hydrogenotrophic pathway converts CO_2 and H_2 into methane. This process is influenced by factors such as fiber digestibility, population dynamics of microorganisms, and inhibitory effects on methanogens. Substrates, such as chitin and lipids inherent in insect protein meals, may further inhibit methanogenic activity (Thirumalaisamy et al., 2020; Jayanegara et al., 2020; Sagala et al., 2024), although further studies are needed to elucidate these mechanisms.

IVDMD and IVOMD were comparable between insect-based protein meals and the control. This result shows that the inclusion of insect proteins does not adversely affect ruminal digestibility. The observation further suggests that substituting MBM with insect protein meals is safe concerning rumen fermentability *in vitro*. This is consistent with previous studies reporting that the inclusion of DSPM, at replacement levels of 25–50% for soybean meal, did not compromise digestibility parameters (Rashmi et al., 2018; Rashmi et al., 2022).

Rumen pH values remained stable across all dietary treatments, consistently falling in the optimal range of 6.7-6.8 for ruminal fermentation. The stability ensures effective microbial activity and adequate production of VFA, thereby maintaining the buffering capacity and general fermentation dynamics of the rumen. This result was consistent with the previous study (Van Dung et al., 2013; Purbowati et al., 2014; Ridla et al., 2023), which showed the critical importance of maintaining an optimal rumen pH for maximal cellulolytic bacterial activity and fiber digestion.

The incorporation of insect-based protein sources, particularly DSPM and DFGM, led to an increase in ruminal NH_3-N production. This increase is due to the rapid proteolysis of the protein sources, resulting in an abundant supply of ammonia for microbial protein synthesis. The results are in agreement with Pengpeng and Tan (2013), who documented increased NH_3-N concentrations in response to high-protein diets. Similarly, Rashmi et al. (2022) observed that substituting 20% MBM with DSPM increased NH_3-N, a consequence of the high degradability of DSPM proteins. Other studies reported comparable benefits, including improved nitrogen retention and energy use (Wu et al., 2021).

The inclusion of insect protein meals and MBM led to a significant reduction in total VFA production compared to 100% Napier grass (P1). This is primarily due to lower levels of fermentable carbohydrates, as shown by reduced nitrogen-free extract (NFE) in the supplemented diets, which is 46.54% in P2 and 41.71-43.02% in P3-P6 vs. 52.01% in P1.

Higher crude protein and fat contents, particularly in P3 and P5, can suppress fiber-digesting microbes, limiting VFA synthesis. Moreover, non-carbohydrate components, such as chitin and minerals, may disrupt microbial activity. These changes shifted the fermentation pattern, reflected in higher acetate-to-propionate ratios, showing less efficient energy production.

Total VFA production is a key indicator of ruminal carbohydrate fermentation, including both structural and non-structural carbohydrates (Xue et al., 2019). Diets with reduced carbohydrate content, particularly lower levels of fermentable fiber and soluble carbohydrates, can lead to decreased total VFA concentrations. According to Cao et al. (2021), fiber degradation is strongly linked to acetate formation. Although MBM and insect protein meal diets had lower fiber content, there was an increased acetate:propionate ratio and reduced propionate proportions, suggesting altered fermentation dynamics. This change may be attributed to the presence of chitin and lipids, particularly in FFGM and DFGM. Chitin and the derivative chitosan have been reported to impair digestibility and suppress propionate formation, thereby reducing total VFA production (Sagala et al., 2024). Grasshoppers typically contain 5.3%-14% chitin, depending on species and sex (Erdogan and Kaya, 2016), contributing to the observed effects.

The results of this study show the potential of defatted insect meals, such as DSPM and DFGM, as viable alternatives to conventional protein sources in ruminant nutrition. Insect-based protein meals can improve the nutritional quality and environmental sustainability of livestock feed formulations. This is shown by the enhanced crude protein content, reduced lipid and fiber concentrations, and favorable effects on ruminal fermentation dynamics, including reduced methane emissions and stable rumen pH.

The inclusion of insect-based protein meals, such as Silkworm pupae and Javanese bird grasshopper, shows promising results *in vitro.* This result suggests that meals as a potential alternative protein source to replace conventional MBM. However, further *in vivo* studies are necessary to validate the effectiveness under practical feeding conditions, particularly in terms of intake, nutrient digestibility, and animal performance. The relationship between gas production and the risk of bloat should also be investigated, as excessive fermentation gases, specifically methane, may impact rumen health and general digestive function. Ensuring that feed meets animals' nutritional requirements is important for accurate evaluation of dietary outcomes.

