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Evaluation of nutritive values of various non-conventional protein sources as potential feed ingredients for ruminants

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Abstract. *Samadi, Wajizah S, Pratama SM, Jayanegara A. 2023. Evaluation of nutritive values of various non-conventional protein sources as potential feed ingredients for ruminants. Biodiversitas 24: 4069-4078.* The purpose of this study was to evaluate the nutritive values of various non-conventional protein sources as potential ingredients for ruminants, including chemical composition, *in vitro* digestibility, rumen fermentation, and methane emissions. Seven various non-conventional protein sources; grasshopper meal (GM), earthworm meal (EWM), fish by-product meal (FbPM), centipede meal (CM), snail meal (SM), ant eggs meal (AEM), mealworm meal (MWM) and one conventional protein source; soybean meal (SBM) were used in this study. All samples were dried at the temperature of 60ºC for 24h and ground by the use of a hammer mill to pass a 1 mm sieve. The ground samples were utilized for further analysis, including chemical composition, in vitro digestibility, rumen fermentation, and methane emissions. Incubation was conducted with three replications in the water bath and temperature was maintained at 39ºC for 48h. All data were statistically analyzed using SPSS version 16 and the difference between treatments was stated ($P<0.05$). The results of the study indicated that all the samples contained various CP. The NDICP and ADICP in samples were low. All samples had high IVDMD and IVOMD, the highest in FbPM ($P<0.01$). Methane emission of the samples was lower than SBM ($P<0.01$).

Keywords: Insect, methane, non-conventional, rumen fermentation

INTRODUCTION

The demand for animal products was expected to increase by up to 58% for meat and 70% for milk in 2050 compared to the demand for animal products in 2010 and the major part of the increase was in developing countries (Makkar 2018). Due to the high demand for animal products, the supply chain of feedstuffs to fulfill animal nutrient requirements for maintenance and production should be continuously available. Furthermore, fuel-feed-food competition becomes a crucial issue also to provide feedstuffs for animals. Therefore, alternative animal feedstuffs as novel feed resources should be provided. The use of agricultural and agro-industrial by-products as animal feed has been well documented (Samadi et al. 2016; Pratama et al. 2019; Samadi et al. 2020). Alternative sustainable protein-rich ingredients such as insects are highly promising to be a future protein resource for animal feed. Insects can grow and reproduce quickly with relatively high feed conversion efficiency (Oonincx et al. 2015). Another advantage of insects as animal feed, they can feed on waste biomass to produce highly valuable feed for animals.

Indonesia is one of the richest biodiversity in the world and provides a lot of potential animal species to be alternative non-conventional protein sources for animal feed. Non-conventional protein sources for animal feed define as protein resources that have been not traditionally utilized for animal feed, such as grasshoppers, earthworms, centipedes, snails, ant eggs, mealworms and other potential species. These non-conventional protein sources can be used as a component diet for ruminant and non-ruminant animals. Traditionally, soybean meal (SBM) and fish meal (FM) have been utilized as animal feed for a long time ago. Due to the high cost of traditional animal feed and food-feed competition, non-conventional feed can be a good alternative as a protein source for animal feedstuffs. Makkar et al. (2014) reported that some insects, such as black soldier fly larvae, contain very high lipids/oils and defatted meals can be used as animal feed. Insects also contain beneficial bioactive compounds functioning as immune-modulator to other animals (Zhou et al. 2022) and anti-microbial functions (Xia et al. 2021). Research conducted by Jayanegara et al. (2017) by using several insects as ruminant feed concluded that insects meal can be utilized as an alternative protein in animal feed and in vitro studies indicated that insects produced low methane emission and digestibility compared to conventional feed.

Besides insects which are non-conventional animal feed as aforementioned above, other animals such as grasshopper meal (GM), earthworm meal (EWM), fish by-product meal (FbPM), centipede meal (CM), snail meal (SM), ant eggs meal (AEM), mealworm meal (MWM) also were high potency to be used as alternative protein sources. Some of these non- conventional animal feeds have been studied in non-ruminant animals such as snails in which heat processed snails meal was recommended in the diet of meat and egg-type chickens with the amount of 10%

(Diarra 2015). However, researches relating to non-conventional feed as alternative protein sources in ruminant animals were still limited. It is, therefore, highly important to investigate the nutritive values of various non-conventional protein sources as potential ingredients for ruminants, including chemical composition, in vitro digestibility, rumen fermentation, and methane emissions.

MATERIALS AND METHODS

Sample collection and preparation

Various non-conventional feed used in this study includes grasshopper meal (GHM, *Melanoplus sanguinipes*), earthworm meal (EWM, *Lumbricus rubellus*), fish by-product meal (FbPM, *Canthidermis maculate*), centipede meal (CM, *Scolopendra cataracta*), snail meal (SM, *Pomacea canaliculata*), ant eggs meal (AEM, *Solenopsis invicta*), mealworm meal (MWM, *Tenebrio molitor*) (Figure 1). Samples materials were collected from commercial products sold in the markets such as poultry shops, except SM which was obtained from rice fields in the area of Banda Aceh. The fish by-product meal sample which was used in this study was a by-product of *C. maculate* which was abundant and available in fish processing factories. Soybean meal was used in this study as a reference to conventional feed in ruminant diets. Snail meal and fish by-product meal after collection were kept at the temperature of -20ºC before further process. All samples were dried at a temperature of 60ºC for 24 h. Dried samples were ground in a hammer mill to pass a 1 mm sieve. All ground samples were used for further analysis, including chemical composition determination and in vitro fermentation analysis.

