In Vitro Digestibility and Rumen Fermentation of *Sargassum* sp. Seaweed with Different Drying Methods and Palatability in sheep

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ABSTRACT
This study aims to determine the digestibility and rumen fermentation of seaweed flour from Sargassum sp. with different drying methods on the weaned thin tail rams. Seaweed flour Sargassum sp. were made with three drying methods, namely sun-drying, 55^0C oven and -20^0C freeze dryer. Rumen fluid from 2 Bali fistula cows, 5 weaning rams for palatability test were used in *in vitro* digestibility experiments. A completely randomized design with a factorial pattern with two treatment factors and 5 replications were used for Rumen fermentation test. The first factor was drying methods (S1: sun-drying, S2: oven-drying 55^0C and S3: freeze dryer -20^0C). The second factor was *Polyethylene glycol* (PEG: P0=without PEG and P1=addition of PEG). The results showed that the drying method of *Sargassum* sp. with the addition of PEG had a significant effect ($P<0.05$) on dry matter digestibility, NH_3 and CH_4 production, but had no effect ($P>0.05$) on the digestibility of organic matter pH, VFA and microbial protein synthesis. The highest dry matter digestibility was 82.41%; The highest NH_3 production was 28.14 mg / 100 ml; the lowest CH_4 production was 8.83% in sun-drying with the addition of PEG. The highest palatability value was 7.2 g in sun-dried *Sargassum* sp. Thus, the sun-drying method can be optimally utilized to evaluate *in vitro* digestibility and rumen fermentation and palatability in sheep.

Keywords: *Sargassum sp., rumen fermentation, digestibility, palatability*

1. INTRODUCTION

Seaweed is a macroalgae plant that has great potential as one of the raw materials to produce food, feed, chemicals, and energy which is always increasing throughout the world. Based on the colour pigments, seaweed consists of three types, namely red seaweed (*Rhodophyceae*), brown seaweed (*Phaeophyceae*), and green seaweed (*Chlorophyceae* [1]. *Sargassum* sp. is a species of brown seaweed that lives in temperate, subtropical, and tropical waters throughout the world [2]. In Indonesia *Sargassum* sp. spread throughout the waters from the waters of West Indonesia to the waters of East Indonesia [3].

Seaweed is generally high in minerals content compared to the carbohydrates and protein. Seaweed minerals were 10-20 times higher than that of plants on land with fat content 1-5% DM. Mineral in *Sargassum* sp. is 14-35% DM and crude protein is 6-11% [4]. Fresh seaweed contains 75-85% water and 15-25% organic and mineral components [5]. Fresh *Sargassum* sp. seaweed is easily damaged after a few days of harvesting. It is necessary to dry the *Sargassum* sp. after harvesting, and before undertaking the evaluation of *in vitro* digestibility and rumen fermentation as well as palatability in sheep. The purpose of drying is to reduce water activity, inhibit microbial growth, and help to maintain the quality and reduce storage volume [6].

Postharvest handling of *Sargassum* sp. include the drying method by sun-drying, oven-drying, and freeze-drying. Researches that have been done solely looking at the effect of sun-drying, oven-drying, or sun-drying and oven-drying; for example a study on the sun-drying

of *Sargassum* seaweed for sheep feed in Mexico [7]; oven-drying at 60°C for *in vitro* protein digestibility observations in India [8]. *Sargassum* flavicans seaweed was tested on its total gas and methane gas production *in vitro* method with freeze-drying -55°C for 48 hours in Townsville, Queensland, Australia [10]. Study on sun-drying, oven, and microwave) [11], and Sun-drying, oven, and freeze dryer of *Sargassum* hemyphyllum seaweed on proximate composition, macro, and micro mineral content, and vitamin C was also conducted in Tung Ping Chau, Northeastern Hong Kong. [12]. Additionally, Lalopua *et al.* [13] used red seaweed from Wael village, West Seram Regency, Maluku Province with sun-drying for 3 days on phenol content with the highest yield of hexane extract (15.93%). Based on these problems, this research was done to compare the methods of sun-drying, oven, and *freeze dr*yer for *Sargassum* sp. from Sepanjang Beach Gunungkidul Yogyakarta on *in vitro* digestibility, and rumen fermentation as well as palatability in weaned thin-tailed rams.

