Research Article

The Effect of Swamp Forages Combination in Rations on Rumen Fermentability Characteristics and *In Vitro* Methane Production

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Abstract | Swamp forage is an alternative feed for ruminants due to as a source of nutrients and phytochemicals (tannins and saponins), including water mimosa, giant molests, and water chestnuts. The objective of this study was to evaluate the effect of swamp forages combination in the ration on the characteristics of rumen fermentability and methane production. The design used was completely randomized design (CRD) with 4 treatments and 4 replications. The treatments given were A (guinea grass and concentrate, 7:3), B (guinea grass, water mimosa and concentrate, 4:3:3), C (guinea grass, giant molest and concentrate, 4:3:3), D (guinea grass, water chestnut, and concentrate, 4:3:3). The value of dry matter digestibility (DMD), organic matter digestibility (OMD), pH, N-Ammonia (N-NH_3), total volatile fatty acids (TVFA), partial VFA, and methane production, total bacteria, and protozoa were the variables observed. The results showed that the combination of swamp forage could increase ($p<0.05$) DMD, OMD, TVFA, partial VFA, and total bacteria, while N-NH_3, methane production, and protozoa decreased. It was concluded that the combination of water mimosa in the ration was the best composition in increasing DMD, OMD, total bacteria, and rumen fermentability and reducing methane production.

Keywords | Guinea grass, *In vitro*, Methane production, Phytochemicals, Swamp forage

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INTRODUCTION

The strategy in ruminant feed management is determined by the quality and quantity of forage provided so that it contributes to livestock productivity. The problem that occurs is that in general, ruminant livestock businesses are kept traditionally only relying on natural forage. Natural forage available fluctuates depending on the season, is low quality, and contains high methane gas, causing a lot of energy loss. Ideally, the feed given to support the productivity of ruminants is feed that contains balanced nutritional content, so that it can meet the requirement of rumen microbes and animal hosts (Adegoke and Abioye, 2016; McDonald et al., 2010). Swamp forage can be used as an alternative feed source to overcome this problem. Rao et al. (2015) stated that local forage supplements in cattle production systems in the tropics can improve the quality and characteristics of rations and can contribute to rumen microbial activity, reduce methane gas emissions, and improve ruminant performance.

High productivity swamp forages, potentially as a source of phytogenic feed, such as water mimosa, giant molest, and water chestnut so that they can improve rumen characteristics, nutrient availability and reduce methane emissions. Magdalene et al. (2013) reported that several phytogenic active components that affect the digestibility value of the diet, rumen ecology, and livestock performance include essential oils, flavonoids, saponins, and tannins. Chanwitheesuk et al. (2005) reported the phytochemical content of water mimosa; tannin 2.10%, total phenolic 10.4%, total carotene 0.32%, total xanthophyll 0.106%, vitamin C 1.29%. Meanwhile, Rosyidah et al. (2018) stated that water chestnut contains phenolic compounds, flavonoids, tannins, and terpenoids. Baehaki et al. (2018) reported that giant molest contains phytochemical compounds, namely tannins, saponins, steroids, and triterpenoids. Tan et al. (2011) proved that the addition of lamtoro leaf extract (10 - 30/500 mg) which contained pure condensed tannins, showed that the higher the addition of pure condensed tannins, the lower the emission of methane, TVFA, protozoa populations, and methanogenic bacteria. Patra et al. (2012) stated that the addition of saponins in the ration can increase the population of fiber-digesting bacteria thereby increasing the digestibility of the feed. Furthermore, Zain et al. (2020) stated that the effect of tannins and saponins on the legume *Gliricidia sepium* can reduce methane gas emissions and increase rumen fermentation efficiency and feed digestibility. Valenzuela-Grijalva et al. (2017) reported that the main concept of the use of phytogenic supplementary feeds in ruminants is the ability of phytogenic feeds to modify the rumen ecosystem, so increase the microbial population, improve rumen fermentability characteristics and livestock productivity.