Chemical composition analysis

Proximate analysis was conducted to determine nutritive values of various protein source samples, i.e. dry matter (DM), organic matter (OM), ash, crude protein (CP), crude lipid (CL), crude fiber (CF), and nitrogen-free extract (NFE) using AOAC methods (Latimer Jr 2023). Brief procedures to determine each component for chemical analysis are as follows; DM content was obtained by keeping the samples in an oven at the temperature of 105ºC for 24h. Ash content was determined by burning 300-500 mg samples at the temperature of 500ºC for 3h in a furnace. OM content was determined by subtracting between DM and ash.

CP content was obtained by the standard Kjeldahl's method with the following procedures destruction, distillation and titration. CF content was performed by boiling 0,3-0,5 g samples in 3 ml acid and 10 ml alkali solutions for 30 min each. CF content was determined by extracting the content of lipids with Soxhlet's apparatus. NFE was calculated according to the following equation; NFE=100% - (the content of ash+CP+CL+CF). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were performed based on the method of Van Soest et al. (1991); according to these methods, 100 mg samples were boiled in neutral detergent and acid detergent solutions for 1 h each respectively. In this study, NDF analysis was performed without using α-amylase and sodium sulfite. The determination of neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP) contents was according to the method of Licitra et al. (1996) by using residual from NDF and ADF from the previous analysis. All sample analysis was conducted in duplicate.

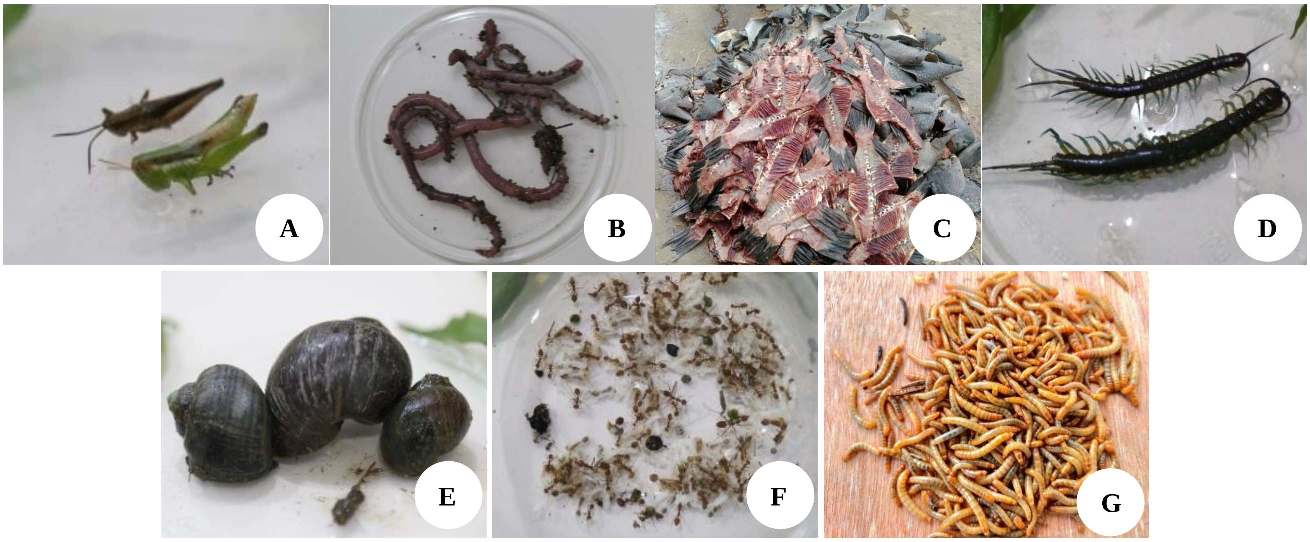

Figure 1. Several types of non-conventional protein feed sources. A. Grasshopper (GM), B. Earthworm (EWM), C. Fish by Product (FbPM), D. Centipede (CM), E. Snail (SM), F. Ant Eggs (AEM), and G. Mealworm (MWM)

