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The Use of Water Hyacinth Leaves Supplementation in Ruminant Feed on Methane, Protozoa, VFAs and Fatty Acids Ruminal Fluid in Vitro

Erwin Hubert Barton Sondakh*, Jerry Kalele, Nancy Tuwaidan

Faculty of Animal Science, Sam Ratulangi University, Manado, Indonesia
***Email: ehb_sondakh@unsrat.ac.id**

Abstract. This study aims to determine the effect of water hyacinth leaves supplementation in ruminant feed parameters of fermentation, and fatty acids ruminal fluid in vitro. The experiment consists of six treatments, R0: no water hyacinth + 30% concentrate; R1: 1% water hyacinth leaves + 29% concentrate; R2: 2% water hyacinth leaves + 28%; R3: 3% water hyacinth leaves + 27% water hyacinth leaves; R4: 4% water hyacinth leaves + 26% concentrate; R5: 5% water hyacinth leaves + 25% concentrate. The fermentation was conducted at 39^{o}C for 72 hours using gas production technique. The results indicated that giving of 4% water hyacinth leaves and 26% concentrate of MCFA could reduce the number of protozoa and methane production ($P<0.05$) and increase propionate acid. It can be concluded that use of 4% water hyacinth leaves and 26% concentrate could reduce methane gas production and quantity of protozoa and also could increase propionate acid in rumen fermentation *in vitro*.

Keywords: Water hyacinth leaves, ruminant, methane, gas test, in vitro

Abstrak. Penelitian ini bertujuan untuk mengetahui pengaruh suplementasi daun eceng gondok pada pakan ruminansia terhadap parameter fermentasi dan asam lemak cairan rumen secara in vitro. Perlakuan dalam penelitian ini terdiri dari R0: daun eceng gondok + 30% concentrate; R1: 1% daun eceng gondok + 29% konsentrat; R2: 2% daun eceng gondok + 28% konsentrat; R3: 3% daun eceng gondok + 27% konsentrat, R4: 4% daun eceng gondok + 26% konsentrat; R5: 5% daun eceng gondok + 25% konsentrat. Setiap perlakuan menggunakan 70% rumput gajah. Fermentasi dilakukan menggunakan Hohenheim Gas Test (HGT) pada suhu 39^{o}C selama 48 jam. Pada akhir fermentasi dilakukan pengukuran gas metan, protozoa, VFA dan asam lemak cairan rumen. Data dianalisa menggunakan rancangan acak lengkap dan dilanjutkan dengan Duncan test. Hasil penelitian menunjukkan bahwa pemberian 4% daun eceng gondok dan 26% konsentrat dapat menurunkan produksi gas metan dan jumlah protozoa ($P<0.05$) serta meningkatkan asam propionat. Kesimpulan penggunaan 4% daun eceng gondok dan 26% konsentrat dapat digunakan sebagai pakan ternak ruminansia karena dapat menurunkan gas metan, protozoa dan meningkatkan asam propionat pada fermentasi in vitro.

Kata kunci: daun eceng gondok, ternak ruminansia, methane, gas tes, in vitro

Introduction

Animal husbandry is faced with two big challenges, namely how to reduce methane emissions from livestock and how to meet animal food needs, which includes the need for animal protein. According to Steinfeld et al. (2006), livestock contributes the most methane in the atmosphere, around 35%. The impact of the accumulation of methane in the atmosphere leads to global warming and climate change. There needs an effort to take action to reduce methane emissions produced by livestock.

The studies have been carried out to reduce methane emissions originating from livestock. Several ingredients can be used to reduce methane: coconut dregs as a defaunation agent and the results were able to reduce methane emissions by 17.38% (Sondakh et al., 2017), virgin coconut oil (Sondakh et al., 2015), fish oil (Sondakh et al., 2017). Although these studies have effectively reduced methane, they are challenging to implement because they use raw materials that compete with human needs. Apart from that, the use of oil in ruminant feed is minimal because oil can interfere with rumen bacteria, thus reducing feed digestibility. Several feed supplementations can reduce methane besides the use of fat. According to Aboagye and Beauchemin (2019), metabolite compounds such as tannin can reduce methane because of tannin's function as an antimicrobial.

Several plants contain tannin, including the water hyacinth plants (Eichornia crassipes). The disturbance caused by the water hyacinth plant is that it can spread over a large area and cover the surface of the water, it can reduce the light entering the water body which results in a reduction in the dissolved oxygen content in the water (Wijaya et al., 2015). If not controlled, it will affect the biota that lives in the lake. Rorong and Suryanto (2010) stated that water hyacinths contain high levels of tannin and saponin. Thus, water hyacinth can be used as ruminant feed to inhibit methane production in the rumen. Another benefit of tannin is that it can bind rumen protein and if this happens then tannin can be used to hold digested protein in the rumen and can function as rumen-undegraded protein (Zhou et al., 2019). Based on the background, this research seeks to measure rumen fermentation parameters using water hyacinth plant substrate by in vitro method.