2. MATERIAL AND METHODS

2.1. Material

Sargassum sp. seaweed was taken from Sepanjang Beach Gunung Kidul Regency, Yogyakarta at low tide and then cleaned from dirt or other materials. The drying process of *Sargassum* sp. consisted of three methods. The first method was sun-drying for three days from 07.00 to 14.00 h. The second method was oven-drying at 55°C for four days. The third method was freeze dryer drying at -20°C for 21 hours. After drying, the *Sargassum* sp. was milled using a Willey mill with a diameter of 1 mm. Rumen fluid was taken from two fistula Bali cattle fed elephant grass (*Pennisetum purpureum*) and concentrated with 17% crude protein and 70% TDN composition. The concentrate given was from Puspeta with a crude protein content of 15.53% and TDN 52.07%. the chemicals for *in vitro* digestibility analysis were main element solution, trace element solution (trace), resazurin solution, buffer solution, reduction solution, and PEG (*Polyethylene glycol*). The palatability test of *Sargassum* sp. using 5 weaned male thin tails with an average body weight of 11.8 kg.

2.2. Methods

Experimental research on rumen fermentation and determination of *in vitro* gas digestibility test used a factorial completely randomized design with 2 factors and 5 replications. The two factors were the methods of drying seaweed *Sargassum* sp. (S) consisted of S1: sun-drying, S2: 55°C oven-drying, S3: *freeze dryer*-drying -20°C; and *Polyethylene glycol* (PEG) (P) which consisted of P0: without the addition of PEG P1: The addition of PEG with 36 tubes. Tools such as water bath and heater, thermometer, analytical balance with an accuracy of 0.001 g, magnetic stirrer, Erlenmeyer, micropipette, measuring cup, syringe, hose, candle, test tube, pH meter Hanna brand, centrifuge, funnel, oven 55°C, oven 105°C, crucible cup, glass wool, automatic pipette, 36 syringes, piston, and tube were all prepared beforehand. The method of sample preparation was the same with the second factor (the addition of PEG and without PEG); the only difference was the addition of 200 mg of PEG for each treatment.

Rumen fluid was taken at 06.30 AM before the fistulated Bali cattle were feed using a modified suction pipe with a hose then filtered with three layers of gauze. The filtered rumen fluid was then put in a thermos that has been previously given warm water with a temperature of 39°C to maintain aerobic conditions and then dispatched to the Animal Food Science Laboratory, Faculty of Animal Science, Universitas Gadjah Mada for subsequent tests.

Buffer solution was prepared by adding 474 mL of distilled water in an Erlenmeyer flask, 237 mL of buffer and macro minerals each, 0.12 mL of micro mineral solution and 1.22 mL of resazurin solution. The temperature of the solution mixture was measured with a thermometer at 38 to 39°C and then added to a reducing solution (2 mL NaOH, 285 mg Na_2S dissolved in 47.5 mL distilled water). After the color changed to silver, 474 mL of rumen fluid was added (ratio of distilled water and rumen fluid 1:1). The rumen fluid was then filled in an *in vitro* tube as much as 30 mL using an automatic pipette. Observations of gas production were carried out starting from 0, 2, 4, 8, 12, 24, 36, 48, and 72 hours. Gas accumulation was reduced when gas production had reached the maximum measurement limit[14].

2.2.1. Observation variable

DM and OM degradation. Blummel *et al.* [15] calculated dry matter degradation (DMD) and organic matter degradation (OMD) as follows:

$$\%\ \mathrm{DMD} = \frac{\mathrm{SDM} - (\mathrm{DMr} - \mathrm{Dumbo\ blank})}{\mathrm{SDM}} \times 100\% \quad (1)$$

$$\%\ \mathrm{OMD} = \frac{\mathrm{BOs} - (\mathrm{DMr} - \mathrm{Wra})}{\mathrm{SDM}} \times 100\% \quad (2)$$

Where: DMD = Dry Matter Degradation (%); DOM = Degradation of Organic Matter (%); SDM = Sample Dry Matter (mg); DMr = Dry matter residue (mg); SOM = Sample Organic Matter (mg); Wra = Weight of residual ash (mg); DMb = Dry Matter blank

The production of CH_4 gas at 72 hours carried out the release of gas and gas samples were taken with a plastic syringe as much as 10 mL to analyze the content of CH_4 gas, then the remaining gas was released.