In vitro rumen fermentation

In vitro rumen fermentation was measured according to the procedure of Theodorou et al. (1994) in which samples were incubated in a buffered rumen fluid mixture. Rumen fluid was obtained in the morning before feeding from a rumen-fistulated Friesian Holstein cow raised at the Laboratory of Nutrition and Dairy Science, Nutrition and Feed Technology Department, IPB Bogor. Before use, four layers of gauze were used to filter rumen fluid. A 125 ml serum bottle was prepared to be filled up with 0.75 g sample and 75 ml buffered rumen fluid with the ratio of rumen fluid: buffer was 1:4 v/v. Then, followed by an incubation process in the water bath with a temperature of 39ºC for 48 h. Serum bottles were sealed with butyl rubber stoppers and aluminum crimp seals. The measurement of gas production was performed periodically at 2, 4, 6, 8, 12, 24, 36, and 48h after incubation using a syringe. Manual shaking was carried out each time gas production was measured. Methane measurement was conducted according to the method of Fievez et al. (2005) at each time of gas production measurement. To separate supernatant and residue, serum bottles were centrifuged after 48h incubation. Total volatile fatty acid (VFA) and ammonia concentration and pH were measured using a supernatant obtained after 48h incubation, as described in Jayanegara et al. (2016). The method of Tilley and Terry (1963) was applied in which residue was performed for further incubation for another 48h in 75 ml pepsin-HCl 0.2N. The values of in vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) were calculated based on the difference between DM and OM residual from initial DM and OM before the incubation process. The in vitro incubation was conducted in three runs and two serum bottles were used in each run. Due to this procedure, a randomized complete block design was applied for treatment in the experimental unit in which runs were considered as the blocks (replicates). As a control, two bottles containing buffered rumen fluid without any substrate were also incubated and considered as blanks.

Data analysis

All data were statistically analyzed by using analysis of variance as the following model

$$Yij = \mu + \alpha i + \beta j + \varepsilon ij$$

Where, Yij is the observed measure, μ is the overall mean, αi is the treatment effect, βj is the block. Differences between treatments were stated ($P<0.05$) and continued with a post-hoc test by using Duncan Multiple Range Test (DMRT). The statistical analysis was performed by using SPSS software version 9.1. Data on gas production kinetics were calculated according to a modified equation of Ørskov and McDonald (1979) as follows:

$$GP = b\,(1 - e^{-ct})$$

Where, GP is gas production (ml/g DM), b is gas production potential (ml/g DM), c is gas production rate (/h) and t is incubation period (h).

RESULTS AND DISCUSSION

Chemical composition

Chemical composition and fiber fraction from various non-conventional feed used in this study include grasshopper meal (GHM, *Melanoplus sanguinipes*), earthworm meal (EWM, *Lumbricus rubellus*), fish by-product meal (FbPM, *Canthidermis maculate*), centipede meal (CM, *Scolopendra cataracta*), snail meal (SM, *Pomacea canaliculata*), ant eggs meal (AEM, *Solenopsis invicta*), mealworm meal (MWM, *Tenebrio molitor*) were presented in Table 1 and 2. Organic content in this study was relatively high, with a range of 46.07 to 85.35%. Based on chemical composition, non-conventional feed in this study was classified as protein source feed due to high protein content except for CM and AEM, consisting of 10.73% and 14.23% CP respectively. Some feed sources were higher than conventional protein sources, such as FbPM, EWM and SM. Jayanegara et al. (2017) evaluated insect's meals as animal feed and found that some insects, such as Jamaican field cricket and mealworm, contained high levels of protein (60%). In our study, the highest protein was FbPM and EWM with the protein of 43.01% and 38.99%. Jonas-Levi and Martinez (2017) stated that different development stages of sample feed (larvae and adult) influenced the chemical composition of insects, and the study concluded that the calculation for nitrogen content of insects for animal feed by using conversion factor should be similar for all insect species and insects development stages. In this study, fish by-product meal contained 41,88% ash. The high ash content in this study was caused by fish bone that was included in the fish by-product. Ween et al. (2017) identified fishmeal ash content was 22.4%, it much lower than this study. The highest extract ether was MWM (21.94%) and it was similar to the findings of Jayanegara et al. (2017) in which mealworm contented 20.3% of EE. Sánchez-Muros et al. (2014) stated that insects contained a high amount of lipid and most of the lipid was in the form of polyunsaturated fatty acids (PUFA).

Crude fiber content in non-conventional feed in this study was low with the highest of 5.95 % in MWM. Different from research conducted by Pratama et al. (2019) by using agro-industrial by-products in Aceh Province contented high crude fiber content. Research conducted by Jayanegara et al. (2016) and Samadi et al. (2018) found that crude fiber content was still high. Insects' exoskeleton affects the fiber, NDF and ADF content. It can be assumed the low crude fiber content in this study was caused by a low exoskeleton. Abidin et al. (2020) stated that the main component of an insect's exoskeleton was a long-chain polymer of an N- acetylglucosamine, which was considered as fiber. The highest content of nitrogen free extract (NFE) was observed in centipede meal (CM) and the lowest content of nitrogen free extract (NFE) in this study was found in fish by-product meal (FbPM). The FbPM contained high CP. NFE content of feed ingredients is highly dependent on other components, such as ash, crude protein, crude fiber and ether extract. NFE is a soluble carbohydrate including monosaccharides, disaccharides and polysaccharides that are easily soluble in acid and alkaline solutions and have energy-high digestibility (Navarro et al. 2019).

Fiber fraction and protein fraction in fiber

The quality of a feed can be seen from the various constituent nutrients, such as energy, protein, fiber, and other secondary compounds. One of the components of feed fiber that are resistant to digestion is an acid detergent insoluble crude protein (ADICP) and neutral detergent insoluble crude protein (NDICP). The NDICP and ADICP are content of crude protein (Table 2) which is resistant to degradation. The results of NDF analysis on several non-conventional protein source feed ingredients found that the highest NDF content was found in Grasshopper meal (GHM), which was 62.97%, higher than SBM, which was 35.49%, while the lowest was found in Earthworm meal (EWM), which was 18.26%. Based on research conducted by Jayanegara et al. (2017), the NDF content in insects is 81.1%.

