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# Chemical composition with different drying methods and ruminant methane gas production of *Palisada perforata*

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**Abstract.** *Hidayah N, Noviandi CT, Astuti A, Kustantinah. 2024. Chemical composition with different drying methods and ruminant methane gas production of* Palisada perforata. *Nusantara Bioscience 16: 37-42.* Indonesia is a tropical country with a large diversity of seaweed, but a few studies analyzed it as an ingredient or supplement for ruminant feed. Evaluation of the nutrient content and phenolic compound with different drying methods (freeze-drying and shade-drying) and ruminant gas production from *Palisada perforata* (Bory) K.W.Nam to know about the potential for ruminant feed and methane mitigation were the goals of this investigation. The nutrient content, kinetic, and methane gas production were analyzed descriptively; meanwhile, the phenolic compound was analyzed with T-tests for the differences among treatments, using 4 replications. The result showed that the nutrient content of *P. perforata* had higher Organic Matter (OM), Crude Protein (CP), and Nitrogen-Free Extract (NFE) with the freeze-drying method (83.47 vs. 52.85%DM, 19.33 vs. 16.05%DM, and 54.16 vs 26.80%DM, respectively) and the mineral content was higher with shade-drying method (16.53 vs 47.15%DM). The shade-drying method decreased almost 50% of the phenolic compound compared to the freeze-drying method. The kinetic gas production of *P. perforata* had easily degraded, potentially degraded, and total degraded and fermented fractions at 5.88, 24.91, and 30.80 mL/200 mgDM, respectively; the methane gas production in 24 and 48 hours incubation at 1.80 and 3.01 mL/gDM. The study concluded that the freeze-drying method is better than the shade-drying method to dry *P. perforata* and this species' potential as ruminant feed and methane mitigation.

**Keywords:** Nutrient content, *Palisada perforata*, phenolic compound, ruminant methane gas production

## INTRODUCTION

Recent research on improving feed management is interested in using seaweed as a feed ingredient or additional feed for ruminant livestock (Roque et al. 2019; Min et al. 2021). Min et al. (2021) stated that seaweed is rich in polysaccharides, amino acids, vitamins, secondary metabolites, and minerals, which are important for livestock metabolic functions. Therefore, using seaweed can increase feed conversion efficiency, growth rate, health, and productivity of ruminants (Belanche et al. 2016; Pirian et al. 2017; Gaillard et al. 2018; Roque et al. 2019). Seaweed is also very efficient in reducing $CH_4$ emissions in ruminant livestock (Kinley et al. 2016, 2020; Machado et al. 2016; Li et al. 2017; Roque et al. 2019; Choi et al. 2021; Min et al. 2021; Mihaila et al. 2022) because it contains metabolite compounds; there is halogen (such as bromoform) and phlorotannin compounds which are not present in terrestrial plants, phenolic compound, and others. Hagaggi and Abdul-Raouf (2022) reported that *Cystoseira myrica* (S.G.Gmelin) C.Agardh was extracted with methanol and ethyl acetate for *Catenococcus thiocycli* Sorokin, 1994 had the highest quantities of flavonoids (2,164.7 and 1,418.4 µg quercetin equivalent/mg extract), respectively. A notable quantity of saponins and tannins (778 µg diosgenin equivalent/mg extract and 606 µg catechol equivalent/mg extract, respectively) were present in the *C. myrica* extracted with ethyl acetate.

Studies on using various seaweed species as feed ingredients or additional feed for ruminants and their effectiveness in reducing enteric $CH_4$ from subtropical seaweed species are still minimal. The genus of *Asparagopsis* is the most vigorous additive in decreasing enteric CH4 production (40-98%) with low levels (0.2-2% Organic Matter (OM)) (Machado et al. 2016; Li et al. 2017; Kinley et al. 2020; Mihaila et al. 2022). Indonesia is one of the tropical countries that produces the largest seaweed in the world (38.7%) after China (47.9%) (FAO 2018), with a fresh seaweed harvest of almost 10 million metric tons in 2019 (van der Heijden et al. 2022). Erniati et al. (2016) stated that seaweed diversity in Indonesia is the largest than in other countries. Waters et al. (2019) stated that Indonesia is a good place to grow different kinds of seaweed because it has a tropical climate, 64,000,000 $km^2$ of ocean area, and 110,000 km of coastline.

