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Effects of Different Dosages and Methods of Saponin Preparation from *Mucuna pruriens* Leaves on *In Vitro* Feed Digestibility

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ABSTRACT

The *Mucuna pruriens* is commonly used in traditional medicine for anti-inflammatory, antibacterial, neuroprotector, antidiabetic, and anti-cancer purposes. The bioactive compounds, such as flavonoid, tannin, and saponin, could improve feed digestion efficiency in ruminants' rumen. The current study aimed to evaluate the effects of different dosages and the two methods of saponin preparation from *Mucuna pruriens* leaves on *in vitro* feed digestibility parameters. A randomized block design with nested arrangements (2×5×3) was used in this study. Two methods of obtaining saponins from *Mucuna pruriens* leaves, including meal (MPLM) and extract (MPLE) of *Mucuna pruriens* leaves, were compared. The nested treatments of the preparation methods were the dosages of the saponin as feed additives in feed samples, involving 0%, 0.025%, 0.050%, 0.075%, and 0.10%. There were 15 samples in each group (five-level dosage and three repetitions). The feed contained 40% forage and 60% concentrate. The obtained results indicated that saponin preparation from *Mucuna pruriens* leaves (MPLM and MPLE) significantly affected dry matter, organic matter, and crude fiber rumen degradability (r-DMD, r-OMD, r-CFD, respectively), as well as NH_3, volatile fatty acid, propionate, butyrate concentrations, acetate-to-propionate (A/P) ratio, acetate, and propionate percentage. However, there was no significant impact on protozoa population, acetate concentration, butyrate percentage, *in vitro* dry matter digestibility (IVDMD), and *in vitro* organic matter digestibility (IVOMD). The MPLM saponin revealed significantly higher values on digestibility parameters except for protozoa, A/P ratio, and acetate percentage. The MPLM saponin dosage of 0.05% showed the highest values for r-DMD (56.48%), r-OMD (56.51%), and r-CFD (54.64%), total Volatile fatty acid (77.71 mM), propionate (21.57 mM), propionate percentage (27.76%), IVDMD (65.95%), and IVOMD (65.86%), but lowest in A/P ratio (2.04). In conclusion, the findings of the present study suggest that the MPLM saponin at a dosage of 0.05% holds promising potential for enhancing the fermentation profile in ruminants.

Keywords: *In vitro*, *Mucuna pruriens*, Nutrient digestibility, Rumen fermentation, Saponin

INTRODUCTION

In commercial feedlots, where ruminants are raised for high productivity, feed antibiotics, such as monensin, have been traditionally used (Ogunade et al., 2018). Monensin reduces protozoa, fungi, and methanogen bacteria; however, its use is constrained due to increasing awareness of its impact on human health and concerns regarding the development of resistance (Shen et al., 2017). Current research endeavors focus on developing natural feed additives as alternative antibiotics. The ongoing research is primarily centered on the exploration of secondary metabolites, such as saponin (Unnawong et al., 2021), tannins (Patra and Saxena, 2011), flavonoids (Gohlke et al., 2013), and polyphenols, as rumen modifiers (Vasta et al., 2019). Klevenhusen et al. (2011) found that the secondary metabolites decreased the acetate and ammonia concentration, acetate-to-propionate ratio, and methane production while increasing propionate *in vitro*. Additionally, they have antimicrobial properties against bacteria, fungi, and protozoa.

The *Mucuna pruriens*, also known as the velvet bean, belongs to the *Fabaceae* family with approximately 150 species of annual and perennial legumes (Lampariello et al., 2012). *Mucuna* is a multipurpose legume that plays an essential role in soil fertility, soil structure improvement, soil protection against erosion, and weed control (Buckles et al., 1998), especially in smallholder farmers and when rotated with the maize, it contributes to improvements in water productivity (Masikati et al., 2014). *Mucuna pruriens* seeds are commonly used as raw materials for making tempeh, a traditional Indonesian food, especially in Wonogiri, Central Java (Handajani, 2001). The product is popularly called "*koro benguk tempeh*," when used as a dish, and some small and medium enterprises further process the tempeh to produce chips (Winarni and Dharmawan, 2017). *Mucuna* is not only used as tempeh raw materials but also as an ingredient for making nuggets, cookies for school-age children, and vegetable milk (Mang et al., 2016). Besides, *Mucuna pruriens* has the potential to serve as a fiber source in new dietary food products, function as an antioxidant (Encalada

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and Campos, 2021), have hypolipidemic potential (Dimitry et al., 2022), and could affect fertility (Daramola et al., 2015).