Acid detergent fiber (ADF) is a fiber fraction in a feed that only has cellulose and lignin without hemicellulose. The high NDF content in feed ingredients is thought to be due to the chitin content found in the exoskeleton. Chitin is a group of carbohydrates belonging to structural homoglycans which are composed of N-acetyl glucosamine monomers (2-acetamide-2-deoxy-D-Glucose) (Kabir et al. 2022). These chitin monomers are bound by glycosidic bonds at the β (1-4) position. The molecular structure of chitin is in the form of a long straight chain similar to cellulose and differs only in the group attached to the number 2 carbon atom.

In cellulose, the group attached to the number 2 carbon atom is a hydroxyl group (OH), while in chitin it is an acetamide group ($NHOCH_3$) so that chitin becomes a polymer of N - acetylglucosamine units. According to (Carrillo-Díaz et al. 2022) the constituent components of NDF are hemicellulose, lignin which dissolves in alkalis, lignin which does not dissolve in alkalis, fiber which bonds with nitrogen, cellulose and minerals which dissolves in detergents. Neutral detergent fiber (NDF) describes the number of structural carbohydrates contained in the cell wall consisting of hemicellulose, cellulose, and lignin (NRC 2016).

Results of the ADF analysis showed that the highest ADF value was found in the fish by-product meal 40.17% and the lowest ADF value was found in the feed ingredient earthworm meal 5.93%. The ADF content in this study was lower compared to research conducted by Pratama et al. (2022) namely 82.27%. The FbPM is a mixture of bones and a little bit of meat that is still in the fish bones. Bone is a connective tissue consisting of cells, fibers, and filling material. The presence of fiber as a constituent of bone is thought to be the cause of the high ADF content in FbPM. According to NRC (2016), cellulose and lignin are the constituent components of ADF.

Neutral detergent insoluble crude protein (NDICP) is a protein in NDF residue, including rumen degradation-resistant protein available for livestock. In addition, it is a component of the digestible fiber of NDF. The results showed that snail meal (SM) had the highest NDICP value, meaning that the protein content of snail meal was resistant to rumen degradation. Faridah et al. (2018) revealed that snail meal contains 51 85% crude protein with 12% of the crude protein fraction being pure protein. (Tóthová et al. 2017) explained that pure protein compounds belong to the group that is insoluble and degrades more slowly (fraction B_2).

Table 2. Fiber fraction of non-conventional protein feed sources (GHM: Grasshopper Meal, EWM: Earthworm Meal, FbPM: Fish by Product Meal, CM: Centipede Meal, SM: Snail Meal, AEM: Ant Eggs Meal, MWM: Mealworm Meal, SBM: Soybean Meal) as potential feed for ruminant animals

Feed sources of protein	NDF (%DM)	ADF (%DM)	NDICP (%CP)	ADICP (%CP)
GHM	62.97	24.37	0.60	0.26
EWM	18.26	5.93	0.47	0.17
FbPM	53.81	40.17	0.93	0.09
CM	50.88	23.08	1.01	0.15
SM	35.92	24.30	1.91	2.07
AEM	38.17	23.74	0.96	0.22
MWM	36.45	29.18	0.41	0.46
SBM*	35.49	25.72	0.83	1.39

Note: *: Control, ADF: Acid detergent fiber, ADICP: Acid detergent insoluble crude protein, NDF: Neutral detergent fiber, NDICP: Neutral detergent insoluble crude protein

Table 1. Nutritive value of non-conventional protein feed sources (GHM: Grasshopper Meal, EWM: Earthworm Meal, FbPM: Fish by Product Meal, CM: Centipede Meal, SM: Snail Meal, AEM: Ant Eggs Meal, MWM: Mealworm Meal, SBM: Soybean Meal) as potential feed for ruminant animals

Feedstuff	DM (%)	OM (%)	Ash (%DM)	CP (%DM)	EE (%DM)	CF (%DM)	NFE (%DM)
GHM	92.93	83.78	9.15	21.52	7.69	4.87	56.77
EWM	94.23	84.16	10.07	38.99	7.5	0	43.44
FbPM	87.95	46.07	41.88	43.01	1.45	0	13.66
CM	92.79	84.03	8.76	10.73	7.85	5.38	67.28
SM	90.98	61.1	29.88	33.54	2.09	0	34.49
AEM	93.09	83.62	9.47	14.23	9.47	5.1	61.73
MWM	92.72	86.35	6.37	21.52	21.94	5.95	44.22
SBM*	86.25	78.64	7.61	32.34	1.32	1.01	57.72

Note: *: Control, DM: Dry Matter, OM: Organic Matter, CP: Crude Protein, EE: Extract Eter, CF; Crude Fiber and NFE: Nitrogen Free Extract