Seaweed in Indonesia has been used as food raw material, especially *Eucheuma cottonii* Weber Bosse, 1913 and *Gracilaria* Greville, 1830 for carrageenan and agar-agar, respectively, which are used in the production of various food items, including crackers, drinks, noodles, jellies, sweets, etc. (van der Heijden et al. 2022). However, until now, the seaweed used and commercialized is still very limited to the *Eucheuma* spp. and *Gracilaria* spp. So, there are still many seaweed species that have not been explored. Haryatfrehni et al. (2015) stated that Gunungkidul, Yogyakarta, Indonesia, has several beaches with the potential for high diversity and availability of natural seaweed sources. *Palisada perforata* (Bory) K.W.Nam is one of the seaweed species abundant in Gunungkidul coastal areas, with the potential to evaluate the chemical composition (nutrient content and phenolic compound) as an alternative feed ingredient or additional feed for ruminant livestock (Figure 1).

However, it is crucial to investigate the different drying methods to evaluate the chemical composition of *P. perforata* because seaweeds are high in water content. Kustantinah et al. (2022) reported that some tropical seaweed species had water content higher than 70% and even up to 85%, depending on the species, as Badmus et al. (2019) stated. Hence, the drying process is very important to investigate. Moreira et al. (2015) found that the total polyphenol content and antioxidant activity decreased as the drying temperature increased. So, this study aims to evaluate the effect of different drying methods (low temperature: freeze drying and high temperature: shade drying) on the chemical composition of *P. perforate.* This study also investigates ruminant gas production from the best drying method to observe seaweed degradation in the rumen and potential methane mitigation.

## MATERIALS AND METHODS

### The *P. perforata* collection and drying method

This sample was collected by hand picking in October 2022 from Gunungkidul, Yogyakarta, Indonesia, and identified with the morphological method in the Plant Systematics Laboratory, Biology Faculty, Universitas Gadjah Mada, Yogyakarta, Indonesia. After water rinsing to remove any remaining sand or grit, the seaweed samples were dried using a freeze drier (Buchi, Lyovapor, L-200) for the freeze-drying method (-20°C for 3 days). The sample was spread on the bamboo shelf and left to dry under the roof, which was indirectly exposed to the sunlight for the shade drying method (25-30°C for 4 days). The dry samples were pulverized in a hammer mill to a fine powder (80-100 mesh) and placed in sealed plastic bags in a freezer for further analysis.

### Chemical composition analysis

Powder samples, comprising nutritional content and phenolic component analysis, were employed for the chemical composition examination.

### Nutrient content analysis

Dry Matter (DM), ash, Organic Matter (OM), Crude Protein (CP), Ether Extract (EE), Crude Fiber (CF), and Nitrogen-Free Extract (NFE) were the components of the nutrient content analysis, which was conducted following AOAC (2005). After the sample was dried at 105°C to determine the DM concentration, the residue was burned at 550°C to the analyzed ash concentration. The amount of OM as [100-ash], following N analysis using the Kjeldahl method, CP values were determined as N × 6.25. After the material was extracted using the Soxhlet technique and dried at 105°C, the amount of ether extract was determined. In the meantime, the CF was assessed using the boiling sample in a solution of sulfuric acid ($H_2SO_4$), and it was further boiled for 30 minutes at 300°C using a solution of sodium hydroxide (NaOH) before being dried at 105°C. The formula for calculating NFE was [100-(ash+CP+EE+CF)].

### Phenolic component analysis

Flavonoids, phenol, and tannin were among the phenolic compounds analyzed. The procedure was followed by Abdulrazak and Fujihara (1999), who specified methanol as the solvent in preparing the extracts. In summary, during the extraction process, 200 mg of dried seaweed powder was dissolved in 10 mL of methanol solvent and incubated for 90 min at 130 rpm and 30°C in a platform incubator shaker series (Innova 42, New Brunswick, Eppendorf AG, DE).

**Figure 1**. *Palisada perforata* (Bory) K.W.Nam

Next, for phenolic analysis, the mixture was centrifuged at 3000 rpm for 20 minutes at 4°C, and the supernatant was moved to another tube as much as possible without disturbing the residue. The phenol and tannin were assessed using the Folin-Ciocalteau method (Makkar 2003); we utilized solutions of tannic acid (20-100 µg/mL) from Sigma–Aldrich as a standard with the curve (y= 0.0108x+0.0102, $r^2$= 0.99). The absorbance was measured at a wavelength of 725 nm. Next, Arvouet-Grand et al. (1994) adopted the Dowd method for measuring flavonoid concentration. The absorbance was measured at 415 nm using quercetin (Sigma-Aldrich) solutions in the 20-100 µg/mL range for the standard curve (y= 0.002934x-0.032, $r^2$= 0.99).