Materials and Methods

The materials used in this research were rumen fluid, resazurin solution, buffer solution, standard solution, glass wool, vaseline, elephant grass, concentrate, and water hyacinth plants. The equipment included a fermenter, CO2tube, syringe, pipette (10-mL), phenoliect, spectrophotometer, gas chromatograph.

Method

The research treatment consisted of R0: no water hyacinth + 30% concentrate; R1: 1% water hyacinth leaves + 29% concentrate; R2: 2% water hyacinth leaves + 28% ; R3: 3% water hyacinth leaves + 27% water hyacinth leaves; R4: 4% water hyacinth leaves + 26% concentrate; R5: 5% water hyacinth leaves + 25% concentrate. Each treatment uses 70% elephant grass forage with 4-repetitions, and the analysis was done in a variance completely randomized design.

Procedure

The ruminal fluid of the ruminants was prepared using a fistula. The forage and concentrate substrates were put into a syringe according to the treatment. Insert 30 mL of rumen fluid mixed with elemental solution into the syringe, contains substrate for anaerobic fermentation at a temperature of 390C for 48 hours (Yusiati et al., 2008). After fermentation for 48 hours, the gas was taken from the syringe using a 10mL-pipette and put into phenoliect to analyze the gas components (Lopez et al., 1996). The fermentation liquid was filtered to separate undegraded feed ingredients, then centrifuged at 3,000 g speed for 15 minutes to separate the supernatant from precipitates.

The supernatant was taken and centrifuged again at 10,000 g for 15 minutes to separate the supernatant from the microbial sediment. The pH value was measured using a pH meter (AOAC, 2005). Protozoal numbers were counted (Diaz et al., 1993). VFA concentrations were determined using GC (Doreau et al., 1993). NH3 concentration was analyzed following Charney & Marbach (1962) method. Fatty acid profiles were analyzed with GC (AOAC, 2005).

Data Analysis

All research variables were analyzed in a completely randomized design and tested further using the polynomial orthogonal test (Steel and Torrie, 1980)

Result and Discussion

The data observations of the use of water hyacinth leaves on methane gas production, pH, protozoa, VFAs in vitro are presented in Table 1.

Methane

The results showed that water hyacinth leaves in the feed affected the formation of methane gas (P<0.01). Excessive water hyacinths would reduce the amount of methane gas. In this case, 4-5% water hyacinth leaves showed less methane gas production than 0-2% water hyacinth. Furthermore, 4% water hyacinth leaves plus 26% concentrate supplemented into sheep feed was sufficient to reduce methane gas by 9.53% compared to no water hyacinth leaves. If water hyacinth was increased to 5% with 25% concentrate in the feed formula, the methane content was reduced to 9.93%.

The decrease in methane gas was probably due to the role of tannins in water hyacinth leaves because tannin is a defaunation agent that can inhibit protozoa activity. According to Wahyuni et al., (2014) and Saminathan et al., (2017), tannins can decrease the number of protozoa in rumen fermentation in vitro. This is confirmed in the present study in which the number of protozoa decreased. Afzalani et al. (2019) stated condensed tannin extract from sengon leaf flour (*Albizia falcataria*) up to 2% can reduce methane gas production in vitro rumen fermentation. Defaunation of protozoa resulted in a decrease in methane production (Sondakh et al., 2015). Accordingly, tannins in feed substrates interfere with methane production as observed in vitro fermentation. Cieslak et al. (2016) stated that tannin can suppress methane production. The higher the water hyacinth leaves, the lower the methane production.

Number of Protozoa

The results observed a gap in the number of protozoa ($P<0.05$) in feed containing water hyacinth leaves compared to feed without water hyacinth during in vitro rumen fermentation. Supplementing 5% water hyacinth and 25% concentrate (R5) had the greatest effect on reducing the number of protozoa.

The number of protozoa in feed added with 0% - 2% was around 1,193 - 1,456 protozoa/mL, while the R4 and R5 treatments had no significant effect on the number of protozoa. The number of protozoa decreased along with increasing water hyacinth leaf content in the feed. The lowest number of protozoa in the R5 treatment was due to high levels of water hyacinth, namely 5%. Water hyacinth is seen as the cause of a decrease in the number of protozoa, and can be categorized as defaunation or anti-protozoa agent. According to Cieslak et al. (2017) and Rorong and Suryanto (2019), water hyacinth contains a lot of tannins which, according to Cieslak et al. (2017) and Macáková et al., (2014), can function as antiprotozoals.

