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In vitro profiles of a diet containing concentrate with rice bran and *Arenga pinnata* by-product fermented with *Pleurotus ostreatus*

Sulistyowati, E.[1*], Sari, S. W.[2], Faza, A. N.[2] and Wiryawan, I. K. G.[2]

[1]Department of Animal Husbandry, Faculty of Agriculture, Universitas Bengkulu, Bengkulu, Indonesia; [2]Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia.

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Abstract The results showed that the ration containing concentrated with the fermented rice bran and *Arenga pinnata* by- product (Arenga sago dregs) significantly affected ($p<0.05$) pH and IVDMD. There were not significantly affected ($p>0.05$) on NH3, VFA, IVOMD, protozoa population levels, total bacteria, partial VFA, or methane gas. However, the addition of ration with concentration containing 10% rice bran and Arenga sago dregs fermented by Oyster mushroom (*Pleurotus ostreatus*) increased microbial protein synthesis by about 9.35 mg ml^{-1} – 15.82 mg/L. Feeding a diet containing 15% fermented rice bran-Arenga sago dregs can be tolerated and is found to be optimal, as the digestibility values of dry matter, organic matter, total VFA, and NH_3 concentrations are found to be a normal limits.

Keywords: Arenga sago dregs, Rumen fermentability, *P. ostreatus*

Introduction

In vitro fermentation is a method used to evaluate feed quality for ruminants, such as dairy goats. Feedstuffs such as Arenga pinnata by-products (Arenga sago dregs) and rice brands high in fibre require treatment, such as fermentation with *Pleurotus ostreatus.* This fungus has been shown to degrade lignocellulosic materials. It has been widely utilised in the bioconversion of lignocellulosic waste for animal feed, such as in coffee husk (Badarina *et al*., 2013) and Durio rind (Sulistyowati *et al*., 2016). As in previous research, *Durio zibethinus* Murr rind flour fermented with *P. ostreatus* for 8 weeks did not affect in vitro dry matter digestibility (IVDMD) or N-NH3 production; however, it significantly improved *in vitro* organic matter digestibility (IVOMD) (Hartono *et al*., 2015). Sulistyowati *et al*. (2020) reported that, as a fibre fraction, lignin content was low in 5% fermented Durio, whereas in vitro characteristics increased with higher fermentation levels in the concentrate. Therefore, 5% of fermented Durio

*Corresponding Author: Sulistyowati, E.; **Email:** esulistyowati@unib.ac.id

could be incorporated into a concentrate and potentially applied to dairy goats.

The *Arenga pinnata* tree is primarily planted in hilly areas. In 2004, the area of this plant was approximately 60,482 Ha, distributed across 14 provinces in Indonesia (Effendi, 2010). Thus, the plant can be utilised as a sugar producer from the fluid of its flowers and as a source of flour from its trunk through appropriate processing. The *A. pinnata* by-product, known as dregs, which is ground into meal for feed, has been tested and found to contain crude protein (1.55%) and crude fibre (30.18%), as reported by Sulistyowati *et al*. (2020). Witno *et al*. (2024) report that sago dregs contain 65.7% starch; the rest is crude fibre, crude protein, fat, and ash. Oyster mushrooms have high protein content, namely between 17.5 -27%, with low fat, 1.6-8%, and high dietary fibre content of 8-11.5%, which can be used as a healthy food ingredient (Tjokrokusumo *et al*., 2015). The fermentation process can be used to increase crude protein and reduce crude fibre, for example, by using white oyster mushrooms (*Pleurotus ostreatus*).

The fermented *A. pinnata* by-product with *P. ostreatus* exhibited an improved crude protein content of 11.62% and a decreased fibre content of 24.31% (Sulistyowati *et al.,* 2025). The previous results showed that, on average, increasing the level of fermented *A. pinnata* (10-20%) in concentrates resulted in increases in pH (from 7.10 to 7.23), N-NH3 (from 8.83 to 11.61 mM), and VFA (from 87.18 to 89.21 mM). This pH was slightly higher, whereas N-NH3 and VFA were within the normal range for rumen dairy cows. On the other hand, the IVDMD and IVOMD decreased with increasing levels of fermented *A. pinnata* in the concentrates, as reported by Sulistyowati *et al*. (2024). This white-rot fungus produces lignolytic enzymes, such as laccases and peroxidases, as reported by Yakin and Mulyono (2017). Rice bran, as another feedstuff, can be utilised in ruminant feed concentrates.

Rice bran contains 5.34% crude protein, 2.79% fat, and 26.43% crude fibre (Mila and Sudarma, 2021). This feedstuff contains cellulose (50%), lignin (25%), and silica (15%), as reported by Dewi and Saputra (2024). The fermentation of rice brand in the rumen for 8 days showed a significant effect ($P<0.05$) on the digestibility of dry matter and organic matter, as reported by Tandang *et al*. (2024).

Therefore, an opportunity is presented to combine these two feedstuffs, Sago dregs and rice bran, and then ferment them with *P. ostreatus* for utilisation as an ingredient in a concentrate for ruminants, such as dairy goats. The objective was to evaluate the profiles (pH, IVDMD, IVOMD, VFA total and VFA fraction, N-NH_3, protozoal population, methane gas production, and microbial protein) of a complete ration containing this concentration in *in vitro* fermentation with dairy goat rumen fluid.

Materials and methods

This research began with the fermentation of Arenga sago dregs, conducted at the Department of Animal Husbandry, Faculty of Agriculture, University of Bengkulu (UNIB), from August to September 2024. *In vitro* testing took place from September to October 2024 at the Dairy Animal Nutrition Laboratory, Department of Nutrition Science and Feed Technology, Faculty of Animal Husbandry, Bogor Agricultural University.

Fermentation of Arenga sago dregs

The fermentation process was began by collecting the Arenga sago dregs, drying them in the sun, and grinding them into a coarse flour. The palm dregs, rice bran, and CaCO3 are used at 28.2%, 70.4%, and 1.4%, respectively, with 20 litres of water added, and the mixture is composted overnight. This substrate is packaged in 300g bags and then sterilised at 120°C for 4 hours. Subsequently, the substrate temperature in the bags is allowed to reach approximately 40°C, and the bags are inoculated with 3.5% P. ostreatus as a starter culture. The bags are fermented for 4 weeks until the hyphae are evenly developed. The fermented product is then opened, air-dried, and used as a concentrate. The formulation is based on research by Sulistyowati *et al*. (2020), as shown in Table 1.