### In vitro gas production evaluation

A Bali bull and cow (350 and 290 kg body weight and 5 years old) were employed as donors of ruminal fluid for the in vitro incubations, and this provided the rumen fluid for in vitro feeding. The feed was in the 60:40%DM fresh Napier grass and beef cattle concentrate ratio. Clean water was constantly accessible, and feed was offered twice, between 8 a.m. and 5 p.m. The National Research Council (NRC) guidelines for feeding cattle based on maintenance needs are followed. Rumen fluid was taken before morning feeding, then filtered and added to the media, according to Menke and Steingass (1988). All the experimental procedures were approved by the Animal Care Committee at the Faculty of Veterinary Medicine Universitas Gadjah Mada, Indonesia (Approval number: 052/EC-FKH/Eks./2022).

The powdered seaweed samples were used as substrate and put into 100 mL glass syringes (Haberle Labortechnik, Lonsee, Germany) with 200 mgDM. They were mixed with 30 mL of rumen liquor-buffer solution (1:2 ratio vol/vol) and incubated for 72 hours at 39°C under anaerobic conditions. Next, to adjust the gas production values for gas release from endogenous substrates, a total of 12 glass syringes were used in the in vitro incubation runs: 4 glass syringes for the samples, 4 glass syringes for blanks (without substrate) to correct the gas production values for gas release from endogenous substrates, and 4 glass syringes for standard (the seaweed substrate replaced with Pangola grass) as an indicator in vitro incubation process; the gas production was determined at 2, 4, 8, 12, 24, 48, and 72 hours. The Neway program (Chen 1994) was used to calculate the gas production using the formula $Y= a+b(1-e^{-ct})$, where Y is the total gas produced in time t, a and b are the easily degraded and potentially degraded fractions, c is the rate at which the b fraction is producing gas, and the total fraction fermented and degraded (a+b fraction) is the total fraction; the Fievez et al. (2005) method measured methane emissions from gas samples. After a 24-hour incubation period, 10 mL samples were collected from the aliquot and placed in a vacuum tube to analyze methane gas production (Kang Jian, China). Gas chromatography (GC 14B, Shimadzu Crop., Kyoto, Japan) with a Paropak column (50 m × 0.2 mm × 0.3 µm) and an FID detector were used to measure the methane emissions from gas samples.

### Research design and statistical analysis

The nutrient content and phenolic compound, kinetic, and methane gas production were analyzed descriptively using a Completely Randomized Design with 4 replications and T-tests for the differences among treatments. Analysis of statistics was executed using IBM SPSS Statistics (26 versions).

## RESULTS AND DISCUSSION

### Nutrient content

The nutrient content of *P. perforata* had higher Organic Matter (OM), Crude Protein (CP), and Nitrogen-Free Extract (NFE) with the freeze drying method (83.47 vs. 52.85% DM, 19.33 vs 16.05% DM, and 54.16 vs 26.80% DM respectively) than the shade drying method. Meanwhile, the mineral content was higher with the shade-drying method than the freeze-drying method (16.53 vs 47.15% DM) (Figure 2).

The lower CP in the shade drying method might be due to the high drying temperature that caused protein degradation. Ullah et al. (2023) stated that the protein may become more denaturized due to the high-temperature drying processes, which could make the protein less extractable. Hamid et al. (2018) also reported that the nutrients in seaweed are decreased when dried at high temperatures. Meanwhile, as a low heat-drying treatment, the freeze-drying method will not break down the protein formation, so it is lower in the Maillard reaction process than high heat-drying (Boateng and Yang 2021). The same result was reported by Regal et al. (2020); the CP content of *Asparagopsis taxiformis* (Delile) Trevis. with oven-drying (high temperature at 60°C for 72 h) was lower than freeze-drying (low temperature at -40°C and 4 × 10-4 mbar for 48 h). One possible reason for the reduced protein in oven-dried *A. taxiformis* could be that there was dripping during the 72 hours in the oven (while the water is freeze-dried, making this dripping impossible).