Measurement of CH_4 gas using gas chromatography. *In vitro* pH measurement of rumen fluid used a digital Hanna pH meter that had been calibrated to pH 7 by dipping the pH

meter rod into the rumen fluid solution which would then be read the pH on the monitor screen. For each new rumen fluid pH measurement, the pH meter stem should be rinsed with distilled water [16].

Measurement of NH_3 content was done using spectrophotometry [17]. As much as 0.4 mL of rumen buffer fluid was taken after 72 hours of incubation plus 0.2 mL of solution A (10% Sodium tungstate) and 0.2 mL of solution B (H_2SO_4 1 N) then centrifuged at 3000 g for 15 minutes, then centrifuged again at 10,000 g for 10 minutes. Then as much as 10 µl of the supernatant was diluted it with 10 l of distilled water, add 2.5 mL of solution C (phenol solution) and 2.5 mL of solution D (5% sodium hypochlorite). The solution mixture was heated for 30 minutes at 40°C then the absorbance was read by spectrophotometry at a wavelength of 630 nm. Calculation of NH_3 levels using the standard curve equation Y=0.030X + 0.170 (R20=0.884), Y= Absorbance of the sample, and X = NH_3 content (mg/100 mL).

The VFA concentration was measured by the General Laboratory steam distillation method [10]**.** The procedure for measuring VFA was to prepare a distillation by boiling water into a cooler or condenser. Next, enter 5 ml of the sample and 1 ml of 15% H_2SO_4 into the distillation. VFA production was captured in an Erlenmeyer flask with 5 ml of 0.5N NaOH. The liquid was accommodated up to 250 ml, then 2 drops of phenolphthalein (pp) indicator were added and titrated using 0.5 N HCl. The calculation of total VFA production was:

$$\text{VFA Total} = \frac{(B-S) \text{ x Normality HCl x} \frac{1000}{5}}{\text{SDMg sample x DM sample}} \times 100\% \quad (3)$$

Where: B = Volume of blank titration, S = Sample titration volume.

Microbial protein synthesis. Identification of the microbes in the rumen fluid was measured using the modified stepwise centrifugation principle [18,19]. The rumen fluid sub-samples were filtered using two layers of gauze. An amount of 5 mL of rumen fluid was centrifuged at 3000 g for 15 minutes to separate rumen microbes and feed components. Next 1.5 mL of the supernatant was taken and placed it in an Eppendorf, and centrifuge again for 15 minutes at a speed of 10,000 g. Then the precipitation was taken and mixed with 15 mL NaOH and heated in a water bath while the water was boiling for 10 minutes, then removed and homogenized with a vortex mixer and then analyzed for protein content using the Lowry method with spectrophotometry [19].

Quantitative palatability test. Samples of *Sargassum* sp. seaweed flour of Sun-dried, oven, and freeze dryer were given as much as 100 g to each sheep (5 heads) with a feeding time of 30 minutes before the first feed were given in the morning, after they were being fasted for 4 hours. The fasting time was from 04.00 to 08.00 AM. The duration of administration was 30 minutes; and the observations were recorded. Then the next observation was one hour after the morning feed were given to the sheep. The observation procedure was the same as the initial observation. Observations were lasted for 3 days, for 3 treatments; every day one treatment of *Sargassum* sp. on the same sheep, so that each sheep experienced all treatments. Criteria of palatability were: Dislike (0) 0% when seaweed flour *Sargassum* sp. was consumed, slightly liked (-) <25% when *Sargassum* sp. seaweed flour were consumed, liked enough (+) 25-49% of *Sargassum* sp. seaweed flour were consumed, liked (++) 50-74% *Sargassum* sp. seaweed flour were consumed and very liked (+++) when 75 – 100% *Sargassum* sp. seaweed flour were consumed.