CONCLUSIONS

In conclusion, insect-based protein meals, such as DSPM and DFGM, showed strong potential as replacements for MBM in ruminant diets. These insect meals exhibited comparable ruminal digestibility and maintained optimal rumen fermentation parameters without adverse effects. The total gas and methane production was also reduced, thereby promoting both improved fermentative efficiency and environmental sustainability. Insect protein meals could improve the use of nutrients with higher crude protein levels and lower fiber and lipid contents. Further studies were required to evaluate the long-term effects on animal performance, health, and nutrient metabolism in practical applications.

AUTHOR CONTRIBUTIONS

Sri Ayu Agung Purnami contributed to designing and implementing the study, analyzing the results, and drafting the manuscript
Muhammad Ridla contributed to designing the study, analyzing the results, drafting the manuscript, supervising the study, and revising the final manuscript.
Nahrowi contributed to designing the study, analyzing the results, supervising the study, and revising the final manuscript.
Ki Ageng Sarwono contributed to designing and implementing the study, analyzing the results, drafting the manuscript, supervising the study, and revising the final manuscript
Sri Utami contributed to implementing the study, analyzing the results, and revising the final manuscript
Sih Kahono contributed to implementing the study, analyzing the results, and revising the final manuscript
Roni Ridwan contributed to implementing the study, analyzing the results, and revising the final manuscript
Rusli Fidriyanto contributed to implementing the study, analyzing the results, and revising the final manuscript
Ainissya Fitri contributed to implementing the study, analyzing the results, and revising the final manuscript
Rohmatussolihat contributed to implementing the study, analyzing the results, and revising the final manuscript
Dudi Firmansyah contributed to implementing the study, analyzing the results, and revising the final manuscript

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial organization regarding the material discussed in the manuscript.

REFERENCES

Ahmed, E., Nishida, T., 2023. Optimal inclusion levels of cricket and silkworm as alternative ruminant feed: a study on their impacts on rumen fermentation and gas production. Sustainability. 15(2), 1415.
Association of Official Analytical Chemists, 2005. Official methods of analysis, 18th ed. AOAC, Washington D.C.
Cao, Y., Wang, D., Wang, L., Wei, X., Li, X., Cai, C., Lei, X., Yao, J., 2021. Physically effective neutral detergent fiber improves chewing activity, rumen fermentation, plasma metabolites, and milk production in lactating dairy cows fed a high-concentrate diet. J. Dairy Sci. 104, 5631–5642.
Erdogan, S., Kaya, M., 2016. High similarity in physicochemical properties of chitin and chitosan from nymphs and adults of a grasshopper. Int. J. Biol. Macromol. 89, 118–126.
Estetika, Y., Endrawati, Y.C., 2018. Silkworm productivities of *Bombyx mori* L. BS-09 race in tropic. J. Ilmu. Produksi. dan. Teknol. Has. Peternak. 6, 104–112. (In Indonesian)
Gao, M., El-Sherbiny, M., Szumacher-Strabel, M., Cieślak, A., Yanza, Y.R., Irawan, A., Xie, B., Cao, Z.J., Fusaro, I., Jalal, H., Abd El Tawab, A.M., Liu, Y.B., 2024. Cutting-edge exploration of insect utilization in ruminant nutrition—feature and future: a systematic review and meta-analysis. Front. Vet. Sci. 11, 1–15.
Herliatika, A., Permatahati, D., Pujiawati, Y., Tresia, G.E., Wina, E., 2021. Utilization of cricket (*Gryllus bimaculatus*), black soldier fly (*Hermetia illucens*), mealworm (*Tenebrio molitor*), and silkworm (Samia ricini dan Bombyx mori) as alternative feed protein sources. Indones. Bull. Anim. Vet. Sci. 31, 185. (In Indonesian)

Hlasny, J., 2024. A cause of bovine spongiform encephalopathy (BSE) related to the feeding of meat and bone meal (MBM) to British cows can be ruled out based on known circumstances. Sér. A. 1, 32–42.

Jayanegara, A., Gustanti, R., Ridwan, R., Widyastuti, Y., 2020. Fatty acid profiles of some insect oils and their effects on in vitro bovine rumen fermentation and methanogenesis. Ital. J. Anim. Sci. 19, 1311–1318.

Jayanegara, A., Novandri, B., Yantina, N., Ridla, M., 2017a. Use of black soldier fly larvae (*Hermetia illucens*) to substitute soybean meal in ruminant diet: An in vitro rumen fermentation study. Vet. World. 10, 1439–1446.