Mucuna is an essential medicinal plant used to remedy various diseases, such as diabetes, arthritis, dysentery, and cardiovascular diseases (Nadkarn, 2001). It has high concentrations of L Dopa (4-7%), making it a potential alternative for treating Parkinson's disease and offering an alternative to conventional medicine with long-term side effects (Katzenschlager et al., 2004). The L Dopa is present in roots, stems, leaves, and seeds (Lampariello et al., 2012). *Mucuna pruriens* leaves as an extract had secondary metabolites, such as flavonoids, tannins, saponins, anthraquinones, terpenoids, flavonoids, and cardiac glycosides (Agbafor and Nwachukwu, 2011). *Mucuna pruriens* leaf extract has a significant antimicrobial effect against fungal and bacterial species (Mastan et al., 2009) and the potential to control protozoa (Ekanem et al., 2004). Given the significant contribution of protozoa to carbohydrate breakdown in the rumen and their predatory capacity, the *Mucuna pruriens* would be useful as a feed additive for antiprotozoal agents in ruminant productions (Williams et al., 2020). Modifying microbial composition by adding some feed additive decreases methane production and fermentation efficiency (Castillo-González et al., 2014). Therefore, this study aimed to evaluate the effects of different dosages and the two methods of saponin preparation from *Mucuna pruriens* leaves on *in vitro* feed digestibility parameters.

MATERIALS AND METHODS

Ethical approval

The Ethics Committee has approved all research procedures of Brawijaya University Malang, East Java, Indonesia, with letter number 141-KEP-UB-2022.

Materials

Materials used in this experiment included meal (MPLM) and extract (MPLE) of *Mucuna pruriens* leaves prepared from fresh leaves collected from Wajak, Malang, East Java, Indonesia. For MPLM, 10 kg leaves were aerated in shading and then dried in the oven at 60°C for 24 hours. The oven-dried *Mucuna pruriens* leaves were milled into a fine powder using a mechanical grinder and then stored in an air-tight plastic bag. The preparation of MPLE saponin involved the extraction of fine powder from *Mucuna pruriens* using methanolic solvent by microwave-assisted extraction (MAE) method (Wang et al., 2012). The MAE procedure employed a modified microwave sharp type R21D0SIN with the power 450 W, voltage 220-240 volt/50 Hz, and dimensions 52 cm × 40.7 cm × 32 cm (length × width × height), equipped with a close vessel unit. The modified microwave featured a thermostat to control the temperature, which did not exceed 40°C. To initiate the extraction, 16.5 g of *Mucuna* leaves powder was placed in a 250 ml round flask and mixed with 100 ml methanolic solvent. The microwave was turned on for 15 minutes at 40°C. A chiller pump was used to flow the coolant liquid in and out of the condenser to keep the temperature in the flask at 40°C. The extract was filtered using the Whatman 1 filter paper. Then, another 100 ml methanolic solvent was added to the filtrate, and the same extraction process was done. The solvent is then evaporated and stored in a sealed bottle.

Methods

The experiment used a randomized block design of nested arrangement (2×5×3) for *in vitro* digestibility laboratory research, following the methodology outlined by Tilley and Terry (1963). The treatments were the saponin dosage level of *Mucuna pruriens* leaves, prepared through two different preparation methods, namely MPLM saponin and MPLE saponin. *Mucuna pruriens* leaves saponin were used at levels of 0%, 0.025%, 0.050%, 0.075%, and 0.10% in the basal diet based on Xu et al. (2010) and Castro-Montoya et al. (2011). The basal diet comprised 40% maize forage (*Zea mays*) and 60% concentrate in dry matter basis. The nutrient content of the feed ingredients and *Mucuna pruriens* leaves are presented in Table 1.