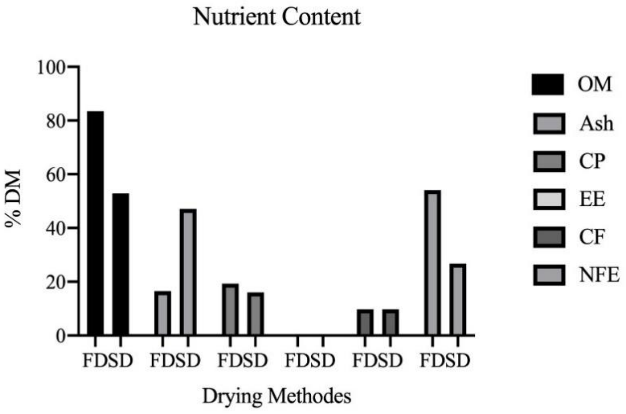

**Figure 2**. Nutrient content of *P. perforata* with different drying methods. FD: Freeze Drying, SD: Shade Drying, OM: Organic Matter, CP: Crude Protein, EE: Ether Extract, CF: Crude Fiber, NFE: Nitrogen-Free Extract

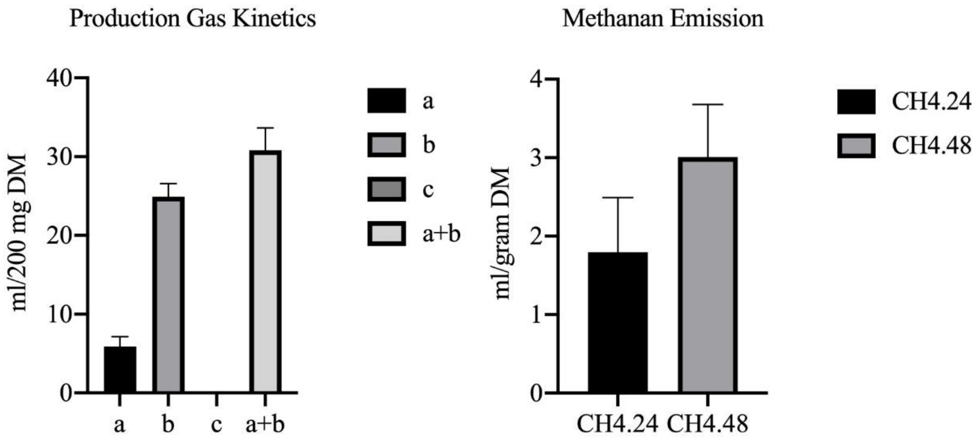

**Figure 3**. Kinetic and methane gas production of *P. perforata* dried with freeze-drying method. a: Easily degraded fraction, b: potentially degraded fraction, and a+b: Degraded and fermented fractions, CH4.24: Methane gas production that incubated 24 hours, CH4.48: Methane gas production that incubated 48 hours

**Table 1**. The phenolic compound of *P. perforata* with different drying methods (mg/g DM)

| Drying methods | Phenol | Tannin | Flavonoid |
|---|---|---|---|
| Freeze drying | $0.80^b$±0.11 | $0.76^b$±0.12 | $4.26^b$±0.13 |
| Shade drying | $0.45^a$±0.03 | $0.42^a$±0.04 | $3.70^a$±0.34 |
| P- Value | 0.00 | 0.00 | 0.00 |

However, the freeze-drying method had lower mineral content than the shade-drying method. This condition might be due to the high temperature not affecting the mineral content of *P. perforata*. Uribe et al. (2018) reported the same result: the ash content in dried *Ulva* spp. Linnaeus, 1753 was higher with the high temperature (Solar Drying (SD) and Convective Drying (CD), with the average ash content of 19.65 g/100 mg DM) than with low temperature (Freeze Drying (FD) and Vacuum Drying (VD) that average ash of 17.69 g/100 mg DM). Paga et al. (2021) explained that *Sargassum* sp. C.Agardh, 1820 that dried with the sun-drying method (high temperature) had a higher mineral content (P <0.01) than the freeze-drying methods (low temperature) based on macro minerals like cobalt (30.62 vs. 22.98 ppm) and micro minerals like calcium (38.48 vs 20.98 g/kgDM) and magnesium (13.20 vs 11.10 g/kgDM). Therefore, the sun-drying method (high temperature) can optimally preserve the fresh *Sargassum* sp. without compromising the mineral composition.