Volatile Fatty Acids (VFAs)

The treatments did not have a significant effect, especially on acetate and butyrate, whereas only propionate showed an effect by the treatment ($p<0.05$). The addition of water hyacinth leaves caused a significant difference in the amount of propionate formed, where propionate increased when 4-5% of water hyacinth leaves were given. The difference in propionate profile was closely related to the water hyacinth content in the diet. When giving 4% water hyacinth leaves, there was an increase in the amount of propionate by 21.07% compared to without water hyacinth.

Table 1. The average of methane, pH, Protozoa, VFA given water hyacinth leaves in vitro

Parameters of fermentation	R0	R1	R2	R3	R4	R5
Methane (%)	42.26^{a}	41.53^{a}	41.17^{a}	40.45^{ab}	38.23^{b}	38.06^{b}
pH	6.87	6.89	6.85	6.87	6.87	6.89
Protozoa /mL	6745^{a}	6548^{a}	6482^{a}	6237^{ab}	5757^{bc}	5289^{c}
Acetate (µmol)	9.34	9.21	9.16	8.89	8.81	8.64
Propionate (µmol)	2.61^{a}	2.74^{a}	3.04^{a}	2.89^{a}	3.16^{b}	3.34^{b}
Butyrate (µmol)	0.76	0.79	1.23	0.64	0.94	1.46

Note: Different superscripts on the same row indicate significantly different ($P<0.05$)

Table 2. The average of fatty acid profile of rumen fluid given water hyacinth leaves

Fatty acids	Perlakuan					
	R0	R1	R2	R3	R4	R5
	---------------------- g/100 g crude fat ----------------------					
Laurate (C12:0)	0.26	0.21	0.14	0.32	0.14	0.23
Myristate (C14:0)	1.98	1.66	1,67	1.43	1.24	1.53
Palmitate (C16:0)	17.63	17.28	18.65	17.23	19.02	17.48
Stearate (C18:0)	26.35	26.42	28.33	29.64	28.67	27.23
Oleate (C18:1)	15.23	16.31	16,24	15.80	14.63	17.42
Linoleate (C18:2)	7.11	6.82	6.52	7.35	6.57	6.84
Linolenic (C18:3)	0.86	0.71	0.67	0.89	0.74	0.79
Total Fatty Acids	69.42	69.41	72.22	72.66	72.01	71.52
SFA	46.22	45.57	48.79	48,62	49.07	46.47
UFA	23.20	23.84	23.43	24.04	21.94	25.05

Note: SFA (saturated fatty acids); UFA (unsaturated fatty acids).

If the water hyacinth leaves were increased by 5%, the propionate would increase by 21.86%. According to Wei-lian et al. (2005), the total concentration of VFAs decreased due to defaunation, but there was an increase in molar propionate. The increase in propionate content is closely related to methane production in rumen fermentation

Propionate is formed from 2 pathways, namely through the acrylate and succinate pathways. Both pathways need hydrogen to form propionate (Martinko and Madican, 2005). Judging from the lack of methane formed (Table 1), a lot of hydrogen was used to form propionic acid. Meanwhile, the condition of acetic and butyric acid in the feed up to 5% water hyacinth has not caused changes in the content of these volatile acids.

Fatty Acids Profile of Rumen Fluid

The data observations of the use of water hyacinth leaves as a source of tannin on fatty acids profile are presented om Table 2. Table 2 illustrates the effect of giving water hyacinth leaves with different concentrations on the fatty acid profile of rumen fluid. Treatments given up to 5% had no effect on changes in rumen fluid fatty acids. Tannins can function as fat protection (Majewska et al., 2022). There was no change in rumen fluid fatty acids when water hyacinth leaves were added to the feed. Tannins in water hyacinth can inhibit the rate of fat biohydrogenation. The fat content of feed can be protected by the presence of tannin (Cappucci et al. 2021). According to Rufchaei et al. (2022), water hyacinth plants which contain tannins can protect fat and protein in the rumen. The presence of tannin causes no change in the fatty acid content of rumen fluid in rumen fermentation of both feed without water hyacinth and feed with a water hyacinth content of 1 - 5%.

Conclusions

It can be concluded that use of 4% water hyacinth leaves and 26% concentrate could reduce methane gas production and quantity of protozoa and increase propionate acid in rumen fermentation *in vitro*.

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