2021_NVEO

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Submission date: 07-May-2023 05:12PM (UTC+0700)
Submission ID: 2086377599
File name: In-Vitro-Rumen-Methanogenesis.doc (514K)
Word count: 3176
Character count: 17598

Nat. Volatiles & Essent. Oils, 2021; 8(6): 2081-2089

NVEO

Natural Volatiles &
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In Vitro Rumen Methanogenesis Inhibition Ability Of Brown Seaweed From Nusakambangan Coast, Cilacap, Indonesia

Caribu Hadi Prayitno[1], Nur Hidayat[2], Munasik[3]

Faculty of Animal Science, Jenderal Soedirman University, Indonesia

Abstract The objective of this study was to examine the effects of Sargassum sp. brown seaweed supplementation on methane gas production, methanogen, and total gas as the main indicator of anti methanogenesis agent. The materials used in this study: Sargassum sp. brown seaweed meal (from Nusakambangan Coast, Cilacap, Indonesia), sheep rumen fluid, and laboratory equipment. Completely Randomized Design (CRD) was used in the experimental research of 6 treatments, each with four replications. The applied treatments were PO = rumen fluid with basal feed (CP = 11.8% and TDN = 60%); PI = PO + 0.03% monensin; P2 = PO + 1% Sargassum sp.; P3 = PO + Sargassum sp.; P4 = RO + 3% Sargassum sp.; and P5 = RO + 4% Sargassum sp. The result of the experiment showed that the supplementation of Sargassum sp. decreased total gas, methane gas concentration and methanogen, increased dry matter digestibility and organic matter digestibility, and did not significantly affect (P>0.05) protozoa. Supplementation of 2% Sargassum sp. decreased methane gas by 82.95% and decreased methanogen by 75.17%. In conclusion, Sargassum sp. brown seaweed meal was an effective anti methanogenesis agent.

Keywords Sargassum sp., Anti-methanogenesis, Methanogen

1. Introduction

Global warming issue has been the concern of the global citizen because of the severe impact on human beings. Methane is a gas that contributes to global warming with 28-fold strength compared to C02. Some researchers from the livestock sector have attempted to reduce methane emission especially from the ruminants [1,2,3]. Methanogenesis in ruminant cattle is due to carbohydrate fermentation which turns into volatile fatty acid (VFA), C02 and H2 [4]. C02 and 1-12 resulted from fermentation are converted into methane by the methanogen. The present study investigated the potential of brown seaweed Sargassum sp as an anti-methanogenesis agent in sheep cattle. Phlorotannin in Sargassum sp will bind the methanogen protein-enzyme which then lyses the membrane cell of methanogen. This approach was conducted in vitro to identify

the ability of Sargassum sp. in inhibiting methanogenesis through the concentrate of total gas, methane gas, methanogen population, protozoa population and the end product of fermentation.

2. Materials and Methods

The materials used in this study were the ruminal fluid of sheep that had been adapted with Sargassum sp. seaweed meal for 1.5 months, Sargassum sp. seaweed meal, 0.03% monesin, field grass, concentrate as basal feed with 60:40 forage and concentrate. The equipment used consisted of a measurement set for total gas and methane gas (0,2,4,8,12,18,24,30,36 and 48 hours) that described by Inthapanya et al methanogen [61, optical microscope model CX23LEDRFS1, counting chamber Sedgewick-Rafter, incubator, and autoclave Prestige Medical 2100 Classic. Media Paynter and Hungate [7] (per 100 ml: 1.3 g agar; 0.05g KH2P04,• 0.05 g 1<2HP04; 0.05 g (NH4)2 SOG 0.10 g NaCl; 0.05 g MgS04; 0.013 g CaC12.2H20; 0.1% ml resazurin solution, and 70 ml aquadest, 30 ml rumen liquid, 0.5 g NaHCOg, 0.048 g NapSOg and 0.03 g L-metionin HCl), and ingredients for protozoa coloring (methylgreen formalin saline, per 100 ml 35% formaldehyde solution 10 ml; 90 ml aquadest; 0.03 g methyl green and 0.8 g NaCl).

Table 1. The composition of the experimental ration

No	Feedstuff	P0	P1	P2	P3	P4	P5
1	Field grass	60	60	60	60	60	60
2	Concentrate	40	40	40	40	40	40
	- Tapioca by-product	47.5	47.5	47.5	47.5	47.5	47.5
	Pollard	25	25	25	25	25	25
	- Coconut waste	7.72	7.72	7.72	7.72	7.72	7.72
	- Palm waste	11	11	11	11	11	11
	Soybean meal	1.57	1.57	1.57	1.57	157	1.57
	- Limestone	1.43	1.43	1.43	1.43	1.43	1.43
	- Salt	1	1	1	1	1	1
	Molasses	4.5	4.5	4.5	4.5	4.5	4.5
	Mineral	0.28	0.28	0.28	0.28	0.28	0.28
3	Sargassum sp.			1	2	3	4
4	Monensin		0.03				

A completely randomized design was used for six treatments each with four replicates.