Table 1. Formula of concentrate containing fermented rice bran and Arenga sago dregs for dairy goat

Ingredients (%)	FRSC-0	FRSC-5	FRSC-10	FRSC-15
Fermented rice bran+ sago dregs*	0	5	10	15
Rice bran	30	25	20	15
Ground corn	30	30	30	30
Soybean meal	35	35	35	35
Palm oil	2	2	2	2
Mineral mix	0.5	0.5	0.5	0.5
C. xanthorhiza powder	0.5	0.5	0.5	0.5
Yeast (*S. cerevisiae*)	0.5	0.5	0.5	0.5
NaCl	0.5	0.5	0.5	0.5
CaCO3	0.5	0.5	0.5	0.5
TSP	0.5	0.5	0.5	0.5

*Modification Sulistyowati *et al*. (2020)

In vitro fermentation

Firstly, goat rumen fluid and buffer solution were mixed in a 1:2 ratio. The mixture was supplied with CO_2 gas and incubated at 39°C. Then, 0.5 g of each sample was placed in a fermentor tube, followed by 40 ml of McDougall's solution and 10 ml of rumen fluid. CO_2 was introduced into the tube for 30 seconds, after which the tube was sealed with a rubber stopper. The tube was placed in a shaker bath and fermented for 4 hours. The rubber stopper was removed, and two drops of saturated $HgCl_2$ were added to inhibit microbial activity and stop fermentation. The tube was centrifuged at 3000 rpm for 15 minutes, and the supernatant was collected for VFA and NH3-N analyses and for pH measurement using a pH meter. The method used was that described by Tilley and Terry (1963).

N-NH_3 (Ammonia) concentration measurement

Ammonia analysis was performed using the Conway Microdiffusion method (Millar, 1966). Ammonia concentration can be calculated using the formula:

$$\text{Ammonia concentration (mM)} = \frac{\text{ml H2SO4} \times \text{N H2SO4x1000}}{\text{Sample weight (g)} \times \text{\% DM of sample}}$$

Total and partial VFA measurement

The VFA analysis was performed by steam distillation (Millar, 1966). The VFA can be calculated using the formula:

$$\text{VFA concentration (mM)} = \frac{(\text{a} - \text{b}) \times \text{N HCl x} \left(\frac{1000}{5}\right) \text{ml}}{\text{Ration weight (g)} \times \text{\% DM of ration}}$$

Note: a = volume of blank titrant (ml); b = volume of sample titrant (ml)

A Bruker® Scion 436-GC gas chromatograph and SHO-40 with an auto-injection system were used in the partial Volatile Fatty Acid (VFA) analysis. Acidified rumen samples were mixed with 0.003 grams of sulfosalicylic acid dehydrate in a microtube. The microtubule mixture was centrifuged for 10 minutes at 12,000 rpm at 7 °C. The microtubule mixture was centrifuged for 10 minutes at 12,000 rpm at 7 °C. The supernatant was placed in a stoppered tube. The tube containing the supernatant was put into a Bruker® SHO-40.

Methane gas calculation

Methane gas estimation using the partial VFA proportion approach. The methane gas estimation can be done using the following formula:

CH4 = 0.45 Acetate − 0.275 Propionate + 0.40 Butyrate.

***In vitro* dry matter (IVDMD) and organic matter digestibility (IVOMD)**

Digestibility was measured using the Tilley and Terry (1963) method. The formula used is as follows:

$$\mathbf{IVDMD}\ (\% = \frac{\text{Sample DM (g)} - [\text{DM residue (g)} - \text{blank DM (g)}] \times 100}{\text{Sample DM (g)}}$$

$$\mathbf{IVOMD}\ (\%) = \frac{\text{Sample OM (g)} - [\text{OM residue (g)} - \text{blank OM (g)}] \times 100}{\text{Sample OM (g)}}$$

Microbial protein synthesis (MPS)

The MPS analysis used the Makkar *et al*. (1982) method, followed by the method of Lowry *et al*. (1951) to measure microbial N content. After this process, the solution was measured at 650 nm using a spectrophotometer. The obtained absorbance values were then entered into a standard absorbance graph to get the MPS content.

MPS content (mg/ ml) = (mg/ml^{-1} sample) x Dilution factor

Total bacterial population

The rumen bacterial population was calculated by serially diluting rumen fluid and then culturing it on Brain Heart Infusion (BHI) agar. BHI medium was prepared by mixing 3.7 g of BHI flour, 0.05 g of glucose, 1 ml of CMC (1%), 0.05 g of starch, 0.05 g of cysteine-HCl, 0.05 ml of resazurin, and 100 ml of distilled water. The mixture was heated until the colour changed from yellowish-brown to reddish-brown. It was then cooled and anaerobically conditioned with CO_2, resulting in a colour change back to yellowish brown. Five ml of the medium was transferred to a Hungate tube containing 0.15 g of Bacto agar, and the mixture was sterilised in an autoclave at 121 °C for 15 minutes.

The sample was previously diluted by adding 0.05 ml of rumen fluid to 4.95 ml of the initial dilution medium. The dilution was then carried out using the same procedure. A 0.1 ml sample was taken from each dilution tube and inoculated onto agar media. The bacteria grown on BHI agar were incubated at 39 °C for 24 hours to count colonies. The bacterial population was calculated using the following formula: x = xth dilution.

$$\text{Bacterial population} = \frac{\text{Total Colony}}{0{,}05 \text{ X } 10^{-x} \text{ X } 0{,}1}\ \text{CFU/ml}$$

Total protozoan population

Protozoan population calculations are performed in a counting chamber by counting the total number of protozoa present. Rumen fluid, treated similarly to the total bacterial sample, is collected, mixed with TBFS solution at a 1:1 ratio, and then stored in a film bottle. Two drops of the mixture are placed in a counting chamber with a thickness of 0.1 mm, which has the smallest square area of 0.0625 mm² and comprises 16 squares, with the area of each square read. Protozoan population calculations (C) are performed under a microscope at 100x magnification. The protozoan population can be calculated using the formula: DF (dilution factor).

$$\text{Protozoa/ml rumen fluid} = \frac{100 \text{ x n x DF}}{0.2 \text{ x } 0.0625 \text{ x } 16 \text{ x } 16} \text{ (log cell ml}^{-1}\text{)}$$

Experimental design and data analysis

The experimental design used was a Completely Randomised Design (CRD) with four treatments and four replications. The dry matter ration contained 52% tofu dregs, 18% green fodder, and 30% concentrate. The treatments were as follows:

FRSC- 0: Ration with concentrate without (0%) fermented rice bran + Arenga sago dregs
FRSC-5: Ration with concentrate containing (5%) fermented rice bran + Arenga sago dregs
FRSC-10: Ration with concentrate containing (10%) fermented rice bran + Arenga sago dregs
FRSC-15: Ration with concentrate containing (15%) fermented rice bran + Arenga sago dregs

The data were analysed using analysis of variance (ANOVA) in SPSS 26. If significantly different results were obtained, a Duncan's Multiple Range Test (Lentner and Bishop, 1986) was performed.