Acid detergent insoluble crude protein (ADICP) is a protein bound to the ADF fraction of feed or it can also be said to be an insoluble protein fraction that is not available to animals due to heat damage. The results of this study indicated that snail meal had the highest ADICP value, meaning that snail meal had the most protein bound to the cell wall and a protein that was more resistant to rumen degradation compared to other feed ingredients. The NDICP and AIDCP were also lower compared to Jayanegara et al. (2016). Compared to Pratama et al. (2022) by using several forage in Aceh Province, the NDICP value in this study was higher and ADICP was lower.

pH values, IVDMD and IVOMD

The value of pH, in vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) of various non-conventional protein sources as potential feed ingredients for ruminants after rumen fermentation are presented in Figures 2, 3, and 4 respectively. Based on the statistical results, non-conventional feed sources were significantly different (P>0.01) for pH, IVDMD, and IVOMD. The value of pH had an important role in the degradability process of feedstuff in the rumen and supported the growth of rumen microbes. In our study, the agro-industrial by-product with the highest rumen pH was grasshopper meal at 6.82, while the lowest rumen pH was observed with snail meal at 6.63 (Figure 2). The ideal pH standard according to (Kitkas et al. 2022) is 6-7. The high pH of grasshopper meal is due to its low digestibility (Figure 2). Low digestibility serves as an indicator of suboptimal fermentation, resulting in reduced production of organic acids (Vargas et al. 2023). Organic acids function to maintain the balance of the population of microorganisms in the rumen. According to (Suiryanrayna and Ramana 2015), organic acids can accelerate the decrease in pH. Added by Kara et al. (2018) organic acids (formic and propionic acids) affect protein degradation from complex molecules to simple and soluble molecules. To guarantee microbial growth, the rumen pH must be maintained between 6 and 7. The degree of acidity (pH) of the rumen has a reciprocal relationship with the process of breaking down feed protein and deamination. The optimum pH value for proteolysis and deamination is 6-7, the optimum pH for maximum protease enzyme activity is 7.4 and the rumen fluid pH value varies from 5 to 7.7 (Mirahsanti et al. 2022).

Figure 3 shows IVDMD in the range of 77.96 - 96.57%. The highest IVDMD was shown in fish by-product meal, namely 96.57%. The high digestibility of dry matter in fish by-product meal is influenced by CP content (Table 1). IVDMD is affected by the protein content of the feed because each protein source has different solubility and resistance to degradation Wang et al. (2016). High dry matter digestibility in ruminants indicates the high digestibility of nutrients, especially those digested by rumen microbes. The higher digestibility percentage of the feed affects better quality. (Gebremariam and Belay 2021) stated that the factors that affect the digestibility value of dry matter rations are the proportion of feedstuff, chemical composition, ration protein level, and percentage of fat and minerals. The lowest dry matter digestibility value was found in mealworm meal, namely 77.96%. The low digestibility of the Mealworm Meal is due to the high EE content (Table 1). The high content of EE causes a hydrogenation process in the rumen, which converts unsaturated fats into saturated ones and the high-fat content can interfere with cellulolytic microbial activity, thereby reducing the rate of fermentation of crude fiber in the rumen (Debi et al. 2019). The digestibility of dry matter in this study was higher than that of a study conducted by (Rahman et al. 2016) which used fish meal, which was 87.0%.

Figure 4 shows IVOMD in the range of 75.92 - 94.2%. The highest IVOMD value was found in the fish by-product meal and the lowest in the mealworm meal. The organic matter digestibility value is in line with the dry matter digestibility value. According to Maranatha et al. (2020), which states the digestibility of organic matter is closely related to the digestibility of dry matter because part of the dry matter consists of organic matter. Ndaru et al. (2022), explained that the digestibility of dry matter can affect the digestibility of organic matter. A decrease in the digestibility of dry matter will result in a decrease in the digestibility of organic matter. The digestibility value of the organic matter in this study was higher than that carried out by Jayanegara et al. (2016), namely 59.7. Karimizadeh et al. (2017) added that the high or low quality of feed can be indicated by the digestibility of the feed, so the higher the digestibility of a type of feed, the higher the quality of the feed.

Volatile Fatty Acid (VFA) and NH_3 concentration

Volatile fatty acid (VFA) concentration is an indicator to control and optimize the anaerobic digestion process (Jin et al. 2016). Table 3 shows the total volatile fatty acid (VFA) Production value and the NH_3 value of each feedstuff tested.

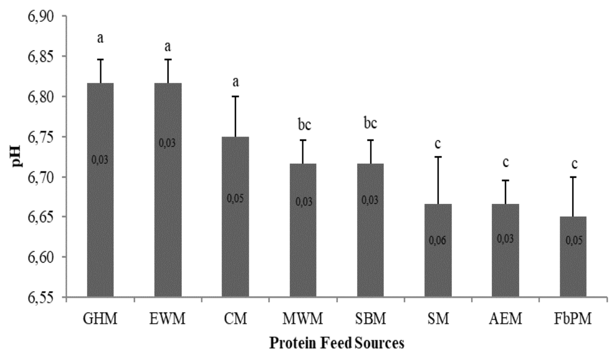

Figure 2. pH values of non-conventional protein feed sources (GHM: Grasshopper Meal, EWM: Earthworm Meal, FbPM: Fish by Product Meal, CM: Centipede Meal, SM: Snail Meal, AEM: Ant Eggs Meal, MWM: Mealworm Meal, SBM: Soybean Meal) as potential feed for ruminant animals after rumen fermentation. Different superscripts in the same column show significant differences (P<0.01)