The saponin content of the leaf meal was 12.41 mg/g. To meet the dosage level of MPLM saponin the leaves meal was added as much as 0 mg, 10 mg, 30 mg, and 40 mg into a 500 mg basal diet in each fermentor tube. Moreover, to meet the dosage level of MPLE saponin, the liquid extract of *Mucuna pruriens* leaves was added as much 0 µL, 80 µL, 160 µL, 240 µL, and 330 µL into 500 mg basal diet in each fermentor tube. The extract had 1504.44 mg/L saponin content. All treatment diets were tested using one-and two-step *in vitro* feed digestibility tests in three runs as replication (Tilley and Terry, 1963). Each treatment diet was placed in a fermentation tube containing a mixture of 10 ml rumen fluid and 40 ml McDougall buffer solution, then incubated at 39°C for 48 hours (one step *in vitro* feed digestibility test) for dry matter (r-DMD), organic matter (r-OMD) and crude fiber (r-CFD) *in vitro* degradability measurement in the rumen.

After the incubation, the fluid or supernatant was carefully removed from the tube into a centrifuge tube without disturbing the solid part (sample residue) at the bottom of the tube. Subsequently, the supernatant in each centrifuge tube was homogenized, and then 5 ml of the supernatant was taken and used as a protozoa cell count sample. Another 5 ml of

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the supernatant was taken and used as the sample to analyze ammonia and volatile fatty acid concentration. The supernatant's sample protozoa cell count was added and homogenized with 5 ml formalsaline 10%. The protozoa cell count was done using a counting chamber (Hausser Scientific, catalog #3800) under a light microscope (Olympus CX 43, USA) at 100×magnification (Park et al., 2019). A supernatant sample for ammonia and VFA concentration analysis was added with a few drops of H_2SO_4 10% until it reached pH 2.5-3.0. The sample was then divided into two aliquots and stored at -20°C temperature in the refrigerator for ammonia and VFA concentration analysis.

The sample residue was centrifuged at 16,000 rpm for 10 minutes, the supernatant was discharged, and the pellet residue was oven-dried at 55°C for 24 hours and finally weighed to determine r-DMD, r-OMD, and r-CFD. Another set of samples underwent an additional 48 hours at 39°C incubation in a 50 ml mixture solution of 3 ml of 20% HCL and 1 ml of 5% pepsin (two steps *in vitro* feed digestibility test) for *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) measurements.

Table 1. The nutrient content of feed ingredients

Nutrient content (% dry matter basis)	Concentrate	Maize forage	*Mucuna* leave
Dry matter	88.68	91.10	87.68
Ash	7.09	11.85	10.43
Crude protein	15.78	10.00	27.00
Ether extract	3.74	2.10	2.80
Crude fiber	9.44	30.50	29.48
Nitrogen-free extract	57.61	45.55	30.29
Total digestible nutrient (TDN)*	69.59	59.27	70.70

* TDN: 1.6899 + 1.3844(CP) + 0.7526(NFE) – 0.8279(EE) + 0.3673(CF)

Analysis of rumen metabolite

Total VFA, including acetate, propionate, and butyrate concentrations, were analyzed based on Li et al. (2014) using gas chromatography (Agilent Technologies 7820A GC system. Santa Clara. USA). The concentrations of ruminal ammonia nitrogen (NH_3-N) concentrations were measured using Chaney and Marbach (1962) methods.

Statistical analysis

The data were analyzed using SPSS software version 25. The obtained data were subjected to the analysis of variance (ANOVA) for a randomized block design with a nested arrangement. Duncan's Multiple Range Test (DMRT) was conducted to compare the mean values between treatments, aiming to determine any significant effect of the treatments ($p < 0.05$).

RESULTS AND DISCUSSION

Effect of saponin dosage on rumen fermentation profiles

The effect of *Mucuna pruriens* leaves saponin dosages, either MPLM or MPLE saponin, on the parameters of the *in vitro* digestibility test are presented in Table 2.