## Phenolic compound

The shade-drying method decreased (P <0.01) almost 50% of the phenol and tannin compound and 13.15% of the flavonoid content of *P. perforata* than the freeze-drying method. The phenolic compound from the freeze-drying method at 0.80 mg tannic acid per g of dry matter for phenol, 0.76 mg tannic acid per g of dry matter for tannin, and 4.26 mg quercetin per g of dry matter for flavonoid. Meanwhile, the shade drying method at 0.45 mg tannic acid per g of dry matter for phenol, 0.042 mg tannic acid per g of dry matter for tannin, and 3.70 mg quercetin per g of dry matter for flavonoid (Table 1).

Kamiloglu et al. (2016) stated that the drying process directly exposed to the sun had the lowest phenol content. This could be because the sample was exposed to the atmosphere for a longer period, which caused the phenolic compounds to oxidize and cause degradation. In comparison, the low-temperature drying methods had higher retention of bioactive compounds and better antioxidant activities (Meng et al. 2018). The same result reported by Paga et al. (2021) reported that the phenolic compound (phenol, tannin, and flavonoid) of *Sargassum* sp. was higher with freeze dry than with sun dry method (9.43 vs. 6.61%, 1.22 vs. 0.89%, and 7233.03 vs 2393 mg/kg respectively). The same result reported by Neoh et al. (2021) that *Sargassum polycystum* C.Ag. extracted with cold water had total phenol and flavonoid compound, with freeze dry method (25.33 mg PGE/gDE and 12.12 mg RE/gDE) higher than sun dry method (22.76 mg PGE/gDE and 7.30 mg RE/gDE).

## Kinetic gas production and methane emission

The dried *P. perforata* methods had different results for nutrient and phenolic compounds. The freeze-drying method had a higher nutrient content and phenolic compound than the shade-drying method. So, the freeze-drying sample was used for the in vitro gas production evaluation to know about the rumen degradability of *P. perforata*. The result showed that the kinetic gas production of *P. perforata* had easily degraded, potentially degraded, and degraded and fermented fractions at 5.88, 24.91, and 30.80 mL/200 mgDM, respectively, and methane gas production in 24 and 48 hours incubation at 1.80 and 3.01 mL/gDM (Figure 3). This result on the several compounds is higher than the result reported by Hidayah et al. (2023), the *Gracilaria* sp. collected from Kalapa beach, Tuban, East Java, Indonesia at 2.02, 27.60, 29.62 mL/200 mgDM and high methane gas production in 24 hours incubation at 9.81 mL/gDM. The result indicated that *P. perforata* for single feed was easily degraded by rumen microbes and produced low gas methane production.

In comparison with the previous research by Hidayah et al. (2023), kinetic gas production of *P. perforata* had a higher easily degraded fraction (a) but a lower potentially degraded (b) and degraded and fermented fraction (a+b) than *Laminaria* sp. J.V.F.Lamouroux, 1813, *Padina australis* Hauck, *Gracilaria* sp., and *E. cottonii*. This condition might be affected by the CP and NFE of *P. perforata, which are higher than those of* these seaweeds. Jayanegara et al. (2009) reported that gas production ($P <0.05$) had a positive correlation ($r= 0.81$) with crude protein. This is because protein is an easily degraded component in the rumen, except for proteins protected using certain compounds. Meanwhile, the methane gas production incubated for 24 hours was less than that of all these seaweed species. This condition might be due to the higher phenolic compound of P. perforata than all these seaweed species. Lee-Rangel et al. (2022) explained many studies showing that seaweed secondary metabolites can reduce rumen $CH_4$ production during enteric fermentation. Gemeda and Hassen (2015) also reported that there was a significant ($p <0.001$) negative correlation between methane production at 24 hours of incubation with phenolic compounds (total phenol, total tannin, condensed tannin, and hydrolyzable tannin). The study concluded that the freeze-drying method is better than the shade-drying method for chemical composition to dry *P. perforata*. This seaweed species is easily degraded by rumen microbial and low gas methane production, so the seaweed species has potential as ruminant feed and methane mitigation.

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## REFERENCES

Abdulrazak SA, Fujihara T. 1999. Animal Nutrition: A Laboratory Manual. Laboratory of Animal Science, Faculty of Life and Environmental Science, Shimane University, Shimane.

AOAC. 2005. Official Methods of Analysis. 18th ed. AOAC International, Arlington.