Jayanegara, A., Yantina, N., Novandri, B., Laconi, E.B., Nahrowi, Ridla, M., 2017b. Evaluation of some insects as potential feed ingredients for ruminants: Chemical composition, in vitro rumen fermentation and methane emissions. J. Indones. Trop. Anim. Agric. 42, 247–254.

Ji, H., Zhang, J.-L., Huang, J.-Q., Cheng, X.-F., Liu, C., 2015. Effect of replacement of dietary fish meal with silkworm pupae meal on growth performance, body composition, intestinal protease activity, and health status in juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquac. Res. 46, 1209–1221.

Lähteenmäki-Uutela, A., Marimuthu, S.B., Meijer, N., 2021. Regulations on insects as food and feed: a global comparison. J. Insects as Food Feed 7, 849–856. Makkar, H.P.S., Tran, G., Heuzé, V., Ankers, P., 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 197, 1–33.

Moss, A., Jouany, J., Newbold, J., 2000. Methane production by ruminants: its contribution to global warming. Ann. Zootech. 49, 231–253.

Paulin, I.G., Purwanto, M.G., 2020. Nutritional characteristics of Teak Grasshopper (*Valanga nigricornis Burmeister*), Cricket (*Brachytrupes portentosus* L.), and Mealworm (*Tenebrio molitor*) as alternative food sources in Indonesia. Indones. J. Biotechnol. Biodivers. 4, 52–61.

Pengpeng, W., Tan, Z., 2013. Ammonia assimilation in rumen bacteria: a review. Anim. Biotechnol. 24, 107–128.

Purbowati, E., Rianto, E., Dilaga, W.S., Sri Lestari, C.M., Adiwinarti, R., 2014. Characteristics of the rumen fluids, type and number of ruminal microbes in Java and Ongole grade bulls. Bul. Peternak. 38, 21. (In Indonesian)

Putra, L.O., Suharti, S., Sarwono, K.A., Sutikno, S., Fitri, A., Astuti, W.D., Rohmatussolihat, R., Widyastuti, Y., Ridwan, R., Fidriyanto, R., Wiryawan, K.G., 2023. The effects of heat-moisture treatment on resistant starch levels in cassava and on fermentation, methanogenesis, and microbial populations in ruminants. Vet. World. 16, 811–819.

Rahmasari, R., Sumiati, S., Astuti, D.A., 2014. The effect of silkworm pupae (*Bombyx mori*) meal to substitute fish meal on production and physical quality of quail eggs (*Cortunix cortunix japonica*). J. Indones. Trop. Anim. Agric. 39, 180–187.

Rashmi, K.M., Chandrasekharaiah, M., Soren, N.M., Prasad, K.S., David, C.G., Thirupathaiah, Y., Shivaprasad, V., 2018. Effect of dietary incorporation of silkworm pupae meal on in vitro rumen fermentation and digestibility. Indian J. Anim. Sci. 88, 731–735.

Rashmi, K.M., Chandrasekharaiah, M., Soren, N.M., Prasad, K.S., David, C.G., Thirupathaiah, Y., Shivaprasad, V., 2022. Defatted silkworm pupae meal as an alternative protein source for cattle. Trop. Anim. Health Prod. 54.

Renna, M., Coppa, M., Lussiana, C., Le Morvan, A., Gasco, L., Maxin, G., 2022. Full-fat insect meals in ruminant nutrition: in vitro rumen fermentation characteristics and lipid biohydrogenation. J. Anim. Sci. Biotechnol. 13, 138.

Ridla, M., Asikin, N., Jayanegara, A., Samsudin, A.A., 2023. Mitigating methane emissions for rural tropical livestock through selecting appropriate grass types. Livest. Res. Rural. Dev. 35, 7.

Ridla, M., Nahrowi, 2025. Methane mitigation strategies by optimizing nutrient profiles in an eco-friendly mixture of cassava pulp and Indigofera zollingeriana branch silage with strategic protein supplementation. Adv. Anim. Vet. Sci. 13, 198–208.

Rodríguez-Rodríguez, M., Barroso, F.G., Fabrikov, D., Sánchez-Muros, M.J., 2022. In vitro crude protein digestibility of insects: a review. Insects. 13, 1–19.

Sagala, Y.G., Andadari, L., Handayani, T.H., Sholikin, M.M., Fitri, A., Fidriyanto, R., Rohmatussolihat, R., Ridwan, R., Astuti, W.D., Widyastuti, Y., Fassah, D.M., Wijayanti, I., Sarwono, K.A., 2024. The effect of silkworms (*Bombyx mo*ri) chitosan on rumen fermentation, methanogenesis, and microbial population in vitro. Vet. World. 17, 1216–1226.