Results

Nutrient contents of the ration

The nutritional contents of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF), and N-free extract (N-FE) of fermented rice brand and Arenga sago dregs with different levels of a ration were presented in Table 2. The results showed that fermenting the rice brand and Arenga sago dregs with *P. ostreatus* slightly improved the nutritional value of this ration, as indicated by OM, CP, and N-FE at different levels.

Rumen fermentability and in vitro digestibility

In vitro fermentation was determined by measuring the acidity level (pH) and product concentrations in the form of ammonia (NH_3) and total volatile fatty acid (VFA), as well as *in vitro* dry matter (IVDMD) and organic matter digestibility (IVOMD) of the ration containing concentrates with fermented rice brand and Arenga sago dregs at different levels. The effect of treatment on rumen fermentability is presented in Table 3. The pH and IVDMD levels were significantly higher ($P<0.05$) than the others, especially found in the 5- 10% levels of the concentrated feedstuff.

Table 2. Nutrient contents of the ration with concentrate containing fermented rice brand and sago dregs

Nutrients (%)	FRSC-0	FRSC-5	FRSC-10	FRSC-15
Dry matter	85.50	87.23	88.39	88.99
Moisture	14.50	12.77	11.61	11.01
Ash	7.44	6.18	5.41	5.35
Organic matter	81.06	81.05	82.98	83.64
Crude protein	22.63	24.79	23.41	22.16
Ether extract	1.63	1.73	1.84	2.23
Crude fiber	18.35	19.73	20.57	18.93
N-free extract	49.96	47.59	48.78	51.34

Note: **FRSC-0**: ration with concentrate containing 0% fermented rice brand and sago dregs; **FRSC-5**: ration with concentrate containing 5% fermented rice brand and sago dregs; **FRSC-10**: ration with concentrate containing 10% fermented rice brand and sago dregs; **FRSC-15:** ration with concentrate containing 15% fermented rice brand and sago dregs.

Table 3. Dairy goat rumen fermentability and *in vitro* digestibility of ration with concentrate containing fermented rice brand and sago dregs

Variables	FRSC-0	FRSC-5	FRSC-10	FRSC-15	*P-Value*
pH	7.01 ±0.08[a]	7.03 ±0.10[a]	7.04 ± 0.02[b]	7.08 ± 0.01[b]	0.019
NH_3 (mM)	12.24 ±1.55	13.27 ±0.71	11.10 ± 2.26	10.50 ± 1.20	0.105
Total VFA (mM)	124.20 ±10.68	131.63 ± 8.88	140.77 ± 12.79	129.61 ± 10.49	0.235
IVDMD (%)	70.9± 2.33[a]	73.6± 1.65[b]	74.1±1.23[b]	74.3.± 0.75[b]	0.045
IVOMD (%)	70.3± 2.42	73.1± 1.72	73.1± 1.26	73.7± 2.01	0.052

Note: **FRSC-0**: ration with concentrate containing 0% fermented rice brand and sago dregs; **FRSC-5**: ration with concentrate containing 5% fermented rice brand and sago dregs; **FRSC-10**: ration with concentrate containing 10% fermented rice brand and sago dregs; **FRSC-15:** ration with concentrate containing 15% fermented rice brand and sago dregs. There were significant differences among treatments ($P < 0.05$).

Partial volatile fatty acids (VFA) and rumen methane

The effect of treatment on partial Volatile Fatty Acids (VFA) and rumen methane is presented in Table 4. The analysis results showed that the treatments had no significant effects on partial VFA and methane ($P> 0.05$). Acetate (C_2), a milk fat precursor, was found at high levels in a concentrate containing 10% fermented rice brand and Arenga sago dregs, exceeding those in other feedstuffs. On the other hand, propionate (C_3), a precursor of milk production, was high in 5% fermented rice brand and in Aren 'sago dregs in concentrate. The C_2/C_3 ratio was high in the 10% feedstuff. Methane (CH4) production was low at 0% and 15% in fermented rice brand and Arenga sago dregs concentrates.

Table 4. Partial volatile fatty acids (VFA) and rumen methane of the ration with concentrate containing fermented rice brand and Arenga sago dregs

Partial VFA (mM)	FRSC-0	FRSC-5	FRSC-10	FRSC-15	*P-Value*
C_2	53.48±28.48	65.47±14.55	69.46±8.86	53.15±7.64	0.446
C_3	16.96 ± 6.00	21.41 ± 3.01	17.00±5.74	14.18± 1.0	0.854
iC_4	0.89 ± 0.32	0.86 ± 0.09	0.66 ± 0.22	0.66 ± 0.05	0.647
nC_4	6.43 ± 2.17	8.03 ± 0.94	6.27 ± 2.14	5.05 ± 0.67	0.646
iC_5	1.10 ± 0.37	1.06 ± 0.10	0.76 ± 0.23	0.65 ± 0.06	0.575
nC_5	0.93 ± 0.44	0.82 ± 0.09	0.57 ± 0.16	0.47 ± 0.04	0.436
C_2/C_3	3.15 ± 1.70	3.05 ± 1.21	4.08 ± 1.30	3.74 ± 1.28	0.211
CH_4	21.98 ± 12.2	26.79 ± 6.51	29.09±3.34	22.04±3.34	0.454

Note: **FRSC-0**: ration with concentrate containing 0% fermented rice brand and sago dregs; **FRSC-5**: ration with concentrate containing 5% fermented rice brand and sago dregs; **FRSC-10**: ration with concentrate containing 10% fermented rice brand and sago dregs; **FRSC-15:** ration with concentrate containing 15% fermented rice brand and sago dregs. There were no significant differences among treatments (P>0.05).