Based on the potential of swamp forage, it is important to conduct research on the effect of the combination of swamp forage in cattle rations on digestibility, rumen fermentability characteristics, and methane emissions. The objective of this study was to evaluate the effect of swamp forages combination in the ration on the characteristics of rumen fermentability and methane production.

MATERIALS AND METHODS

The swamp forage used consisted of water mimosa, giant molests, and water chestnuts obtained from a swamp area of Tanjung Pering Village, Ogan Ilir Regency, South Sumatra Province, Indonesia, while guinea grass came from the collection garden of the Animal Science Department, Sriwijaya University. Analysis of chemical content and *in vitro* digestibility was carried out at the Feed Nutrition Laboratory, Faculty of Agriculture, Sriwijaya University.

This research used a completely randomized design with 4 treatments and 4 replications. The treatments given were A (guinea grass and concentrate, 7:3), B (guinea grass, water mimosa and concentrate, 4:3:3), C (guinea grass, giant molest and concentrate, 4:3:3), D (guinea grass, water chestnut, and concentrate, 4:3:3).

Chemical quality analysis of feed

Leaf parts of swamp forages were washed with running water and then dried for three days in an oven (60 °C) and ground to pass through a 0.5 mm sieve. Measuring the levels of tannins and saponins in the sample using methanol solvent, put 10 ml of methanol solvent and 0.5 g of plant material into a test tube. Then the tubes were stored at room temperature for 20 minutes. Each sample was centrifuged (Beckman coulter safety centrifuge, microfuge 20R tube uk.1.5 ml) for ten minutes at 3000 rpm, this procedure was carried out twice, then the supernatants obtained were combined and the concentrations of tannins and saponins were measured (Jayanegara et al., 2014; Makkar, 2003).

Measurement of nutrient content was based on proximate analysis according to the procedure (AOAC, 2010) and fiber fraction (Van Soest, 1994), using mashed feed ingredients consisting of guinea grass, water mimosa, giant molest, water chestnut and concentrate (according to treatment). The nutritional content of feed ingredients and treatment rations is shown in Tables 1 and 2.

Table 1: Nutritional composition of Guinea grass (GG), water mimosa (WM), giant molest (GM), water chestnut (WC) and Concentrate (% DM).

Nutrient	GG	WM	GM	WC	CON
Dry matter (DM)	80.24	86.69	82.56	84.38	80.13
Organic matter (OM)	78.52	80.21	78.23	79.31	71.63
Crude protein (CP)	10.25	24.36	19.51	9.28	18.02
Crude fiber (CF)	27,85	16.93	20.25	25.47	10.24
Ether extract (EE)	1.40	2.27	2.51	2.01	8.27
Nitrogen free extract (NFE)	38.48	36.26	35.96	42.55	35.45
TDN	57.86	70.38	66.36	59.89	79.38
Neutral detergent fiber (NDF)	62.83	49.62	68.71	67.43	37.18
Acid detergent fiber (ADF)	43.52	25.51	50.89	48.26	20.85
Hemicellulose	19.31	24.11	17.82	19.17	16.33
Cellulose	33.41	14.26	27.52	26.51	16.64
Lignin	12.85	11.32	23.16	21.67	4.32
Tannin	0.15	2.63	3.04	3.16	Nd
Saponin	1.03	5.81	4.78	3.68	Nd

In vitro Fermentation Simulation Based on the procedure of Tilley and Terry (1963). Rumen fluid for *in vitro* fermentation was obtained from buffalo fistulas that had been fed (70% guinea grass and 30% concentrate). The fermentation medium used consisted of 1 gram of substrate, 10 mL of rumen fluid, and 40 mL of buffer solution (McDougall), then put into a fermenter tube (100 mL). The temperature was maintained at 39°C, before being closed, and CO_2 gas flowed for 30 seconds so that the conditions remained anaerobic. Then incubated for 24 hours, after the incubation time was completed two

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drops of $HgCl_2$ were added to stop microbial activity, and to separate the supernatant and residue, the material was centrifuged at 4000 rpm for 10 minutes. The supernatant was used for the analysis of concentrations of N Ammonia ($N\text{-}NH_3$), total volatile fatty acids (TVFA), partial VFA, and calculation of total bacteria and protozoa.