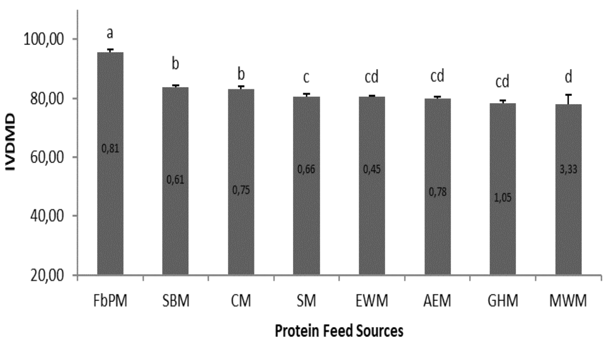

Figure 3. IVDMD of non-conventional protein feed sources (GHM: Grasshopper Meal, EWM: Earthworm Meal, FbPM: Fish by Product Meal, CM: Centipede Meal, SM: Snail Meal, AEM: Ant Eggs Meal, MWM: Mealworm Meal, SBM: Soybean Meal) as potential feed for ruminant animals after rumen fermentation. Different superscripts in the same column show significant differences (P<0.01)

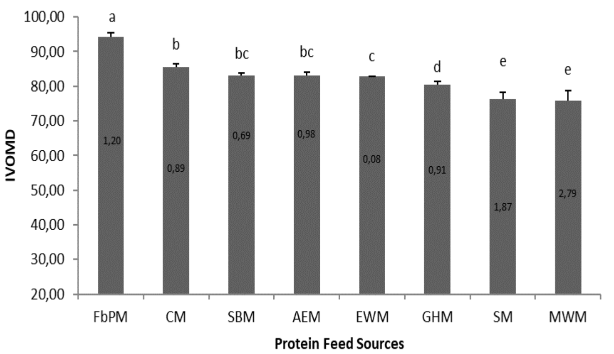

Figure 4. IVOMD of protein feed sources (GHM: Grasshopper Meal, EWM: Earthworm Meal, FbPM: Fish by Product Meal, CM: Centipede Meal, SM: Snail Meal, AEM: Ant Eggs Meal, MWM: Mealworm Meal, SBM: Soybean Meal) as potential feed for ruminant animals after rumen fermentation. Different superscripts in the same column show significant differences (P<0.01)

The production of volatile fatty acids/VFA is an illustration of the increase in soluble carbohydrates and proteins (Agnihotri et al. 2022). Based on the analysis of variance that was carried out in Table 3, the non-conventional protein source feed ingredients used had a very significant effect (P>0.01) on VFA concentrations. The VFA value in this study was in the range of 109.63 - 181.33 mM, this value was enough to meet the needs of rumen microbes to allow the rumen fermentation process to be better. The high production of VFA in feed ingredients is also supported by the high organic matter content in feed ingredients, namely 86.35%. This is consistent with the opinion Jamarun et al. (2020), that the total VFA production can be determined by the amount of organic matter in feed ingredients that can be digested by rumen microorganisms. VFA production can describe the fermentability of a feed because VFA is able to reflect an increase in soluble carbohydrates and proteins. The VFA value of this study was higher than the study conducted by Jayanegara et al. (2016), namely 103-135 mM. (Somanjaya et al. 2022) stated that the feed that enters the rumen is fermented to produce the main products in the form of volatile fatty acid (VFA), microbial cells, methane gas, and CO_2. In the rumen, VFA is mainly produced from the breakdown of carbohydrates by rumen microbes. VFA is used as a carbon skeleton for rumen microbial growth and as a source of energy for livestock. Concentrations that are not different in feed protein will produce VFA that are not different.

The concentration of ammonia/NH_3 is directly proportional to the microbes present in the rumen. Ammonia is one of the products of fermentation activity in the rumen, from the degradation of protein derived from feed and a source of nitrogen which is quite important for rumen microbial synthesis (Tahuk et al. 2016). Based on the analysis of variance in Table 3, it shows that non-conventional protein sources have a very significant effect (P>0.01) on NH_3 concentrations. NH_3 values in this study ranged from 23.32 - 84.69 mM. The highest NH_3 value was found in the Soybean Meal and the lowest in the Centipede Meal. The high NH_3 value in Soybean Meal is directly proportional to the crude protein content (Table 1). The high value of NH_3 in SBM is caused by the faster degradation of protein, causing NH_3 production to increase. The high or low digestibility of protein can increase the degradability and fermentability of feed ingredients in the rumen so that the resulting NH_3 levels are high (Permana et al. 2022). Hackmann and Firkins (2015) explained that the factors influencing the increase in NH_3 production were protein sources in rations that were easily degraded by rumen microbes and high feed energy and high growth of rumen microbes. Soybean Meal produced NH_3 of 84.69 mM with 32.34% crude protein. This is possible due to increased microbial protein synthesis, which will result in a decrease in the amount of N-NH_3 remaining as a result of crude protein degradation. Conversion of ammonia into microbial amino acids requires Adenosine Tri Phosphate (ATP), whereas if the concentration is high enough, then without the need for ATP ammonia is directly incorporated into microbial amino acids.