Rumen microbial population and protein synthesis

The effects of treatment on microbial populations and protein synthesis are presented in Table 5. The analysis showed that the treatments had no significant effects on protozoan and bacterial populations (P > 0.05). On the other hand, the analysis showed that the treatment had a significant effect on microbial protein synthesis (P < 0.05). The treatments (5-15%) were higher as compared to the concentrate without (0%) fermented rice bran and Arenga sago dregs.

Table 5. Rumen microbial population and protein synthesis of a ration with concentrate containing fermented rice brand and sago dregs

Variables	FRSC-0	FRSC-5	FRSC-10	FRSC-15	*P-Value*
Bacteria total (log cell ml^{-1})	8.97 ± 0.32	8.88 ± 0.30	8.87 ± 0.11	8.77 ± 0.11	0.702
Protozoa total (log cell ml^{-1})	6.00 ± 0.14	6.02 ± 0.13	6.01 ± 0.04	5.89 ± 0.06	0.192
Microbial protein (mg ml^{-1})	9.35 ± 1.70^{a}	11.78 ± 1.11^{ab}	15.82 ± 1.72^{ab}	12.7 ± 1.50^{b}	0.000

Note: **FRSC-0**: ration with concentrate containing 0% fermented rice brand and sago dregs; **FRSC-5**: ration with concentrate containing 5% fermented rice brand and sago dregs; **FRSC-10**: ration with concentrate containing 10% fermented rice brand and sago dregs; **FRSC-15:** ration with concentrate containing 15% fermented rice brand and sago dregs. There were significant differences among treatments (P < 0.05).

Discussion

Rumen fermentability

Rumen pH is a key indicator of rumen fermentation activity. The results indicated that the treatment of fermented Arenga sago dregs had a significant effect on rumen pH ($P < 0.05$). According to Suryani *et al*. (2014), the optimal condition for goat rumen microbes to function is a normal pH of 6.0-6.9. Based on the study's results, the resulting pH value was 7.01-7.08. This indicates that the rumen pH results were higher than the normal pH value. Although the pH was slightly outside the optimal range, it was considered unlikely to disrupt rumen microbial activity, thereby maintaining balanced, normal function and ensuring optimal feed digestion (McDonald *et al*., 2010). The pH of rumen fluid plays a vital role in regulating several rumen processes, supporting rumen microbial growth and producing VFA and NH_3 (Usboko *et al*., 2024).

The concentrations of NH3 and VFA are metabolic outcomes in livestock, reflecting the amount of organic matter degraded by rumen microbes and indicating the effectiveness of rumen fermentation. NH3 is produced in the rumen by proteolytic bacteria, which are essential for rumen microbial growth, as NH3 is the primary source of microbial protein synthesis and the means of meeting microbial protein needs (Hoy *et al*., 2023). The NH3 produced in this study ranged from 10.50 to 13.27 mM. Wole *et al*. (2018) reported that the optimal NH3 concentration ranges from 85 to 300 mg/l (6 to 21 mM). Based on the analysis of variance, the treatment effects on ammonia concentration were not significantly different ($P > 0.05$). The results of this analysis are in line with the research conducted by Badarina *et al*. (2013) in the treatment of adding 6% coffee fruit skin fermented with *Pleurotus ostreatus* fungus, where there was an increase to reach optimal NH_3 and a decrease in NH_3 at the highest treatment level.

The highest NH_3 value was observed in the FRSC-5 treatment, which included the addition of 25% rice bran and 5% fermented Arenga sago dregs, as this treatment achieved a balanced protein-to-carbohydrate ratio. The balance of protein and energy in the feed is essential for optimal rumen microbial growth, thereby maximising protein utilisation in the rumen and increasing the effectiveness of microbial fermentation (Fitriyanto *et al*., 2021). The FRSC-15 treatment, with the addition of 15% rice bran and 15% fermented palm sugar dregs, reduced NH3 concentration because crude protein decreased and N-FE increased. Crude protein can indicate the availability of nitrogen (N) to rumen microbes. Protein availability must be balanced with energy sources for a balanced feed. Excess energy relative to N availability reduces fermentation efficiency and rumen microbial growth (Fitriyanto *et al*., 2021).

Prayitno *et al*. (2018) stated that factors influenced to NH_3 production included feed protein content, protein degradability, and the source and proportion of soluble carbohydrates. The extent of rumen crude protein degradation influenced the NH3 concentration. NH_3 concentration increased when rumen crude protein degradation was high. Still, if rumen crude protein degradation was low, the resulting NH3 concentration was likewise low. Microbes can work optimally to break down amino acids, which are then used to build proteins in the body. The rumen pH environment can also reduce microbial activity. It was consistent with the crude protein content of the samples.

Volatile fatty acids are the main product of carbohydrate fermentation by rumen microbes. Rumen microbes fermented carbohydrates to form VFA, which was a ready-to-use energy source for rumen microbes (Wea *et al*., 2015). The VFA concentrations measured in this study ranged from 124.2 to 140.77 mM. This value is still within the normal range, as the normal range of total VFA is 70-150 mM (McDonald *et al*., 2002). Based on the analysis of variance, the treatment did not have a significant effect on VFA concentration ($P > 0.05$). The results of this analysis are consistent with previous research by Sulistyowati *et al*. (2022), which found an increase in VFA in the treatment with concentrate added to 20% Durian Peel fermented by the fungus Pleurotus ostreatus. The higher the concentration of fermented Arenga sago dregs in the concentrate, the higher the VFA concentration. Sairullah *et al*. (2017) stated that the composition of VFA in the rumen varies with differences in physical form, level, feed composition, feeding frequency, and processing. The lower the VFA production, the lower the soluble protein and carbohydrates.

The decrease in VFA from FRSC-10 to FRSC-15 is likely attributable to changes in nutrient digestibility. Microbes use VFA as an energy source to synthesise microbial proteins and support cell growth. The FRSC-10 treatment produced the highest value, at 140.77 mM. This increase in VFA is attributed to the relatively high N-FE content of FRSC-10. The rise in VFA in FRSC-10 indicates more effective carbohydrate fermentation, although the pH remains above the optimal range. Furthermore, the increase in VFA in FRSC-10 indicates more intensive carbohydrate fermentation than in the other treatments.