Table 2: Chemical compositions of the experimental diets (% DM).

Item	Diet			
	A	B	C	D
DM	80.21	82.14	80.91	81.45
OM	76.45	76.96	76.37	76.69
CP	12.58	16.81	15.36	12.29
CF	22.56	19.28	20.28	21.84
EE	3.84	4.07	4.01	3.86
NFE	37,51	36.91	36.82	38.79
TDN	64.32	68.07	66.87	64.93
NDF	55.14	51.17	56.90	56.52
ADF	36.72	31.32	38.93	38.14
Hemicellulose	18.42	19.82	17.97	18.37
Cellulose	28.38	22.63	26.61	26.31
Lignin	10.29	9.83	13.38	12.94
Tannin	0.11	0.85	0.97	1.01
Saponin	0.72	2.16	1.85	1.52

Source: Laboratory of Nutrition and Animal Feed, Faculty of Agriculture, Sriwijaya University, 2021.

Determination of N-NH3 Concentration in Conway modified according to General Laboratory Procedures (1966); Partial VFA content was analyzed using Gas-Liquid Chromatography (AOAC, 2010). Protozoa counting was carried out with a Counting Chamber under a microscope (40×), while total bacteria followed Ogimoto and Imai (1988) procedure. Add 50 mL of pepsin-HCl 0.20% to the incubation residue and incubate for 48 hours. Determination of dry matter digestibility (DMD) and organic matter digestibility (OMD) was carried out by filtering the solution using Whatman No. filter paper. 41, then dried at 60 °C for 48 hours (AOAC, 2010). According to Moss et al. (2000) that methane gas production can be estimated from partial VFA (CH_4=0.45 C_2–0.275 C_3+0.40 C_4).

Data analysis

The data obtained (DDM, ODM, pH, $N\text{-}NH_3$, TVFA, partial VFA, C2/C3 ratio, methane gas, total bacteria, and protozoa) were analyzed by ANOVA and Duncan Multi Range Test, using SPSS 13.0 software.

RESULTS AND DISCUSSION

DMD and OMD

The combination of swamp forages had a significant effect ($p<0.05$) on the value of DMD and OMD is presented in Table 3.

Table 3: Effect of combinations of swamp forage in rations on the digestibility of dry matter (DM) and organic matter (OM).

Treatment	Digestibility	
	DM (%)	OM (%)
A	54.29±0.61[a]	63.91±0.33[a]
B	57.50±0.83[c]	67.58±1.18[c]
C	56.13±0.48[b]	66.23±0.46[b]
D	54.42±0.80[a]	63.99±0.46[a]

Note: A (Guinea grass: Concentrate, 7:3), B (Guinea grass: water mimosa: Concentrate, 4:3:3), C (Guinea grass: giant molest: Concentrate, 4:3:3), D (Guinea grass: water chestnut: Concentrate, 4:3:3). Different superscripts in the same column indicate significant differences (P<0.05).

Post hoc test results showed that the highest DMD and OMD were found in B, namely 57.50% and 67.58%, this indicated that there was an optimal effect of nutrient and phytochemical content (tannins and saponins) in water mimosa (Table 1) to increase the DMD and OMD of rations. This finding is in line with Zain et al. (2020), who stated that the effect of tannins and saponins on the legume *Gliricidia sepium* can increase rumen fermentation efficiency and feed digestibility. Wahyuni et al. (2014) stated that the addition of 1% tannins and 0.6% saponins to feed improved DMD and OMD. The effect of natural saponins has also been observed by Wanapat et al. (2013) that saponins can select several species of bacteria in the rumen by increasing or inhibiting their growth. Furthermore, Patra et al. (2012) stated that the addition of saponins can change the rumen bacterial community by increasing the population of *Prevotella*, *F. Succinogenes*, and *Ruminococcus* flavefaciens selectively and significantly so that it has an impact on increasing feed digestibility..

pH, $N\text{-}NH_3$ and total VFA

The combination of swamp forages had a significant effect ($p<0.05$) on the value of $N\text{-}NH_3$ and total VFA, while pH had no significant effect ($p>0.05$) is presented in Table 4.