Gas production total and kinetics

Gas production indicates the process of feed fermentation by rumen microbes, namely the hydrolysis of carbohydrates into monosaccharides and disaccharides which are then fermented into volatile fatty acids (VFA), especially acetate, propionate and butyrate as well as methane gas (CH_4) and CO_2. Total gas production increases with increasing incubation time.

The results of the gas analysis showed that the longer the feed ingredients were incubated, the higher the gas produced. This indicates that the ability of rumen microbes to digest feed ingredients is increasing. The results of the gas analysis showed that the tested feed ingredients had a very significant effect (P<0.01) on gas production at 12, 24, and 48 hours of incubation.

The highest gas production is found in soybean meal. This is because soybean meal is a feed ingredient with high digestibility. In addition to high digestibility, soybean meal also contains high organic matter with low crude fat content (Table 1). High gas production has a positive correlation with the level of digestibility of feed ingredients (Huang et al. 2017). Gas production was able to be used to predict organic matter digestibility. Amanzougarene and Fondevila (2020) stated that gas production can be used optimally to predict microbial efficiency if the limitation of the batch culture gas production technique was recognized, but the role of the technique was not able to predict nutrient supply.

Based on Figure 5, it can be seen that the volume of total gas production at an incubation time of 2 hours to 12 hours experienced a slight increase. This is caused by environmental adjustments for microorganisms so that they do not produce maximum gas. Gas production at 24-48 hours of incubation increased because microorganisms began to degrade feed from complex forms (carbohydrates) to simple forms (gases).

Gas kinetics on total gas production (Table 4) is determined based on the exponential model of Ørskov & McDonald (1979) where a+b is the maximum gas production and c is the level of gas production. Statistical tests on maximum gas production (a+b) and gas production levels (c) showed highly significant different values (P<0.01). The highest maximum gas production was produced by the Soybean Meal sample which had the same trend as the crude protein content in Table 1. The high crude protein content maximized the microbial protein formation process, thereby increasing the maximum gas production. Gas production can be used as an indicator of in vitro fermentability of a feed. The volume of gas production during incubation has a positive correlation with rumen microbial growth and the amount of fermented feed (Dhakal et al. 2023). Anaerobic fermentation besides producing VFA also produces gas consisting of CH_4 (30-50%), CO_2 (25-45%), a little H_2, N_2, and H_2S. The gas produced in this method comes from direct feed fermentation (CO_2 and CH_4) and comes from indirect gas production through the VFA buffering mechanism, namely in the form of CO_2 gas released from the bicarbonate buffer produced during the fermentation process (Jayanegara et al. 2015).

Methane emission

Methane (CH_4) is a gas formed from the reaction between hydrogen (H_2) and carbon dioxide (CO_2) assisted by methanogenic bacteria (Buan 2018). Statistical tests showed a very significant difference in each treatment (P<0.01) (Figure 6). The CH_4 value of all insects was lower than the soybean meal. The low digestibility of insects causes low production of H_2 gas. H_2 gas is the main substrate for methanogenic bacteria (Jayanegara et al. 2015). The high crude fat content in insects causes a low methane gas content in insects compared to Soyben Meal, especially medium chain fatty acids (MCFA) which are toxic to methanogenic bacteria (Jayanegara et al. 2017). Furthermore, polyunsaturated fatty acids (PUFA) found in insects also play a role in inhibiting hydrogen (Czumaj and Śledziński 2020). Based on a report by Jayanegara et al. (2017), insects contain chitin. The chitin found in insects plays a role in reducing methane gas by inhibiting the supply of hydrogen to methanogens.

Table 3. Volatile Fatty Acid and NH_3 concentration of non-conventional protein feed sources as potential feed for ruminant animals

Feedstuff	VFA (mmol/I)	NH_3 (mmol/I)
GHM	112.98e±7.18	23.32e±0.78
EWM	162.81b±2.47	67.70b±0.29
FbPM	137.14cd±4.50	25.33d±0.54
CM	109.63e±4.11	21.33f±0.53
SM	149.34c±4.83	68.70b±0.74
AEM	129.88d±4.88	23.40e±0.55
MWM	141.98cd±2.65	29.69c±0.52
SBM	181.33a±3.18	84.69a±0.32
SEM	1.578	0.199
P-value	<0.001	<0.001

Note: Different superscripts in the same column show significant differences (P<0.01). GHM: Grasshopper Meal, EWM: Earthworm Meal, FbPM: Fish by Product Meal, CM: Centipede Meal, SM: Snail Meal, AEM: Ant Eggs Meal, MWM: Mealworm Meal, SBM: Soybean Meal)

Table 4. Kinetic gas production of non-conventional protein feed sources as potential local feed for ruminant animals