2.2.2. Statistical analysis

The data obtained were analyzed using analysis of variance (ANOVA) according to 3 x 2 factorial completely randomized design with SPSS (SPSS Windows version, release 22) [20]. Duncan's test (Duncan's New Multiple Range Test (DMRT) was carried out if a significant effect exist between treatments according to the instructions [21].

3. RESULTS AND DISCUSSION

3.1. Dry matter degradation

The results showed that *Sargassum* sp. by sun-drying. oven and freeze drying with and without the addition of PEG had a significant effect (P<0.05) on dry matter degradation (Table 1).

Table 1. Average dry matter degradation according to *Sargassum* sp. drying treatment, and with PEG

PEG	Drying of *Sargassum* sp.			Average
	1	2	3	
P0	52.03±9.66^{a}	71.56±5.50^{bc}	60.33±14.16^{c}	61.31±12.37
P1	82.41±3.81^{b}	46.26±22.80^{bc}	66.98±8.64^{a}	65.21±19.97
Average	67.22±6.74	58.91±0.87	63.65±11.14	63.26±16.17

$^{a.b.c.d.}$ Different superscripts in the same line were significantly different (P<0.05). Descriptions: 1 = Sun-dry. 2 = 55°C oven-dry 3 = *freeze- dry* -20°C. P0 = No PEG. P1 = Addition of PEG

Table 2. Average organic matter degradation according to *Sargassum* sp. drying treatment, and with PEG

PEG	Drying of *Sargassum* sp.			Average^{ns}
	1	2	3	
P0^{ns}	80.77±5.79	87.84±6.26	89.59±2.69	86.07±4.91
P1^{ns}	88.4±1.56	87.03±0.87	94.57±1.82	90.00±1.42
Average^{ns}	84.59±3.68	87.44±3.57	92.08±2.26	88.03±3.17

ns = not significant; Descriptions: 1 = Sun-dry; 2 = 55^{O}C oven-dry; 3 = *freeze- dry* -20^{O}C and P0 = No PEG; P1 = Addition of PEG

The high value of sun-dried dry matter degradation with the addition of PEG is thought to be due to PEG is able to inactivate tannins by forming a complex bond of tannins with PEG. Thus, tannins released bonds with several nutrients such as carbohydrates and proteins from *Sargassum* sp. so that they can be degraded by rumen microbes. PEG is a chemical that has a high affinity for tannins. PEG binds to tannins so they cannot react [22]. The result of this study found that dry matter degradation was lower than that of organic matter. This is due to the dry matter still contains ash while the organic material does not contain ash [23].

3.2. Degradation of organic matter

The results showed that *Sargassum* sp. by sun-drying, oven, and freeze drying with and without the addition of PEG did not affect (ns) on the degradation of organic matter (Table 1). The high degradation of organic matter in freeze dryer-drying is suspected to be the organic matter content of *Sargassum* sp. Freeze-drying remains stable or does not decrease. Chan *et al.* [12] stated that the seaweed *Sargassum hemiphyllum* freeze-drying has the best amount of nutrient content compared to sun-drying and oven-drying. The high organic matter degradation value above 80% is thought to be due to the high N-NH_3 content and carbon skeleton. N-NH_3 ranges from 217.7 to 281 mg/L so that it is optimal for microbial growth. The more microbes, the more degradation of organic matter. The degradation of organic matter is closely related to the degradation of dry matter. The results showed that the OMD was higher in the range of 80.77 – 94.57% than the DMD ranged from 52.03 – 82.41%. Setyadi *et al.* [24][24] reported a higher OMD value than DMD because dry matter still contains ash while organic matter does not contain ash. Garry *et al.* [23] that ash inhibits the degradation of feed dry matter.