Table 4: Effect of combinations of swamp forage in rations on pH, $N\text{-}NH_3$ and TVFA.

Treatment	pH	$N\text{-}NH_3$(mM)	TVFA (mM)
A	6.62±0.18	9.92±0.17[d]	120.25±6.85[a]
B	6.64±0.15	9.30±0.54[c]	137.16±2.25[c]
C	6.71±0.06	7.69±1.75[b]	120.75±2.10[b]
D	6.72±0.61	6.81±0.81[a]	121.95±6.53[b]

Note: A (Guinea grass: Concentrate, 7:3), B (Guinea grass: water mimosa: Concentrate, 4:3:3), C (Guinea grass: giant molest: Concentrate, 4:3:3), D (Guinea grass: water chestnut: Concentrate, 4:3:3). Different superscripts in the same column indicate significant differences (P<0.05).

Rumen pH

The results showed that the treatment of different swamp forages combination could maintain an ideal rumen pH for rumen microbial growth. It was seen that normal rumen pH ranged from 6.62 to 6.72 so it could support microbial growth in the rumen. The results of this study are in line with the research of Nurhaiti and Hidayah (2021) which stated that supplementation of tea leaf saponins in fermented palm fronds produced a pH value ranging from 6.65 to 6.73. Barber et al. (2010), reported that the normal condition of the rumen pH was 6.2-7.00. Furthermore McDonald et al. (2010) reported that rumen pH was maintained (normal) due to a balance of VFA and N-NH3 fermentation products.

N-NH_3

The results showed that all treatments contributed to a decrease in N-NH_3 levels compared to control (A), the lowest content was found in treatment D at 6.81 mM, while the highest was in A at 9.92 mM. Ammonia is produced from protein degradation by rumen microbes, the ammonia produced will be used for microbial protein synthesis (McDonald et al., 2010). The control treatment (A) had the highest ammonia content, this indicated that a small amount of ammonia was used by rumen microbes for their growth. Increasing levels of tannins and saponins in swamp forages cause a progressive decrease in N-NH_3. Tannins and saponins in reducing ammonia in the rumen have different mechanisms, administration in combination provides a more intensive effect. Tannins can slow down the rate of deamination degradation of amino acids into ammonia by binding to protein molecules (Jayanegara et al., 2015). The effect of saponins on lowering ammonia levels is mainly due to their direct antimicrobial effect such as antiprotozoal, saponins also inhibit rumen proteolytic bacteria (Jayanegara et al., 2014).

Jayanegara et al. (2015) stated that tannins and saponins can prevent protein degradation (protein by-pass) thereby contributing to an increase in protein supply in the small intestine and the performance of ruminants. Research by Liu et al. (2019) reported that the addition of tea leaf saponins 2 g/head/day in ewe reduced rumen N-NH3 concentrations compared to the control. The ammonia levels from this study met the optimal limit for N-NH3 requirements for microbial protein synthesis, according to Yuan (2010) reported that the optimum ammonia levels for rumen microbial growth ranged from 4 to 21 mM.

Partial VFA, C2/C3 ratio and Methane Gas

The combination of swamp forages had a significant effect ($p<0.05$) on the value of partial VFA, C2/C3 ratio, and methane gas is presented in Table 5.

Partial VFA

Acetic acid (C2), propionate (C3), and butyrate (C4) are the end products of the structural and non-structural fermentation of carbohydrates by microorganisms in the rumen. This partial VFA is also produced by the fermentation of feed proteins (McDonald et al., 2010). The proportion of acetic acid in A 67.65%, B at 57.53%, C at 60.79%, and D at 61.06% while the proportion of propionic acid produced at A at 18.66%, B at 28.06%, C at 24.96 %, and D 25.42%. The research results of Nurhaiti and Hidayah (2021) reported that the supplementation of tannins and saponins derived from tea leaves in fermented palm fronds obtained acetate proportion values ranging from 61.66% to 66.73% and propionate 15.91% to 19.94%. Acetic acid is the final product of fiber fermentation, while the end product of sugar and starch fermentation produces propionic acid.