As indicated in Table 2, the level of addition of *Mucuna pruriens* leaves saponin in the diet, either prepared as meal or extract, had significant effects on *in vitro* digestibility test parameters including r-CFD, NH_3 concentration, and protozoa population ($p < 0.05$). The addition of MPLE saponin in the diet showed a significant effect on r-DMD and r-OMD ($p < 0.05$). The supplementations *Mucuna pruriens* leaves saponin in the diet, either MPLM or MPLE, had insignificant effects on IVDMD ($p > 0.05$) but IVOMD of MPLM showed a significant effect ($p < 0.05$), while MPLE did not show a significant effect ($p > 0.05$). Wahyuni et al. (2014) reported that adding saponin from *Sapindus rarak* increased IVDMD and IVOMD.

The level of addition of *Mucuna* leaves saponin prepared as extract (MPLE) in the diets had a significant effect on r-DMD, r-OMD, and r-CFD ($p < 0.05$). The crude fiber rumen degradations (r-CFD) decreased with the increasing dosage level. This shows that *Mucuna* extract leaves saponin in the diets suppressed straight crude fiber degradability in the rumen. On the other hand, the addition of MPLM saponin to the diets did not significantly affect r-DMD and r-OMD ($p > 0.05$) but it had a significant effect on r-CFD ($p < 0.05$). The highest values of r-CFD were found in MPLM (54.64%) by 0.05% dosage level and tended to decrease when the dosage level increased. The higher crude fiber degradability in diets prepared with MPLM saponin compared to MPLE may be attributed to the fact that MPLM contains not only saponin but also 27% crude protein (Table 1). This leads to an increase in the crude protein content of the diets. Feed digestibility depends on the rumen microorganism's activity since it plays a role in fermentation, while the rumen microorganism was affected by the material feed substances. Goel and Makkar (2012) reported that saponins modify ruminal fermentation by suppressing ruminal protozoa and selectively inhibiting some bacteria and fiber

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degradation. The rumen microbial population in ruminants with regular diets containing sufficient crude fiber must be fiber-degrading bacteria or fibrolytic bacteria (Chen et al., 2022).

Table 2. Parameters of *in vitro* digestibility test of feeds supplemented with different saponin dosages in the forms of *Mucuna pruriens* leaves meal and extract

Variables	Saponin	Dosage of saponin in the diet					SD
		0%	0.025%	0.050%	0.075%	0.100%	
r-DMD (%)	MPLM	54.67	54.43	56.59	54.46	53.88	0.93
	MPLE	54.67^{b}	45.43^{a}	45.60^{a}	47.98^{a}	45.20^{a}	0.67
r-OMD (%)	MPLM	53.82	54.19	56.51	53.31	53.22	1.02
	MPLE	53.82^{b}	45.25^{a}	45.37^{a}	47.83^{a}	45.05^{a}	0.69
r-CFD (%)	MPLM	50.79^{bc}	52.05^{c}	54.64^{d}	49.12^{ab}	47.41^{a}	0.99
	MPLE	50.79^{b}	49.21^{ab}	48.99^{ab}	46.94^{a}	46.70^{a}	0.50
IVDMD (%)	MPLM	62.15	62.03	65.95	61.76	61.54	0.87
	MPLE	62.15	61.81	61.66	61.51	60.77	0.71
IVOMD (%)	MPLM	61.86^{a}	61.93^{a}	65.86^{b}	61.26^{a}	61.22^{a}	0.88
	MPLE	61.86	61.35	61.66	61.28	60.68	0.74
NH_3 (mM)	MPLM	6.68^{ab}	7.15^{b}	6.31^{a}	6.24^{a}	6.13^{a}	0.15
	MPLE	6.68^{b}	6.23^{b}	5.43^{a}	5.36^{a}	5.27^{a}	0.13
Protozoa (10^3 cell/ml liquid)	MPLM	79.92^{c}	77.64^{c}	76.83^{c}	60.50^{b}	47.06^{a}	3.77
	MPLE	79.92^{b}	63.69^{a}	62.56^{a}	58.06^{a}	56.86^{a}	1.92

Note: Different superscript letters in the same row mean the significantly different ($p < 0.05$). MPLM: *Mucuna pruriens* leaves meal, MPLE: *Mucuna pruriens* leaves extract, r-DMD: Dry matter, r-OMD: Organic matter, r-CFD: Crude fiber degradability in the rumen, IVDMD: *In vitro* dry matter digestibility, IVOMD: *In vitro* organic matter digestibility, NH_3: Rumen ammonia, SD: Standart deviation.