3.3. Rumen fermentation profile

The results showed that *Sargassum* sp. flour by sun-drying, oven, and freeze drying with and without the addition of PEG had a significant effect (P<0.05) on the production of methane gas (CH_4). (Table 2). The low production of methane gas in the sun-drying treatment without PEG could be due to the low crude fiber content of *Sargassum* sp. sun-drying (6.67%) compared to the crude fiber content of oven-drying (8.76%) and freeze-drying (7.71%). The low gas production in the treatment of sun-drying, oven and freeze dyer without PEG is the same as the results of research by Niderkorn *et al.* [22]. It was expected that ruminants produce low methane gas so that a lot of energy from the carbohydrate metabolism process is utilized for the livestock production process and can reduce the contribution to

Table 3. *In vitro* rumen fermentation profile of *Sargassum* sp seaweed by sun-drying, oven, and freeze dryer with PEG and without PEG

PEG	Drying of *Sargassum* sp.			Average
	1	2	3	
Acetate (mM)				
P0	35.18±22.69	163.55±42.30	208.58±121.30	135.77±62.10
P1	40.15±10.15	55.51±23.03	95.09±53.70	63.58±28.96
Average	37.67±16.42	109.53±32.67	151.84±87.50	99.68±45.53
Propionate (mM)				
P0	18.36±12.09	87.30±21.40	120.46±76.03	75.37±36.51
P1	17.87±4.28	18.36±12.09	53.28±29.27	29.84±15.21
Average	18.12±8.19	52.83±16.75	86.87±52.65	52.61±25.86

ns = not significant; Descriptions: 1 = Sun-dry. 2 = 55^{O}C oven-dry 3 = *freeze- dry* -20^{O}C. P0 = No PEG. P1 = Addition of PEG

the accumulation of greenhouse gases in the atmosphere. Shinkai *et al.* [25] stated that methane gas from ruminants is not only a problem for the environment but the loss of 2-15 gross energy of feed that is not utilized for the production process.

Sargassum sp. seaweed. Sun-drying. oven and freeze dryer with and without the addition of PEG had no effect (ns) on the pH of the rumen fluid *in vitro*. The pH range of the treatment was at alkaline pH. presumably due to the activity of degradation of feed protein and non-protein nitrogen (NPN) by microbes. Santoso *et al*. [26] stated that the process of protein and amino acid metabolism results in the release of ammonium (NH_3) and carbon dioxide gas (CO_2) causing an increase in pH to become alkaline

Sargassum sp. seaweed. Sun-drying, oven, and freeze drying with and without the addition of PEG did not affect (ns) on acetate, propionate, butyrate, and VFA. The high concentration of VFA in *freeze*-drying without or with PEG could be due to the amount of nutrient content of *Sargassum* sp. in *freeze* drying remain stable. Chan *et al* [12] stated that the seaweed *Sargassum hemiphyllum* freeze-drying has the best amount of nutrient content compared to sun-drying and oven-drying. The concentration of sun-dried VFA with PEG as a result, of this study was higher (64.25 mM) than without PEG (60.43 mM), the same as the results study of the Niderkorn *et al*. [22]

Sargassum sp. seaweed by Sun-drying, oven, and freeze drying with PEG and without PEG had a significantly effect ($P<0.05$) on ammonia concentration. The value of low ammonia concentration in *Sargassum* sp. In *freeze dryer* drying, it was suspected that the ammonia production is widely used by microbes for protein synthesis in their bodies.

This is in accordance with the results of the highest VFA study in the freeze dryer drying treatment without PEG, namely 371.80 mM. Freeze dryer drying treatment at -20^{O}C results showed alkaline pH, lowest NH_3, and highest VFA compared to sun-drying treatment and 55^{o}C oven. Sretenovic *et al.* [27][28] stated that low ammonia concentration indicates that ammonia is widely used for rumen microbial growth. The optimum concentration of ammonia obtained in this study for rumen microbial growth was 21.78 ± 0.45 – 28.14 ± 1.75 mg/100 mL or equivalent to 217.8 – 281 mg/L. McDonald *et al*. [28] ammonia concentration for microbial growth ranged from 85 – 300 mg/L or equivalent to 2.7 – 14.3 mM rumen fluid.