According to Wijayanti *et al*. (2012), high VFA production in feed is attributed to the presence of readily digestible carbohydrates, such as starch and sugars, which are degraded by rumen microbes into energy sources. The level of feed fermentability influences the production of high or low VFA, the amount of soluble carbohydrates, rumen pH, feed digestibility, and the number and types of bacteria presented in the rumen.

Dry matter and organic matter digestibility

Dry matter digestibility is indicated the proportion of dry matter in the ration that rumen microbes can digest. Dry matter digestibility is indicated feed quality and the animal's ability to utilise a feed. Organic matter digestibility is refered to the proportion of organic matter that is digested by digestive enzymes produced by rumen microbes. As with *in vitro* dry matter digestibility (IVDMD), organic matter digestibility (IVOMD) can also serve as a benchmark for evaluating ration quality.

The results of the analysis of variance showed that the FRSC concentrate treatment had a significant effect ($P < 0.05$) on in vitro dry matter digestibility but no significant effect ($P > 0.05$) on in vitro organic matter digestibility. The dry matter digestibility values in this study ranged from 70.99 to 74.32%. The organic matter digestibility values in this study ranged from 70.26 to 73.65%. The results of the dry matter and organic matter digestibility analysis in this study were higher when compared to the results of Sulistyowati *et al*. (2024) study with a ration of 20% sago dregs fermented with *Pleurotus ostreatus*, which showed dry matter and organic matter digestibility values of 53.84 - 65.93% and 53.16 - 63.68%, respectively. A high digestibility value indicates higher quality and greater nutrient availability for livestock (Tandang *et al*., 2024). Meanwhile, low digestibility indicates that the feed is less able to supply nutrients for basic life and livestock production.

The digestibility of dry matter and organic matter, with the addition of 15% fermented Arenga sago dregs and 15% rice bran, was highest, at 74.32% and 73.65%, respectively. In comparison, the lowest digestibility values for dry matter and organic matter in this study were observed with the addition of 0% fermented Arenga sago dregs and 30% rice bran, at 70.99% and 70.26%, respectively. This condition indicates that providing fermented Arenga sago dregs and rice bran at 15% yields the best effect. According to Tandang *et al*. (2024), the increase in dry matter digestibility is also caused by the increase in IVDM because OM is a component of DM. This is thought to result from the breakdown of DM during fermentation, thereby reducing DM content. The reduction in DM content due to further decomposition implies a decrease in ash content, because DM comprises OM and Ash.

The increase in IVDMD and IVOMD can also be associated with a decrease in crude fibre. Lower crude fibre content facilitates feed digestion; the reduction in crude fibre content in each treatment is proportional to the corresponding increase in IVDMD and IVOMD. According to Fidriyanto *et al.* (2021), increasing the amount of feed fibre can reduce the digestibility of dry matter and organic matter. Meanwhile, a decrease in crude fibre can increase the digestibility of dry matter and organic matter. The cruder fibre contained in a feed, the thicker the cell walls and, consequently, the lower the digestibility of the feed. Feed ingredients with high crude fibre are generally difficult for digestive enzymes to digest, thereby affecting the digestibility of both dry matter and organic matter (Jhena *et al*., 2020).

Partial volatile fatty acids (VFA) and rumen methane

Volatile Fatty Acids (VFAs) are fermentation products of carbohydrates and fats that serve as energy sources and carbon skeletons for rumen microbes (Jamarun *et al*., 2021). The partial proportion of VFA consists of short-chain fatty acids (acetate, propionate, and n-butyrate), branched-chain fatty acids (iso-butyrate and iso-valerate), and n-valerate (Rosmalia *et al*., 2022). Acetic acid is the end product of fibre fermentation; Propionic acid is the end product of sugar and starch fermentation, while Butyric acid is the primary energy source for livestock through the process of gluconeogenesis (Mardalena, 2015). The proportions of acetic, propionic, and butyric acids were not significantly different among treatments ($P > 0.05$). Suwarno (2008) stated that the proportion of acetic acid (C_2) is 50-65%, propionic acid (C_3) 18-20%, butyric acid (C_4) 10%, in addition, isobutyrate (iC_4), isovalerate (iC_5), and valerate (C_5), around 5%, are also produced. The proportion of acetic acid tends to increase in the FRSC-10 treatment, whereas in the FRSC-0, FRSC-5, and FRSC-15 treatments, it is within the normal range. Acetic acid is positively correlated with methane gas production. Acetic acid production increases in tandem with methane production but is negatively correlated with protein and fat content (Danielsson *et al*., 2017). Propionic acid, butyric acid, isobutyrate, isovalerate, and valerate in this study are lower than the standard limit.

The acetic-to-propionic acid (C_2/C_3) ratio serves as a benchmark for the efficiency of ruminant livestock in utilising energy and for the quality of the resulting product. The C_2/C_3 ratio did not differ significantly between treatments ($P>0.05$). In this study, the C2/C3 ratios were FRSC-0 (3.15), FRSC-5 (3.05), FRSC-10 (4.08), and FRSC-15 (3.74). The FRSC-10 treatment exceeded the standard limit, whereas the FRSC-0, FRSC-5, and FRSC-15 treatments were below it. This means that in the FRSC-10 treatment, the proportion of acetate produced was high. The acetate-to-propionate ratio is used as an indicator of energy-use efficiency and product quality (Muchlas *et al*., 2014). The A/P ratio increases with fermentation duration, indicating that more structural carbohydrates are degraded and that acetate production increases while propionate production decreases (Sulistyowati *et al*., 2014). High proportions of acetate and propionate can indicate low energy efficiency; hence, lower proportions of acetate and propionate indicate more efficient energy use (Suwandyastuti and Rimbawanto, 2015). The results of the FRSC-0, FRSC-5, and FRSC-15 treatments indicated that energy use was more efficient and that rumen fermentation produced propionate. However, the FRSC-10 treatment exhibited low energy efficiency, and rumen fermentation produced acetate.