Arvouet-Grand A, Vennat B, Pourrat A, Legret PJJB. 1994. Standardization of a propolis extract and identification of the main constituents. J Pharm Belg 49: 462-468.

Badmus UO, Taggart MA, Boyd KG. 2019. The effect of different drying methods on certain nutritionally important chemical constituents in edible brown seaweeds. J Appl Phycol 31: 3883-3897. DOI: 10.1007/s10811-019-01846-1.

Belanche A, Pinloche E, Preskett D, Newbold CJ. 2016. Effects and mode of action of chitosan and ivy fruit saponins on the microbiome, fermentation and methanogenesis in the rumen simulation technique. FEMS Microbiol Ecol 92: fiv160. DOI: 10.1093/femsec/fiv160.

Boateng ID, Yang XM. 2021. Thermal and non-thermal processing affect Maillard reaction products, flavor, and phytochemical profiles of *Ginkgo biloba* seed. Food Biosci 41: 101044. DOI: 10.1016/j.fbio.2021.101044.

Chen X. 1994. Neway Program International Feed Resources Unit. Backburn, Rowett Research Institute, Aberdeen.

Choi Y, Lee SJ, Kim HS, Eom JS, Jo SU, Guan LL, Seo J, Kim H, Lee SS, Lee SS. 2021. Effects of seaweed extracts on in vitro rumen fermentation characteristics, methane production, and microbial abundance. Sci Rep 11: 24092. DOI: 10.1038/s41598-021-03356-y.

Erniati E, Zakaria FR, Prangdimurti E, Adawiyah DR. 2016. Potensi rumput laut: Kajian komponen bioaktif dan pemanfaatannya sebagai pangan fungsional. Acta Aquatic 3: 12-17. DOI: 10.29103/aa.v3i1.332. [Indonesian]

FAO. 2018. The Global Status of Seaweed Production, Trade and Utilization. Food and Agriculture Organization of the United Nations, Rome.

Fievez V, Babayemi O, Demeyer D. 2005. Estimation of direct and indirect gas production in syringes: A tool to estimate short chain fatty acid production that requires minimal laboratory facilities. Anim Feed Sci Technol 123-124: 197-210. DOI: 10.1016/j.anifeedsci.2005.05.001.

Gaillard C, Bhatti HS, Novoa-Garrido M, Lind V, Roleda MY, Weisbjerg MR. 2018. Amino acid profiles of nine seaweed species and their in situ degradability in dairy cows. Anim Feed Sci Technol 241: 210-222. DOI: 10.1016/j.anifeedsci.2018.05.003.

Gemeda BS, Hassen A. 2015. Effect of tannin and species variation on in vitro digestibility, gas, and methane production of tropical browse plants. Asian Australas J Anim Sci 28: 188-199. DOI: 10.5713/ajas.14.0325.

Hagaggi NSA, Abdul-Raouf UM. 2022. Macroalga-associated bacterial endophyte bioactive secondary metabolites twinning: *Cystoseira myrica* and its associated *Catenococcus thiocycli* QCm as a model. World J Microbiol Biotechnol 38: 205. DOI: 10.1007/s11274-022-03394-2.

Hamid SS, Wakayama M, Soga T, Tomita M. 2018. Drying and extraction effects on three edible brown seaweeds for metabolomics. J Appl Phycol 30: 3335-3350. DOI: 10.1007/s10811-018-1614-z.

Haryatfrehni R, Dewi SC, Meilianda A, Rahmawati S, Sari IZR. 2015. Preliminary study the potency of macroalgae in Yogyakarta: Extraction and analysis of algal pigments from common Gunungkidul seaweeds. Proc Chem 14: 373-380. DOI: 10.1016/j.proche.2015.03.051.

Hidayah N, Kustantinah K, Noviandi CT, Astuti A, Hanim C, Suwignyo B. 2023. Evaluation of rumen in vitro gas production and fermentation characteristics of four tropical seaweed species. Vet Integr Sci 21: 229-238. DOI: 10.12982/vis.2023.018.

Jayanegara A, Sofyan A, Makkar H, Becker K. 2009. Kinetika produksi gas, kecernaan bahan organik dan produksi gas metana *in vitro* pada hay dan jerami yang disuplementasi hijauan mengandung tanin. Med Pet 32: 120-129. [Indonesian]

Kamiloglu S, Toydemir G, Boyacioglu D, Beekwilder J, Hall RD, Capanoglu E. 2016. A review on the effect of drying on antioxidant potential of fruits and vegetables. Crit Rev Food Sci Nutr 56 (sup1): S110-S129. DOI: 10.1080/10408398. 2015.1045969.