Based on Table 2, saponin supplementations either from MPLM or MPLE significantly decreased ammonia concentration and protozoa population ($p < 0.05$), where the lowest values were found at 0.1% dosage level. Saponin affects rumen fermentation by reducing protein degradations, thus decreasing rumen ammonia concentration (Demirtas et al., 2018). Lower ammonia concentration must also indicate the increase of rumen ammonia utilization to increase microbial growth, especially fibrolytic bacteria in the rumen (Jadhav et al., 2016).

The decrease in protozoa population as a result of saponin addition in the diets aligns with findings from Krisnawan (2011), Suhartati et al. (2011), and Hidayah (2016). Saponin is known to have functioned as a defaunation agent for rumen protozoa (Wina et al., 2006). Most researchers reported that saponin decreases the rumen protozoa population as saponins have an antiprotozoal effect in the rumen (Hu et al., 2006; Guo et al., 2008). Several factors influence the effectiveness of saponin use, including the source of saponins, level in the diet, time after saponins feeding or consumption, and types of protozoa (Wina et al., 2006). In addition, Tan et al. (2020) found the different genera of rumen protozoa ciliates appear to be selectively inhibited by tea seed saponins.

Saponin made a bond with the surface sterols protozoa membrane, leading to the rupture of their cell wall (Arum et al., 2013). Another mechanism through which saponins influence the rumen environment involves the modulation of microbial ruminal and ruminal metabolite, although this modulation depends on the basal diet (Wang et al., 2019). The decrease in rumen protozoa population in this research was accordingly followed by an increase in feed degradability and digestibility (Liu et al., 2019). The increase of the fermentations parameter in this research indicates feed efficiency digestions in the rumen. Saponins also could inhibit bacterial (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumonia*, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 14028) and a fungal strain (*Candida albicans*) growth (Mataalah et al., 2012). Wang et al. (2012) reported that some anti-nutritional factors, such as saponin in tea extract, inhibit methanogenesis. The decrease in the ruminal protozoa population decreased methane production (Morgavi et al., 2012). Saponins have a toxic effect on protozoa since some rumen bacteria can hydrolise saponin to their free glycoside fractions (Newbold et al., 1997). However, some bacteria degrads saponin into sapogenin so that it cannot affect protozoa (Ramos-Morales et al., 2014) or the protozoa produce extracellular polysaccharides around the membrane and avoid the degradations by saponin (Wina et al., 2006). This variability may explain why some studies found that saponins do not change the protozoa populations (Kang et al., 2016). It can be the explanation for the increasing protozoa populations on meal saponin supplementations.

Effects of saponin dosage on volatile fatty acids profiles

As indicated in Table 3, the addition of *Mucuna pruriens* saponin to the diet at different dosages either prepared as a meal or extract had a significant effect on total VFA, the concentrations of acetate, propionate, and butyrate, and A/P ratio, as well as the percentages of acetate, butyrate, and propionate ($p < 0.05$). Total VFA production was at its highest level in 0.05% saponin dosage either in the meal form (77.71 mM) or extract (75.76 mM). However, total rumen VFA decreased beyond the 0.05% dosage level.