The treatments consisted of PO = basal ration (CP = 11.8% and TDN = 60%), PI = PO + monensin 0.03%, P2 = PO + sargassum sp. 1%, P3 = PO + Sargassum sp. 2%, P4 = PO + sargassum sp. 3%, P5 = PO + Sargassum sp. 4%.

The data were analyzed using variant analysis with a completely randomized design. The significantly different result were fúrther analyzed using Duncan multiple range test (DMRT) at the levels of 5% and 1%

3. Findings and Discussion

The supplementation of Sargassum sp. Seaweed to sheep feed significantly increased the concentration of total gas. The effect of 48h observation indicated that P2 treatment (2% Sargassum sp.) was the most optimum treatment to inhibit the production of total gas. Ruminal carbohydrate fermentation produced several gasses including C02, CHS and H2 [3,9]. In contrast, Belanche et al [10] reported that 2% Ascophyllum nodosum (ASC) seaweed did not affect the total gas.

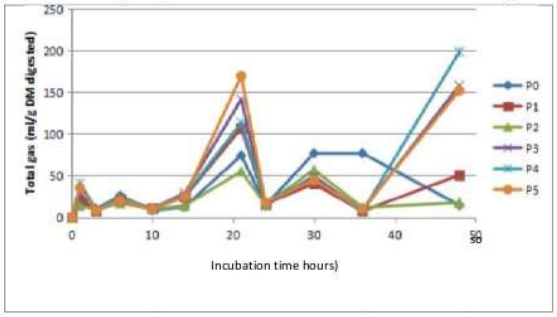

Figure 1. Total gas concentration during incubation time (0-48 h)

The result of this study showed that supplementation of 2% Sargassum sp in sheep feed observation at 4 hours decreased total gas by 25.76% and at the end observation (48 hours) decreased 11.02% of total gas. Level of Sargassum sp above 2% provide a response to total gas that varies. The supplementation of Sargassum sp. seaweed significantly affected the decrease of methane gas concentration. Furthermore, P2 treatment (2% Sargassum sp.) across the incubation hours (0,2, 4, 8,12, 18,24,30,36 and 48 hours) was the optimum level to inhibit methane gas production. The result showed that supplementation Sargassum sp. of 2% decreased methane gas by 88.83% at the 8th hour of observation and at the end of the observation period (48 hours) decreased 82.95%. The ability of Sargassum sp. to reduce methane gas was stronger than monensin (PI). The presence of phlorotanin from Sargassum sp. was thought to be able to suppress methanogen activity.

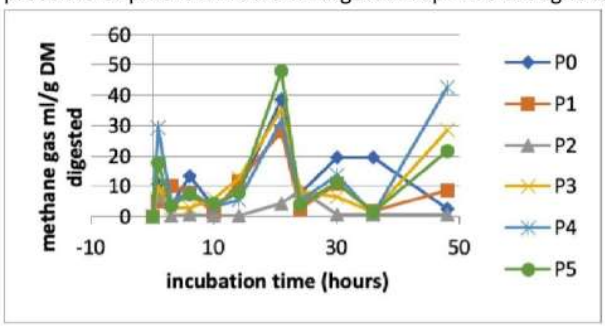

Figure 2. Methane gas concentration during incubation time (0-48h)

Mean and standard deviation of the total protozoa and methanogen and protozoa supplemented with Sargassum sp. are presented in Table 2.

Table 2. Total protozoa and methanogen in rumen fluid of sheep

Treatments	Protozoa (Log/ml) ^{ns}	Methanogen (x10^5 cfu/ml)*	Decrease of methanogen

			(%)
P0	3.41 ± 0,03	2.90[d] ± 3.00	-
		2.90[d] ± 3,00	
P1	3.35 ± 0,11	2.30[c] ± 4,24	20.68
		2.30[c] ± 4,24	
P2	3.43 ± 0,04	2.37[c] ± 1,26	18.28
P3	3.35 ± 0,05	0.72[a] ± 2,63	75.17
P4	3.31 ± 0,08	1.42[b] ± 3,30	51.03
	3.30 ± 0,05	2.80[d] ± 12,73	3.45

*

Note : ns = non significant, = significantly affect show significant difference (P<O,05)

4 values bearing different superscript within column

The graph of total methanogen in the study is presented in Figure 3.