C2/C3 ratio

The combination of swamp forages showed a decrease in the C2/C3 ratio in the rumen, the lowest C2/C3 ratio was found in treatment B at 2.05, while the highest was in treatment A at 3.62. Treatment C was not significantly different from D. The low value of the C2/C3 ratio in B is caused by the effect of phytogenic feed supplementation from swamp forages which stimulates starch-degrading bacteria to form propionic acid, thus allowing the formation of propionic acid to be higher than acetic acid. Castro-Montoy et al. (2011) in a study using Quebracho, mimosa, and tannin feed showed a significant effect on decreasing the proportion of acetate and the C2:/C3 ratio while the proportion of propionate increased descriptively. Furthermore, Jayanegara et al. (2020) reported that

Table 5: Effect of combinations of swamp forage in rations on VFA partial, C2/C3 ratios and Methane gas.

Treatment	C2 (mM)	C3 (mM)	C4 (mM)	Ratio C2/C3	Methane gas (mM)
A	81.34±1.50^{c}	22.45±2.63^{a}	16.46±0.98^{a}	3.62±0.31^{c}	37.02±0.51^{b}
B	79.51±1.84^{b}	38.77±0.63^{c}	20.40±1.19^{c}	2.05±0.04^{a}	33.07±0.71^{a}
C	77/19±1.28^{a}	31.70±0.96^{b}	18.16±1.92^{b}	2.44±0.11^{b}	33.26±0.45^{a}
D	78.99±1.15^{b}	32.94±2.44^{b}	17.53±1.82^{ab}	2.41±0.21^{b}	33.50±0.52^{a}

Note: A (Guinea grass: Concentrate, 7:3), B (Guinea grass: water mimosa: Concentrate, 4:3:3), C (Guinea grass: giant molest: Concentrate, 4:3:3), D (Guinea grass: water chestnut: Concentrate, 4:3:3).). Different superscripts in the same column indicate significant differences ($P<0.05$).

supplementation with various proportions of tannin- and saponin-rich plant extracts in the diet (70% Napier grass and 30% concentrate) resulted in a decrease in the C2/C3 ratio. The value of the ratio C2/C3 has an important meaning in ruminology, a low ratio value will stimulate the formation of body fat (fattening). Propionic acid is glucogenic, it is the main precursor for blood glucose formation (McDonald et al., 2010). In the A treatment with a high C2/C3 ratio, it means that the microbial fermentation pattern leads to the formation of acetic acid, the types of microbes that play a role are cellulolytic and hemicellulolytic microbes.

Methane gas

The partial VFA profile can predict methane gas production through the levels of propionate, acetate, and butyrate produced from rumen fermentation (Jayanegara et al., 2015). Martin et al. (2009) stated that the formation of acetate and butyrate produces H_2, which provides an opportunity for methanogenic bacteria to convert it into methane. While the formation of propionate requires H_2 so that the formation of methane will be reduced, Furthermore, Walles et al. (2017) reported that the C2/C3 and methane ratios correlated positively, meaning that the higher the propionate produced or the lower the C2/C3 ratio, the lower the methane gas produced. The results showed that the combination of swamp forages caused a decrease in methane gas production ($p<0.05$). This was due to the lower treatment C2/C3 ratio compared to the control. The low amount of methane gas produced in the rumen can increase energy and feed efficiency so that it has a positive impact on livestock performance (McDonald et al., 2010).