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Table 3. Volatile fatty acids profile of feeds supplemented with different saponin dosages in the forms of *Mucuna pruriens* leaves meal and extract

Variables	Saponin	Dosage of saponin in the diet					SD
		0%	0.025%	0.050%	0.075%	0.100%	
VFA (mM)	MPLM	61.06^{a}	61.30^{a}	77.71^{b}	72.99^{b}	71.54^{b}	2.01
	MPLE	61.06^{a}	60.40^{a}	75.76^{b}	64.04^{a}	60.49^{a}	1.61
Acetate (mM)	MPLM	34.31^{a}	38.04^{ab}	43.64^{c}	42.51^{bc}	41.58^{bc}	0.93
	MPLE	34.31^{a}	35.60^{a}	46.85^{b}	37.45^{a}	37.20^{a}	1.32
Propionate (mM)	MPLM	16.35^{b}	14.43^{a}	21.57^{d}	19.04^{c}	18.94^{c}	0.82
	MPLE	16.35	15.60	15.93	16.50	15.17	0.38
Butyrate (mM)	MPLM	10.41^{b}	8.83^{a}	12.50^{c}	11.44^{bc}	11.02^{bc}	0.43
	MPLE	10.41^{c}	9.21^{ab}	12.98^{d}	10.10^{bc}	8.13^{a}	0.49
A/P Ratio	MPLM	2.10^{a}	2.65^{b}	2.04^{a}	2.23^{a}	2.21^{a}	0.08
	MPLE	2.10^{a}	2.29^{ab}	2.94^{c}	2.28^{ab}	2.45^{b}	0.13
Acetate (%)	MPLM	56.13^{a}	62.07^{b}	56.17^{a}	58.19^{a}	58.10^{a}	0.75
	MPLE	56.13^{a}	58.85^{b}	61.85^{c}	58.49^{ab}	61.46^{c}	0.74
Propionate (%)	MPLM	26.82^{b}	23.51^{a}	27.76^{b}	26.11^{b}	26.49^{b}	0.56
	MPLE	26.82^{b}	25.87^{b}	21.02^{a}	25.74^{b}	25.11^{b}	0.88
Butyrate (%)	MPLM	17.05^{c}	14.42^{a}	16.07^{bc}	15.69^{abc}	15.41^{ab}	0.26
	MPLE	17.05^{c}	15.28^{b}	17.13^{c}	15.77^{bc}	13.43^{a}	0.42

Note: Different superscript letters at the same row mean significant differences ($p < 0.05$), MPLM: *Mucuna pruriens* leaves meal, MPLE: *Mucuna pruriens* leaves extract, VFA: Volatile fatty acid, A/P ratio: Acetate-to-propionate ratio, SD: Standart deviation.

The normal range of VFA for optimum rumen microbial growth was 80-160 mM (McDonald et al., 2010). However, the VFA value was below the range in the current study. Besides the saponins, *Mucuna pruriens* leaves contained tannins and could form complexes binding with proteins. This process reduces the value of rumen-degradable protein, leading to a decrease in the total VFA concentration due to the decreased proteolysis and less oxidative deamination of feed proteins. There was sufficient ammonia to develop bacteria, but the branched-chain fatty acids were insufficient, so the total VFA decreased. Branched-chain fatty acids consisting of isobutyric acid, two methyl butyrate, and valeric acid are a source of carbon skeletons for bacteria, and these compounds are the result of decarboxylation and deamination of branched-chain amino acids (Nurhaita et al., 2010).

Based on Table 2, the addition of *Mucuna pruriens* saponin in the form of a meal at 0.05% dosage level resulted in high organic matter degradability and digestibility consistent with the findings in Table 3 showing high VFA concentrations. Volatile fatty acid concentrations correlated with dry matter and organic matter digestibility (Noziere et al., 2011). The increase in volatile fatty acids signifies enhanced fermentation of organic matter and higher rumen microbial activity (Madrid et al., 2002). Rumen volatile fatty acid and other carbon skeletons are the end products of organic matter fermentations, including carbohydrates, protein, and lipids that provide energy and carbon skeleton for rumen microbial growth (Dijkstra, 1994). The efficacy of saponins as rumen antiprotozoal is affected by many factors, including the source and the form of saponins (Patra and Saxena, 2009). The effect of *Mucuna pruriens* leaves saponin in the form of either MPLM or MPLE saponin on *in vitro* feed degradability, protozoa population, metabolic products of fermentation in the rumen as well as *in vitro* feed digestibility are presented in Table 4.