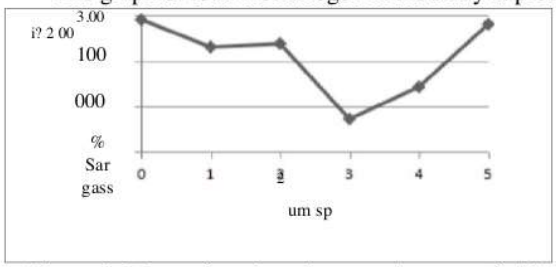

Figure 3. Mean of total methanogen in rumen fluid of sheep

15 The result of the present study showed that the supplementation of Sargassum sp. seaweed meal significantly affected (P<O.05) the decrease of total methanogen. On the contrary, Blanche et al [11] stated that the supplementation of 5% Ascophyllum nodosum brown seaweed did not affect the total methanogen in sacco. However, the result indicated that the supplementation of Ascophyllum nodosum could reduce the total methanogen by 89.90%, from 3.27 Log 103 x ACT with control feed to 0.33 Log 103 x ACT with 5% Ascophyllum nodosum. It indicated that phlorotannin in brown seaweed could affect the growth of methanogen. Shannon and Abdul-Ganam [12] stated that phlorotannin could bind bacteria protein like the enzyme produced by the bacteria and membrane cell that could produce lysis cells. Methanogen was expected to have the same reaction on phlorotannin as the other bacteria.

The result of study showed that the most significant decrease (75.17%) in total methanogen was obtained at 3% Sargassum sp. supplementation. Treatment with 0.03% monensin supplementation as the positive control could decrease 20.68% methanogen. It was assumed that 3% Sargassum sp. supplementation could lower more methanogen than the supplementation of 0.03% monensin. Morgavi et al. [13] stated that ionophore such as monensin was evidenced to inhibit the production of methane gas. Moreover, monensin can hinder Ruminococci but does not affect Fibrobacter succinogenes, so that monensin can be frequently used as anti-methanogen. Therefore, Sargassum sp. is the potential alternative to chemicals to decrease the amount of methanogen and methane gas emission.

The amount of methanogen in the treatment and the supplementation of 4% Sargassum sp. (P5) was relatively similar to the amount of methanogen in control feed (PO). It was expected that the higher the level of Sargassum sp., the lower phlorotannin efficacy to decrease methanogen, which indicated the change in ruminal microbe diversity. The higher the supplementation level, the lower the amount of fibrolytic which does not produce H2, and therefore the methanogen amount increased, It was in line with Mot-van et al. [141, that the population of fibrolytic bacteria had a positive correlation with the amount of methanogen in the rumen of several cattle such as cow and sheep. It was supported by Wang et al [151, that supplementing 500 ug/ml phlorotannin could affect the diversity of particular species of fibrolytic bacteria by decreasing the amount of Fibrobacter succinogenes

(H2-producing fibrolytic bacteria) but did not affect Ruminococcus flavefaciens (H2-producing fibrolytic bacteria). Morgavi et al [13] claim that increasing the number of bacteria that did not produce 1-12 like F. succinogenes could be the alternative to decrease the emission of methane gas without affecting fiber digestibility and to increase the efficiency of fiber digestibility my microbe. Patra et al [16] stated that the contributing factor to the ability of tannin to decrease the production of methane gas and the amount of methanogen was the difference in tannin structure where the condensed tannin had more significant potential. Jayanegara [17] stated that the structure of tannin compound is different across plant types which accounts for the non-toxic properties in several tannins at a certain level.

The result of the research indicated that the supplementation of 4% Sargassum sp. brown seaweed meal did not significantly (P>O.05) the population of protozoa. It was in contrast with the result of Belanche et al. [10] that the addition of 2% Ascophyllum nodosum brown seaweed in vitro could decrease protozoa activity up to 23%. Koivikko [18] informed that the abundance of phlorotannin in brown seaweed could reach 15% from the dry weight. The trend of total protozoa is presented in Figure 4.

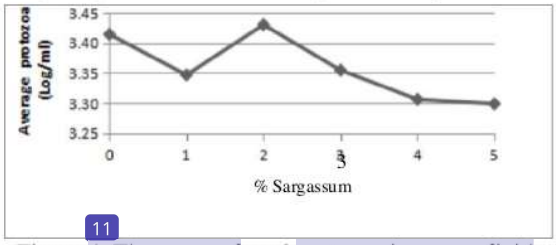

Figure 4. The mean of total protozoa in rumen fluid of sheep

Total dry matter of Sargassum sp. in this research was 8.[illegible]% and assumed to contain low phlorotannin and therefore less effective to decrease protozoa. Cahyaningrum et al [19] reported that the content of phlorotannin in seaweed Sargassum polycystum obtained from Poktunggal beach in Yogyakarta was 0.61±0.27 mg PG E/g extract. In this research, protozoa were presumed to adapt with the low phlorotannin.