The results of Hassanat and Benchaar (2013), found that the addition of up to 40% acacia (5% tannin), or quebracho (10% tannin) caused methane gas production to decrease compared to the control. According to Ramírez-Restrepoa et al. (2016) that the various effects of tannins on methane gas may be due to the chemical structure and concentration from which tannins are obtained. Furthermore, Jayanegara et al. (2020) reported that the addition of various proportions of tannin- and saponin-rich plant extracts to feed (70% Napier grass and 30% concentrate) reduced protozoa populations and methane production. Patra et al. (2012) argued the decrease is probably due to the inhibitory effect of methane production from methanogens, protozoa, and other hydrogen-producing microbes. The effect of saponins on methane gas was reported by Valenzuela-Grijalva et al. (2017) who observed that tea saponins could inhibit the activity of protozoa by affecting the integrity of cell membranes. It is known that methanogenesis has a positive correlation with protozoa so the biological properties of saponins can be used to suppress methane production, by reducing the number of rumen protozoa, and modulating rumen fermentation patterns (Kumar et al., 2014; Valero et al., 2014).

Total bacterial and protozoa

The combination of swamp forages had a significant effect ($p<0.05$) on the values of total bacterial and protozoa is presented in Table 6.

Table 6: Effect of combinations of swamp forage in rations on total bacterial and protozoa.

Treatment	Total bacterial (10^9cfu)	Protozoa (10^5cfu)
A	5.05 ± 0.41^a	5.56 ± 0.97^b
B	7.76 ± 1.19^c	3.92 ± 0.49^a
C	6.73 ± 0.85^b	4.16 ± 0.46^a
D	6.44 ± 0.26^b	4.43 ± 0.87^a

Note: A (Guinea grass: Concentrate, 7:3), B (Guinea grass: water mimosa: Concentrate, 4:3:3), C (Guinea grass: giant molest: Concentrate, 4:3:3), D (Guinea grass: water chestnut: Concentrate, 4:3:3). Different superscripts in the same column indicate significant differences ($P<0.05$).

Supplementation of phytogenic swamp forages in the ration increased the total population of rumen bacteria, this was evident from the high DMD value, the levels of NH_3 and total volatile fatty acids were still within optimal limits so that both were sufficient to support an increase in microbial protein synthesis. This increase in digestibility was in line with the increase in microbial protein synthesis (Zain et al., 2020). The high total number of treated bacteria (B) was due to the activity of the phytogenic feed which stimulated the rumen system thereby increasing the rumen microbial population. Besides that, the high total population of rumen bacteria was also followed by a low population of protozoa.

The results showed that phytogenic feed supplementation had an impact on reducing protozoa populations. This happens because the feed contains saponins which have a dead effect which allows protozoa cell lysis. The results of the study are in line with the findings of Ramírez-Restrepo et al. (2016) stated that the saponin content of tea leaves has the potential as an antiprotozoal. Saponins can react with the cholesterol-membrane of protozoa, which causes an increase in cell wall permeability, thus killing the protozoa (Wallace et al., 2017). Cieslak et al. (2014), the use of saponin sources derived from the roots of *S. officinalis* can reduce protozoa populations. finally, Nurhaita and Hidayah (2021) reported that supplementation of tea leaf powder up to 6% (1.43% tannins and 12.10% saponins) in Fermented Palm Leaves (FOPF) reduced the protozoa population.

CONCLUSIONS AND RECOMMENDATIONS

It was concluded that the combination of water mimosa in the ration was the best composition in increasing dry matter digestibility, organic matter digestibility, total bacteria, and rumen fermentability and reducing methane production. This research recommends a different combination of swamp forage should be tested in an *in vivo*.

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NOVELTY STATEMENT

We believe that this study is the first in Indonesia, evaluating the effect of phytochemicals from swamp forages in ruminant rations on digestibility, rumen fermentation characteristics and methane gas *in vitro*.

AUTHOR'S CONTRIBUTION

Riswandi: Initial concept, formulate, and research design.
Asep Indra M. Ali and Muhakka: Involved in conducting the experiment, supervising the trial design, and data analysis.
Afnur Imsya and Agus Wijaya: Contributed in checking the data analysis and revising the manuscript.
Finally, all authors have read and approved the final version of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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