Protozoa are positively correlated with CH_4 production because they produce H_2, which is then converted to CH_4 by methanogens (Besharati *et al*., 2022). Rumen protozoa have symbiotic relationships with methanogens, both ecto- and endosymbiotically, and together they account for nearly half of rumen biomass (Newbold *et al*., 2015). Reducing the number of rumen protozoa can increase the availability of microbial protein to the host by up to 30% and minimise CH_4 production by up to 11% (Benchaar, 2023). Therefore, the removal of ciliate protozoa from the rumen can potentially reduce enteric CH_4 production in the rumen and reduce energy losses associated with methanogenesis in the rumen (Tuwaidan *et al*., 2024). Based on the results of the analysis of variance, the treatment effect was not significant ($P < 0.05$) on total methane production. The study results showed that methane gas production in FRSC-5 and FRSC-10 was high, whereas FRSC-0 and FRSC-15 had the lowest methane gas production. This was positively correlated with the protozoa obtained in this study: FRSC-5 and FRSC-10 had high protozoa populations, whereas FRSC-0 and FRSC-15 had low protozoa populations. The FRSC-0 and FRSC-15 treatments were the most effective, producing the lowest methane emissions, compared with FRSC-5 and FRSC-10. The lower the methane production by livestock, the higher the quality of the feed provided.

Rumen microbial population and protein synthesis

Bacteria are the dominant rumen microorganisms. Based on their functions, rumen bacteria can be classified into 7 main groups: cellulose-digesting, hemicellulose-digesting, starch-digesting, sugar-digesting, lactate-consuming, methane-forming, and proteolytic (Purbowati *et al*., 2014). Based on the results of the analysis of variance, the treatment effect was not significantly different ($P < 0.05$) across the total population of rumen bacteria. The results of this study indicated a total bacterial population ranging from 8.77 to 8.97 log CFU/ml in rumen fluid. McDonald *et al*. (2010) reported that the normal bacterial population ranges from 9 to 10 log CFU/ml. The bacterial population in this study was lower than the one indicated by McDonald's. This is because the rumen pH is higher than the normal rumen pH, which corresponds to Susi's research, which is 7.01–7.08. Research by Yanuartono *et al*. (2019) indicates that the optimal rumen pH value for bacterial growth ranges from 5.5 to 7. The type of feed, feeding frequency, and digestion duration can influence this pH value. This condition indicates that providing 30% rice bran has the best effect.

Protozoa are the second-largest group of rumen microorganisms, after bacteria (Miltko *et al*., 2020). Based on the results of the analysis of variance, the treatment effect was not significantly different ($P < 0.05$) on the total rumen protozoa population. The results of this study revealed a protozoa population of 5.89-6.02 log cells/ml in the rumen fluid. The protozoal population in this study remained within the normal range, according to Fares *et al*. (2019), which is approximately 4-6 log cells/ml of rumen fluid. The highest protozoal population was observed in goats fed the ASAF5 ration. The high protozoa population in goats fed the FRSC-5 ration was attributable to its highest CP content among all treatments. The PK consumptions of goats treated with FRSC-0, 5, 10, and 15 were, respectively, 18.82, 20.2, 19.16, and 17.67 g/h. Protozoa play an essential role as protein producers because they consume bacteria, thereby producing protozoan proteins that are more easily digested and have higher biological value (Yanuartono *et al*., 2019). In addition to preying on bacteria to meet their protein needs, protozoa also consume feed protein that enters the rumen, so that the conditions in the rumen fluid of goats treated with FRSC-5 allow protozoa to grow.

A ration containing fermented Arenga sago dregs resulted in a lower protozoan population compared to a feed containing rice bran. The total protozoan population in rice bran indicates that rice bran can stimulate rumen protozoan growth due to its higher soluble sugar content. The high protozoan population in the rumen is attributable to their adaptation to feed on rich sources of soluble sugars (Yanuartono *et al*., 2019).

Based on the results of the analysis of variance, the treatment effect was significant ($P < 0.05$) on the total population of microbial protein synthesisers (MPS). The highest MPS value was observed in the FRSC-10 treatment, which consisted of a concentrate containing 20% rice bran, 10% fermented rice bran, and Arenga sago dregs. The lowest MPS concentration was observed in the FRSC-0 treatment, which consisted of a concentrate with 30% rice bran, 0% fermented rice bran, and Arenga sago dregs. The high digestible organic matter content in goats fed the FRSC-10 and FRSC-15 rations met microbial nutrient requirements, resulting in increased growth and the highest microbial protein synthesis (Suryani *et al*., 2014). This aligns with the digestible organic matter content reported by Susi for FRSC-10 and FRSC-15, which were 74.07%.

Rations with higher FRSC content will increase protein, which is very important for the formation of microbial protein synthesis, so that rations given more FRSC, such as FRSC-10 and FRSC-15, can produce a greater amount of microbial protein synthesis. This is because fermentation of Arenga sago dregs with white oyster mushrooms can reduce anti-nutritional properties such as phytic acid, tannins, and saponins contained in rice bran and Arenga sago dregs, as stated by Dona and Triani (2015), which states that tannin content can protect proteins so that rumen microbes do not completely digest proteins. The growth and development of rumen microbes require NH_3 for protein synthesis, so it is closely related to the activity and population of rumen microbes (Leo *et al*., 2023). Rumen microbes degrade feed protein into peptides and amino acids; some of these amino acids are further degraded to ammonia. Rumen microbes utilise ammonia to synthesise microbial proteins.

Rations of fermented rice bran and palm waste (Arenga sago dregs) can be supplemented with up to 15% without affecting the digestibility of organic matter, NH3, and total VFA, and have a significant effect on dry matter digestibility and pH. Feeding rice bran and palm sugar waste fermented by white fungus (Pleurotus ostreatus) at up to 10% can be tolerated and is optimal, as it yields digestibility values for dry matter, organic matter, and total VFA, as well as NH3 concentrations, that are within normal limits. The 10% group achieved the highest microbial protein synthesis (MPS). Feeding with higher digestible organic matter, such as 10% and 15%, supported better microbial growth. The C_2/C_3 ratio indicated lower energy efficiency. The highest protozoan population was observed in the 5% group, which correlated with higher protein consumption. Overall, the 0% and 15% concentrations of fermented rice bran and palm waste in the concentrate exhibited lower methane production, indicating improved feed quality.

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Conflicts of interest

The authors declare no conflicts of interest in relation to the International Journal of Agricultural Technology (IJAT) publication.

References

Badarina, I., Evvyernie, D., Toharmat, T., Herliyana, E. N. and Darusman, L. K. (2013). Nutritive value of coffee bran fermented with *P. ostreatus* as ruminant feed. Media Peternakan, 36: 58-63.