The non-significant effect of the supplementation of Sargassum sp. on the population of protozoa may partly due to the 9-37% of protozoa that formed a symbiotic relationship with methanogen [4]. Phlorotannin does not explicitly lyse the membrane if protozoa like saponine The mechanism of phlorotannin is similar to that of tannin in the plant in the land regarding binding and sedimenting the protein of bacteria cell which causes bacteria lysis [12]. Similarly, Laia [201 reported that protozoa population was not the dominant factor in methanogenesis because only a small fraction of methanogen which forms a symbiosis with protozoa; therefore when an inhibitor agent is given to the methanogen, it has a non-significant effect on the number of protozoa.

The supplementation of Sargassum sp. brown seaweed on the concentration of Volatile Fatty Acids (VFA) varied across treatments. The mean VFA (Table 2) of treatments RI, R2, R3, R4 and R5 was 190.5 mM; 200.5 mM; 227.5 mM; 240.5 mM and 172 mM, respectively. The data showed that treatment R4 (3% Sargassum sp.) had the highest VFA and R5 (4% Sargassum sp.) had the lowest VFA. Qomariyah et al [21] stated that the increase of total VFA was affected by the fermentability of feed by ruminal microbe to degrade the feed. The higher the degradation of carbohydrate and protein infeed, the higher the VFA and ammonia. The result of the analysis of variance showed that the treatments had a significant effect.

Prayitno et al [3] informed that the optimum level of VFA concentration for ruminal microbe growth was 80 — 180 mM. In the present study, treatments RI, R2, R3 and R4 was above the threshold. RI consisted of 60% forage, and 40% concentrate feed without Sargassum sp. brown seaweed showed an excessive VFA. It indicated that treatment feed was easily fermentable in the rumen. Ramadhan et al [22] stated that the concentrate contained a highly digestible carbohydrate, so it was readily fermented by ruminal microbe into

The optimum level is presented from the curve graph (Figure 3). The polynomial orthogonal test indicated that 3% Sargassum sp. resulted in the highest VFA while the 2 % Sargassum sp. was the intersecting line between quadratic, cubic and quartic lines. The supplementation of 1-3% brown seaweed meal showed an increasing total VFA, partly due to the rising number of fiber-degrading bacteria because of the

supplementation of Sargassum sp. brown seaweed. Wang et al [151 informed that the addition of phlorotannin to cattle beef would affect the total bacteria and cellulolytic bacteria.

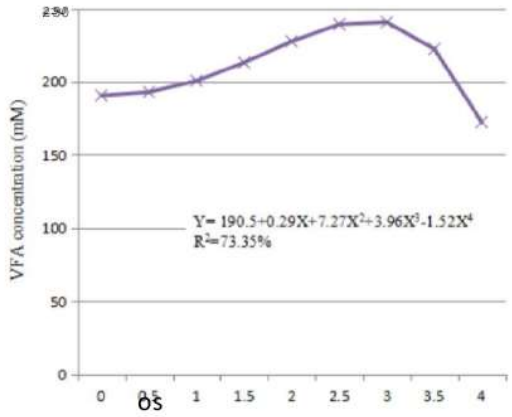

Figure 5. The curve of total VFA concentration (mM)

The highest production of VFA was at 2% supplementation of Sargassum sp. which decreased by 4% due to the decreasing activity of cellulolytic bacteria which lowered carbohydrate degradation. The supplementation of 2% Sargassum sp. was the optimum level because the range of normal VFA concentration in feed supplemented with 3% Sargassum sp. brown seaweed meal was not significantly different from that of 4%. Feed supplemented with 2% Sargassum sp. produced ±227S rnM total VFA. It was following the hypothesis that 2% Sargassum sp. supplementation could increase total VFA. Belanche et al [101 informed that in vitro analysis using the rumen liquid of Friesian-Holstein cow supplemented with Ascophyllum nodosum brown seaweed showed a total VFA which tended to increase although not significant. The result of the supplementation of Ascophyllum nodosum 0%; 0.5%; 2% was 78.7 mM; 79.7 mM; 80.6 mM and 82.1 mM, respectively.

4. Conclusion

Supplementation of 2 % brown seaweed (Sargassum sp.) in sheep feed was an effective anti methanogenesis agent as evidenced by the production of total gas, methane gas, methanogen population, and protozoa population. Supplementation of 2% brown seaweed (Sargassum sp.) in sheep feed produces optimal volatil fatty acids.

Acknowledgments

The authors thank the Higher Education, Directorate of Research and community Service, Ministry of Research, Technology and Higher Education, Republic of Indonesia for the research grant of Applied Research. Special thanks to Mrs Restu who provided valuable help in the Feedstuff Laboratory of Animal Science, Jenderal Soedirman University.

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