Sahib, Q.S., Ahmed, H.A., Ganai, A.M., Farooq, J., Sheikh, G.G., Sheikh, I.U.D., Beigh, Y.A., 2023. Evaluation of silkworm pupae meal based calf starter diet on the performance of crossbred cattle calves. Indian J. Anim. Sci. 93, 903–906.

Samal, L., Pattanaik, A., 2014. Dairy production in India - Existing scenario and future prospects. Int. J. Livest. Res. 4, 105.

Sarwono, K.A., Kondo, M., Ban-Tokuda, T., Jayanegara, A., Matsui, H., 2019. Effects of phloroglucinol and the forage: Concentrate ratio on methanogenesis, in vitro rumen fermentation, and microbial population density. Adv. Anim. Vet. Sci. 7, 164–171.

Sarwono, K.A., Rohmatussolihat, R., Watman, M., Ratnakomala, S., Astuti, W.D., FidriyantoR., Ridwan, R., Widyastuti, Y., 2022. Characteristics of fresh rice straw silage quality prepared with addition of lactic acid bacteria and crude cellulase. AIMS Agric. Food. 7, 481–499.

Sheikh, I.U., Banday, I.M., Shaista Nissa, I.S., Bushra Zaffer, I., Bulbul, I.K., Husbandry, A., Nissa, S.S., Zaffer, B., Bulbul, K., 2018. Utilization of silkworm pupae meal as an alternative source of protein in the diet of livestock and poultry: A review. J. Entomol. Zool. Stud. 6, 1010–1016.

Siemianowska, E., Kosewska, A., Aljewicz, M., Skibniewska, K.A., Polak-Juszczak, L., Jarocki, A., Jędras, M., 2013. Larvae of mealworm (*Tenebrio molitor* L.) as European novel food. Agric. Sci. 04, 287–291.

Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol. 48, 185–197.

Thirumalaisamy, G., Malik, P.K., Kolte, A.P., Bhatta, R., 2020. In vitro evaluation of graded level of Silkworm pupae (Bombyx mori) oil on methane production, fermentation characteristics, and protozoal populations. Vet. World. 13, 586–592.

Toral, P.G., Hervás, G., González-Rosales, M.G., Mendoza, A.G., Robles-Jiménez, L.E., Frutos, P., 2022. Insects as alternative feed for ruminants: comparison of protein evaluation methods. J. Anim. Sci. Biotechnol. 13, 1–8.

Ullah, R., Khan, S., Hafeez, A., Sultan, A., Khan, N.A., Chand, N., Ahmad, N., 2017. Silkworm (Bombyx mori) meal as alternate protein ingredient in broiler finisher ration. Pak. J. Zool. 49, 1463–1470.

Van Dung, D., Ba, N.X., Van, N.H., Phung, L.D., Ngoan, L.D., Cuong, V.C., Yao, W., 2013. Practice on improving fattening local cattle production in Vietnam by increasing crude protein level in concentrate and concentrate level. Trop. Anim. Health Prod. 45, 1619–1626.

Williams, S.R.O., Hannah, M.C., Eckard, R.J., Wales, W.J., Moate, P.J., 2020. Supplementing the diet of dairy cows with fat or tannin reduces methane yield, and additively when fed in combination. Animal. 14, s464–s472.

Wu, X., He, K., Velickovic, T.C., Liu, Z., 2021. Nutritional, functional, and allergenic properties of silkworm pupae. Food Sci. Nutr. 9, 4655–4665.

Xue, F., Sun, F., Jiang, L., Hua, D., Wang, Y., Nan, X., Zhao, Y., Xiong, B., 2019. Effects of partial replacement of dietary forage using kelp powder (Thallus laminariae) on ruminal fermentation and lactation performances of dairy cows. Animals. 9, 852.

Yanuartono, Nururrozi, A., Soedarmanto, I., Purnamaningsih, H., Ramadani, D., 2020. Meat bone meal as an alternative animal feed: a brief review. J. Peternak. Sriwij. 9, 35–54. (In Indonesian).

How to cite this article;

Sri Ayu Agung Purnami, Muhammad Ridla, Nahrowi, Ki Ageng Sarwono, Sri Utami, Sih Kahono, Roni Ridwan, Rusli Fidriyanto, Ainissya Fitri, Rohmatussolihat and Dudi Firmansyah. Utilization of Silkworm (*Bombyx mori*) pupae meal and Javanese bird grasshopper (*Valanga nigricornis*) meal as alternative protein sources to replace meat and bone meal in ruminant feed: An in vitro study. Veterinary Integrative Sciences. 2026; 24(1): e2026018-1-12.