Table 4. Parameters of *in vitro* digestibility test of feeds supplemented with saponin in the forms of powder and extract from *Mucuna pruriens* leaves

Measurement	MPLM	MPLE
r-DMD (%)	54.81 ± 2.90^{b}	47.78 ± 4.29^{a}
r-OMD (%)	54.21 ± 3.03^{b}	47.46 ± 4.12^{a}
r-CFD (%)	58.80 ± 3.05^{b}	48.53 ± 1.87^{a}
NH_3 (mM)	6.50 ± 0.70^{b}	5.79 ± 0.70^{a}
Protozoa (10^3 cell/ml liquid)	68.39 ± 14.02	64.35 ± 6.68
VFA (mM)	68.92 ± 7.52^{b}	66.08 ± 2.49^{a}
Acetate (mM)	40.02 ± 4.16	38.28 ± 5.05
Propionate (mM)	18.07 ± 2.69^{b}	15.91 ± 0.80^{a}
Butyrate (mM)	10.84 ± 1.37^{b}	10.16 ± 1.78^{a}
A/P ratio	2.25 ± 0.26^{a}	2.41 ± 0.32^{b}
Acetate (%)	58.13 ± 2.57^{a}	59.36 ± 2.45^{b}
Propionate (%)	26.14 ± 1.77^{b}	24.91 ± 2.29^{a}
Butyrate (%)	15.73 ± 1.09	15.73 ± 1. 57
IVDMD (%)	62.69 ± 2.42	61.58 ± 2.24
IVOMD (%)	62.42 ± 2.37	61.37 ± 2.11

Note: Different superscript letters at the same row mean significant differences ($p < 0.05$). r-DMD: Dry matter, r-OMD: Organic matter, r-CFD: Fiber degradability in the rumen, NH_3: Rumen ammonia, VFA: Volatile fatty acid, IVDMD: *In vitro* dry matter digestibility, IVOMD: Organic matter digestibility, A/P ratio: Acetate-to-propionate ratio.

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As presented in Table 4, the addition of saponins from MPLM to the diet led to significant differences in r-DMD, r-OMD, r-CFD, NH_3, total VFA, propionate and butyrate concentrations, propionate, and acetate percentage, and A/P ratio ($p < 0.05$). However, there were non-significant differences in protozoa population, acetate concentration, butyrate percentage, IVDMD, and IVOMD ($p > 0.05$). The saponin preparations from MPLM tended to show higher values for the digestibility parameters, NH_3, protozoa populations, and VFA profile except for acetate percentage and A/P ratio. In a study by Wang et al. (1998), it was found that saponin from *Yucca scidigera* extract could affect proteolytic activity and reduce protozoal number but did not affect dry matter degradability and bacterial activity. The other study by Patra and Yu (2014) indicated that saponin from *Quilaja Saponaria* decreased the methane but had no effect on VFA concentrations. Furthermore, a study by Lu and Jorgensen (1987) revealed an increase in digestibility and propionate concentrations using saponin from extracted alfalfa.

CONCLUSION

Mucuna pruriens leaves prepared as meal had better rumen degradability and VFA profile, compared to the extract. The use of MPLM with a source of 0.05% saponin in the diet yielded more favorable outcomes in enhancing the fermentation profile of feed within the rumen compared to MPLE. It is necessary to carry out *in vivo* research to determine *Mucuna pruriens* leaves meal saponin as a ruminant feed additive.

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Authors' contributions
Triana Muhartatik wrote the manuscript and conducted the research, Siti Chuzaemi, Halim Natsir, and Marjuki conceptualized the research, supervised the research, and Marjuki revised the final form of the manuscript. All authors read and approved the final draft of the manuscript.

Competing interests
The authors have declared no conflicts of interest.

Ethical considerations
Before publication in this journal, all the authors conducted checks for ethical issues, such as data fabrication, double publication and submission, redundancy, plagiarism, consent to publish, and misconduct.

Availability of data and materials
All data related to the current study are available in this manuscript.

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