Sargassum sp. seaweed by sun-drying, oven, and *freeze* drying with and without the addition of PEG had no effect (ns) on microbial protein synthesis. The high microbial protein in PEG sun-drying is thought to be due to low protection from the degradation process of feed protein by tannins and sources of N in rumen fluid to produce ammonia (NH_3) which is used for microbial protein synthesis. This is presumably because the tannin content in the sun-dried *Sargassum* sp seaweed is 0.77% (w/w) the lowest with *Sargassum* sp. freeze dryer-drying 1.22% (w/w) and oven-drying 0.89% (w/w). Niu *et al*. [29] stated that Polyethylene glycol (PEG) is a chemical that has a high affinity for tannins, and binds tannins so they cannot react.

Table 4. *In vitro* rumen fermentation profile of *Sargassum* sp seaweed by sun-drying, oven, and freeze dryer with PEG and without PEG

PEG	Drying of *Sargassum* sp.			Average
	1	2	3	
Butyrate (mM)				
P0	6.89±3.58	28.64±6.75	42.75±24.17	26.09±11.50
P1	6.23±1.11	8.66±3.27	23.65±11.96	12.85±5.45
Average	6.56±2.35	18.65±5.01	33.20±18.07	19.47±8.47
VFA (mM)				
P0	60.43±38.36	279.50±70.35	371.80±221.39	237.24±110.03
P1	64.25±15.53	82.53±36.21	172.02±94.93	106.27±48.89
Average	62.34±26.95	181.02±53.28	271.91±158.16	171.76±79.46
Microbial protein (mg/100 mL)				
P0	14.20±0.48	14.06±0.99	11.80±0.22	13.35±0.56
P1	15.71±0.73	15.14±0.46	11.89±0.20	14.25±0.46
Average	14.96±0.61	14.60±0.73	11.85±0.21	13.80±0.51

ns = not significant; Descriptions: 1 = Sun-dry. 2 = 55^{O}C oven-dry 3 = *freeze- dry* -20^{O}C. P0 = No PEG. P1 = Addition of PEG

3.4. The palatability test of Sargassum sp.

Palatability is a description of the nature of feed ingredients that are reflected organoleptically such as appearance, smell, texture, taste (bland, salty, sweet, bitter), and temperature that cause stimulation so that the attractiveness of livestock appears to consume it. Potential of *Sargassum* sp. by the sun drying method, 55°C oven and -20°C freeze drying as a mineral source of feed, can be seen at table 3. In addition to its potential availability and content of nutrients and secondary metabolites, it is necessary to know the level of preference of the livestock being tested for palatability.

The results of the observation of the palatability test of *Sargassum* sp. *seaweed* from sun-drying, oven, and *freeze drying* on weaned thin-tailed rams were the same, namely the amount of consumption was less than 25%. The average consumption of sun-drying before eating was 7 g, after eating 7.2 g; oven drying treatment before eating 5.0 grams, after eating 5.4 g, freeze dryer drying treatment before eating 6.4 g, and after eating 7 g. The average consumption of 25%, was suspected to be due to the *Sargassum* sp. tastes salty or high in mineral content. Forbes [30] stated that one of the factors that cause the low palatability of an animal feed ingredient is the organoleptic nature of the salty taste.

Table 5. The mean palatability test of *Sargassum* sp. with the sun drying method, 55°C oven, and -20°C freeze dryer.

Treatment	Giving time	Palatability
Sun-dry	Before morning feed (four hours fast)	<25%
	One hour after morning feed	<25%
Oven-dry 55°C	Before morning feed (four hours fast)	<25%
	One hour after morning feed	<25%
Freeze dry -20°C	Before morning feed (four hours fast)	<25%
	One hour after morning feed	<25%

Description: The palatability includes little consumed

4. CONCLUSION

The sun-drying method can be used optimally to evaluate digestibility *in vitro* and rumen fermentation as well as palatability in sheep.

AUTHORS' CONTRIBUTIONS

Agustinus Paga was conducted study, analysis data, write and revision of manuscript. Ali Agus, Kustantinah, and I Gede Suparta Budisatria were supervision of the study, write and revision of manuscript.

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