Feedstuff	Gas 12 (ml/g DM)	Gas 24 (ml/g DM)	Gas 48 (ml/g DM)	B (ml/g DM)	c (/h)
GHM	90.50^{b}±2.69	121.50^{b}±3.36	138.00^{c}±3.52	139.53^{c}±3.12	0.087^{cd}±0.003
EWM	48.75^{d}±1.37	59.92^{d}±1.29	77.75^{e}±1.42	73.48^{e}±0.70	0.097^{c}±0.005
FbPM	36.75^{e}±0.52	41.25^{e}±0.52	50.42^{f}±0.36	45.25^{f}±0.59	0.164^{a}±0.003
CM	101.67^{a}±0.66	130.83^{a}±0.60	152.83^{ab}±0.44	156.96^{a}±0.10	0.078^{d}±0.0008
SM	54.67^{c}±2.33	61.50^{d}±1.5	75.50^{e}±3.00	68.99^{e}±2.05	0.148^{b}±0.01
AEM	99.25^{a}±0.43	123.25^{b}±0.66	148.75^{b}±0.38	149.46^{b}±0.0	0.083^{cd}±0.0003
MWM	85.33^{b}±3.87	105.00^{c}±5.45	127.83^{c}±4.63	127.51^{d}±5.41	0.085^{cd}±0.0003
SBM	96.58^{a}±0.71	133.42^{a}±1.02	157.42^{a}±0.08	159.69^{a}±0.48	0.078^{d}±0.001
SEM	0.69	0.85	0.84	0.834	0.002
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Different superscripts in the same column show highly significant differences (P<0.01). Standar Error of Mean, SEM; gas production at 12 hours, Gas 12(ml/g DM); gas production at 24 hours, gas 24(ml/g DM); gas production at 48 hours, Gas 48(ml/g DM). GHM: Grasshopper Meal, EWM: Earthworm Meal, FbPM: Fish by Product Meal, CM: Centipede Meal, SM: Snail Meal, AEM: Ant Eggs Meal, MWM: Mealworm Meal, SBM: Soybean Meal

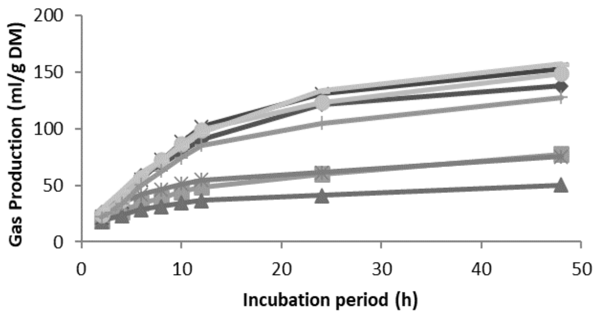

Figure 5. Gas Production of (GHM: grasshopper meal (-◊-), EWM: earthworm meal (-▪-), FbPM: fish by-product meal (-Δ-), CM: centipede meal (-x-), SM: snail meal (-Ж-), AEM: ant eggs meal (-●-), MWM: mealworm meal (-l-) and SBM: soybean meal (---)

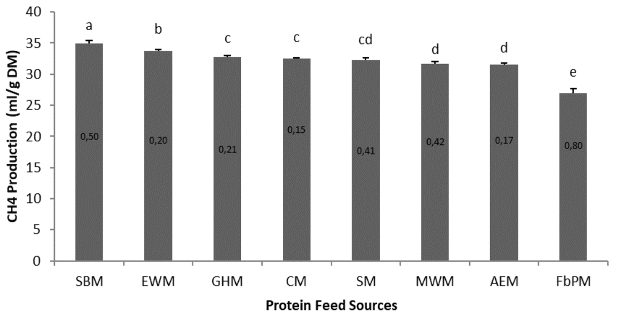

Figure 6. Methane gas production (CH_4) of non-conventional protein feed sources (GHM: Grasshopper Meal, EWM: Earthworm Meal, FbPM: Fish by Product Meal, CM: Centipede Meal, SM: Snail Meal, AEM: Ant Eggs Meal, MWM: Mealworm Meal, SBM: Soybean Meal as potential feed for ruminant animals. Different superscripts in the same column show significant differences (P<0.01)

Even though the methane gas content of all tested feed ingredients was lower than soybean meal, the value range was categorized as high. The high production of CH_4 gas produced can be caused by the high content of NDF crude fiber in the samples. High NDF content will increase methane levels through changes in the proportion of volatile fatty acids (VFA) towards increasing the proportion of acetic acid which produces hydrogen gas (H_2) as a substrate in metagenesis reactions (Al-Sulaimi et al. 2022). CH_4 gas production shows a lot of energy lost in gaseous form which indicates that the feed efficiency is low. Reducing CH_4 gas production can reduce the loss of wasted feed energy thereby increasing the efficiency of feed utilization (Gerber et al. 2013).

In conclusion, non-conventional feed ingredients have the potential to be used as protein source feed ingredients. Insect meals such as Grasshopper Meal (GHM), Earthworm Meal (EWM), Centipede Meal (CM), and Mealworm Meal (MWM) have the potential to be used as animal feed ingredients, especially as a source of protein. Fish by Product Meal (FbPM), and Snail Meal (SM) can also be used as protein source feed ingredients. Non-conventional feed, especially insects, is rich in EE as well and therefore, may supply some essential fatty acid requirements of the animals. All research samples have good digestibility. All the non-conventional have low methane emissions in vitro. Certain treatments or processing methods to remove the exoskeleton fraction or chitin may be required to increase the feeding values of insect meals.

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