Benchaar, C. (2023). The use of plant extracts as dietary supplements in dairy cow nutrition: saponins. Chapter 6. Burleigh Dodds Science Publishing Limited.

Besharati, M., Maggiolino, A., Palangi, V., Kaya, A., Jabbar, M., Eseceli, H., De Palo, P. and Lorenzo, J. M. (2022). Tannin in Ruminant Nutrition: Review. Molecules, 27:8273-8299.

Danielsson, R., Dicksved, J., Sun, L., Gonda, H., Müller, B., Schnürer, A. and Bertilsson, J. (2017). Methane production in dairy cows correlates with rumen methanogenic and bacterial community structure. Frontiers in Microbiology, 8:1-15.

Dewi, S. U. and Saputra, B. (2024). Pengaruh campuran dedak padi sebagai pengganti sebagian agregat halus terhadap kuat tekan beton mutu sedang. TAPAK (Teknologi Aplikasi Konstruksi): Jurnal Program Studi Teknik Sipil, 13:132-138.

Dona, A. dan Triani, H. D. (2015). Produksi NH_3, Protein Bypass dan Sintesis Protein Mikroba dari Pod Kakao yang disuplementasi *Chromolaena odorata*. Jurnal Peternakan Indonesia, 17:171-175.

Effendi, D. S. (2010). Prospek pengembangan tanaman Aren (*Arenga pinnata* Merr) mendukung kebutuhan bioetanol di Indonesia. Perspektif, 9:36-46.

Fares, M. A., Rahmoun, D. E. and Lieshchova, M. A. (2019). Rumen protozoa of adult bovines during autumn in the East of Algeria. Theoretical and Applied Veterinary Medicine, 7:223-228.

Fitriyanto, R., Suhartati, F. M. and Rahayu, S. (2021). Pengaruh penggunaan silase rumput gajah yang diberi singkong terhadap konsentrasi vfa dan N-NH_3 cairan rumen sapi secara *in vitro*. Journal of Animal Science and Technology, 3:272-279.

Fidriyanto, R., Priadi, G., Paradisa, Y. B., Astuti, W. D., Ridwan, R., Rohmatussolihat, R. and Widyastuti, Y. (2021). The use of lemongrass waste as an elephant grass substitute in high-forage feed *in vitro* rumen fermentation: Methane production and digestibility. Agric, 33:103-114.

Millar, R. L. (1966). General laboratory procedures. The American Biology Teacher, 492-502.

Hartono, R., Fenita, Y. and Sulistyowati, E. (2015). *In vitro* dry matter, organic matter, and N-NH_3 production of *Durio zibethinus* fermented with white rod *(Pleurotus ostreatus)* in different incubation times. Jurnal Sain Peternakan Indonesia, 10:87-94.

Hoy, C. P. E., Hartati, E. and Lestari, G. A. Y. (2023). Pengaruh silase pakan komplit berbasis sorgum *Clitoria ternatea* dengan penambahan berbagai level konsentrat mengandung $ZnSO_4$ dan ZnCu Isoleusinat terhadap fermentasi rumen *in vitro*. Animal Agriculture, 1:79-89.

Jamarun, N., Pazla, R. and Yanti, G. (2021). Effect of boiling on *in vitro* nutrients digestibility, rumen fluid characteristics, and tannin content of mangrove (*Avicennia marina*) leaves as animal feed. IOP Conference Series: Earth and Environmental Science, 733:1-6.

Jhena, K., Kleden, M. M. and Benu, I. (2020). Kecernaan nutrien dan parameter rumen pakan konsentrat yang mengandung tepung daun Kersen sebagai pengganti jagung secara *in vitro*. Jurnal Nukleus Peternakan, 7:118-129.

Leo, S., Maranatha, G. and Oematan, G. (2013). Pengaruh level subtitusi rumput (*Bothiriochola pertusa*) dengan kangkung terhadap pH, konsentrasi VFA dan amonia cairan rumen ternak kambing kacang. Journal of Animal Agriculture, 1:13-23.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193:265-275.

Lentner, M. and Bishop, T. (1986). Experimental Design and Analysis. Valley Book Co., VA.

Makkar, H. P. S., Sharma, O. P., Dawra, R. K. and Negi, S. S. (1982). Simple determination of microbial protein in rumen liquor. Journal of Dairy Science, 65:2170-2173.

Mardalena. (2015). Evaluasi serbuk kulit nenas sebagai sumber antioksidan dalam ransum kambing perah Peranakan Etawah secara in Vitro. Jurnal Ilmu-Ilmu Peternakan, 18:14-21.

McDonald, P.; Edwards, R. A.; Greenhalgh, J. F. D., 2002. Animal Nutrition. 6th Edition. Longman, London and New York. 543 p.

McDonald, P., Edwards, R., Greenhalgh, J., Morgan, C., Sinclair, L. and Wilkinson, R. (2010). Animal Nutrition. Seventh Ed. England: Pearson Education Limited.

Mila, J. R. and Sudarma, I. M. A. (2021). Analisis kandungan nutrisi dedak padi sebagai pakan ternak dan pendapatan usaha penggilingan padi di Umalulu, Kabupaten Sumba Timur. Buletin Peternakan Tropis, 2:90-97.

Miltko, R., Kowalik, B., Majewska, M. P., Kędzierska, A., McEwan, N. R. and Bełżecki, G. (2020). The effect of protozoa on the bacterial composition and hydrolytic activity of the roe deer rumen. Animals, 10:467-476.

Muchlas, Kusmartono, M. and Marjuki. (2014). Pengaruh penambahan daun pohon terhadap kadar VFA dan kecernaan secara *in vitro* ransum berbasis ketela pohon. Jurnal Ilmu-Ilmu Peternakan, 24:8-19.

Newbold, C. J., de la Fuente, G., Belanche, A., Ramos-Morales, E. and McEwan, N.R. (2015). The Role of Ciliate Protozoa in the Rumen. Frontiers in Microbiology, 6:1313-1327.

Purbowati, E., Rianto, E., Dilaga, W. S., Lestari, C. M. S. and Adiwinarti, R. (2014). Karakteristik cairan rumen, jenis, dan jumlah mikrobia dalam rumen sapi Bali. Jurnal Buletin Peternakan, 38:21-26.

Rosmalia, A., Permana, I. G. and Despal, D. (2022). Synchronisation of rumen degradable protein with non-fibre carbohydrate on microbial protein synthesis and dairy ration digestibility. Veterinary World, 15:252-261.

Sairullah, P., Chuzaemi, S. and Sudarwati, H. (2017). Effect of Flour and Papaya Leaf Extract (*Carica papaya* L) in feed on ammonia concentration, volatile fatty acids and microbial protein synthesis In Vitro—Journal of Tropical Animal Production, 17:66-73.

Sulistyowati, E., Wiryawan, I. K. G., Badarina, I., Naibaho, S. H., Apreza, Pratama, W. A., Cahyadi, C., Sistanto. and Waspodo, R. J. (2025). Feeding diet containing concentrate with fermented *Arenga pinnata* by-product on lactating dairy cows' nutrient intakes, digestibility, and milk quality. International Journal of Agricultural Technology, 21:713-724.

Sulistyowati, E., Wiryawan, I. K. G., Badarina I. and Romdhoni, M. A. (2024). *In Vitro* Characteristics of a concentrate containing fermented *Arenga pinnata* by-product. The 3rd Iseprolocal (International Seminar on Promoting Local Resources for Sustainable Agriculture and Development). Proceeding. In process.

Sulistyowati, E., Badarina, I. and Mujiharjo, S. (2022). *In vitro* characteristics of concentrate containing different levels of *Durio zibethinus* Murr rind flour fermented with *Pleurotus ostreatus.* IOP Conference Series: Earth and Environmental Science, 1:1-7.

Sulistyowati, E., Badarina, I. and Mujiharjo, S. (2020). *In Vitro* Characteristics of concentrate containing different levels of *Durio zibethinus* Murr rind flour fermented with *Pleurotus ostreatus*. The 4th Animal Production International Seminar. IOP Conf. Series: Earth and Environmental Science, 478:012022.

Sulistyowati, E., Badarina, I., Sucianti, H., Hartono, R. and Mujiharjo, S. (2016). Improved Nutrient Contents of *Durio zibethinus* Murr Rind Powder Fermented with *Pleurotus ostreatus* and Its Addition in PUFA- Concentrate. Jurnal Sain Peternakan Indonesia, 11:9-16.

Sulistyowati, E., Sudarman, A., Wiryawan, K. G. and Toharmat, T. (2014). *In vitro* goat fermentation of PUFA-diet supplemented with yeast and *Curcuma xanthorrhiza* Roxb. Jurnal Media Peternakan, 37:175-181. DOI: 10.5398/medpet.2014.37.3.175

Suryani, N. N., Budiasa, M., Ketut, I., Astawa, A. and Putu, I. (2014). Fermentasi rumen dan sintesis protein mikroba kambing Peranakan Ettawa yang diberi pakan dengan komposisi hijauan beragam dan level konsentrat berbeda. Majalah Ilmiah Peternakan, 17:56-60.

Suwandyastuti, S. N. O. and Rimbawanto, E. A. (2015). Produk metabolisme rumen pada sapi perah laktasi. Jurnal Agripet, 13:31-35.

Suwarno, J. (2008). Pengaruh Rasio Pemberian Pakan yang Berbeda terhadap Produksi VFA dan NH3 Rumen serta Kapasitas Lambung Domba Jantan Lokal. (Skripsi). Fakultas Peternakan Institut Pertanian Bogor, Bogor. Indonesia.

Tandang, M. D., G. Oematan, G. and Lestari, G. A.Y. (2024). Pengaruh lama waktu fermentasi terhadap kandungan asam phytat, kecernaan bahan kering dan bahan organik dedak padi secara *in vitro*. Animal Agricultura, 2:452-463.

Tilley, J. M. A. and Terry, R. A. (1963). A two-stage technique for the in vitro digestion of forage crops. Grass and Forage Science, 18:104-111.

Tjokrokusumo, D., Widyastuti, N. and Giarni, R. (2015). Diversification of processed products of oyster mushroom (*Pleurotus ostreatus*) as healthy food. Seminar Nasional Masyarakat Biodiversitas Indonesia, 1:2016-2020.

Tuwaidan, N. W. H., Sondakh, E. H. B. and Kaunang, C. L. (2024). Strategi mitigasi gas metan pada ternak ruminansia. Zootec, 44:148-173.

Usboko, M. Y. G., Enawati, L. S. and Maranatha, G. (2024). Pengaruh Imbangan Silase Rumput Kume (*Sorghum plumosum var timorense*) dan *Alysicarpus vaginalis* yang Berbeda Terhadap pH, Konsentrasi NH_3 dan VFA residu fermentasi *in vitro*. Animal Agriculture, 1:214-220.

Wea, L., Hartati, E. and Saleh, A. (2015). Pengaruh penggunaan kulit buah kopi terfermentasi *Trichoderma reesei* yang ditambah Zn-Cu isoleusinat terhadap produksi amonia dan volatile fatty acids secara *in vitro*. Jurnal Nukleus Peternakan, 2:1-7.

Wijayanti, E., Wahyono, F. and Surono, S. (2012). *In vitro* digestibility and fermentability of nutrients of complete feed with different levels of bagasse. Animal Agriculture Journal, 1:167-179.

Witno, W., Yumna, Y. and Baso, H. S. (2024). Pengolahan Limbah Ampas Sagu sebagai Pakan Ikan oleh Kelompok Pokdakan Bamba di Desa Walenrang. Jurnal Pengabdian pada Masyarakat, 9:79-91.

Wole, B. Y., Manu, A. E. and Enawati, L. S. (2018). Fermentasi jerami kacang hijau menggunakan cairan rumen kambing dengan waktu yang berbeda terhadap konsentrasi NH3 dan VFA secara *in vitro*. Jurnal Nukleus Peternakan, 5:1-6.

Yakin, E. A. and Mulyono, A. M. W. (2017). Pengaruh lama fermentasi terhadap aktivitas enzim dan lignin pada proses fermentasi kulit buah kakao menggunakan kapang (*Phanerochaete chrysosporium).* Jurnal Ilmu-Ilmu Pertanian, 1:152-157.

Yanuartono, Nururrozi, A., Indarjulianto, S. and Purnamaningsih, H. (2019). The role of protozoa in ruminants and its impact on the environment. Journal of Tropical Animal Production, 20:16-28.

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