Kinley RD, Martinez-Fernandez G, Matthews M K, de Nys R, Magnusson M, Tomkins NW. 2020. Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed. J Clean Prod 259: 120836. DOI: 10.1016/j.jclepro.2020.120836.

Kinley RD, Vucko MJ, Machado L, Tomkins NW. 2016. Evaluation of the antimethanogenic potency and effects on fermentation of individual and combinations of marine macroalgae. Am J Plant Sci 07: 2038-2054. DOI: 10.4236/ajps.2016.714184.

Kustantinah, Hidayah N, Noviandi CT, Astuti A, Paradhipta DHV. 2022. Nutrients content of four tropical seaweed species from Kelapa Beach, Tuban, Indonesia and their potential as ruminant feed. Biodiversitas 23: 6191-6197. DOI: 10.13057/biodiv/d231213.

Lee-Rangel HA, Roque-Jiménez JA, Cifuentes-López RO, Álvarez-Fuentes G, Cruz-Gómez ADI, Martínez-García JA, Arévalo-Villalobos JI, Chay-Canul AJ. 2022. Evaluation of three marine algae on degradability, in vitro gas production, and $CH_4$ and $CO_2$ emissions by ruminants. Fermentation 8: 511. DOI: 10.3390/fermentation8100511.

Li Y, Fu X, Duan D, Liu X, Xu J, Gao X. 2017. Extraction and identification of phlorotannins from the brown alga, *Sargassum fusiforme* (Harvey) Setchell. Mar Drugs 15: 49. DOI: 10.3390/md15020049.

Machado L, Magnusson M, Paul NA, Kinley R, de Nys R, Tomkins N. 2016. Identification of bioactives from the red seaweed *Asparagopsis* *taxiformis* that promote antimethanogenic activity in vitro. J Appl Phycol 28: 3117-3126. DOI: 10.1007/s10811-016-0830-7.

Makkar HPS. 2003. Quantification of Tannins in Tree and Shrub Foliage: A Laboratory Manual. Springer Science & Business Media, Berlin. DOI: 10.1007/978-94-017-0273-7.

Meng Q, Fan H, Li Y, Zhang L. 2018. Effect of drying methods on physico-chemical properties and antioxidant activity of *Dendrobium officinale*. J Food Meas Charact 12: 1-10. DOI: 10.1007/s11694-017-9611-5.

Menke KH, Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. J Anim Res 28: 7-55.

Mihaila AA, Glasson CRK, Lawton R, Muetzel S, Molano G, Magnusson M. 2022. New temperate seaweed targets for mitigation of ruminant methane emissions: An in vitro assessment. Appl Phycol 3: 274-284. DOI: 10.1080/26388081.2022.2059700.

Min BR, Parker D, Brauer D, Waldrip H, Lockard C, Hales K, Akbay A, Augyte S. 2021. The role of seaweed as a potential dietary supplementation for enteric methane mitigation in ruminants: Challenges and opportunities. Anim Nutr 7: 1371-1387. DOI: 10.1016/j.aninu.2021.10.003.

Moreira R, Chenlo F, Sineiro J, Arufe S, Sexto S. 2015. Drying temperature effect on powder physical properties and aqueous extract characteristics of *Fucus vesiculosus*. J Appl Phycol 28: 2485-2494. DOI: 10.1007/s10811-015-0744-9.

Neoh YY, Matanjun P, Lee JS. 2021. Effects of various drying processes on Malaysian brown seaweed, *Sargassum polycystum* pertaining to antioxidants content and activity. Trans Sci Technol 8: 25-37.

Paga A, Agus A, Kustantinah, Budisatria IGS. 2021. Effect of drying methods on the mineral content of seaweed *Sargasum* sp. Livest Res Rural Dev 33 (3): 35.

Pirian K, Jeliani ZZ, Sohrabipour J, Arman M, Faghihi MM, Yousefzadi M. 2017. Nutritional and bioactivity evaluation of common seaweed species from the Persian Gulf. Iran J Sci Technol Trans A Sci 42: 1795-1804. DOI: 10.1007/s40995-017-0383-x.

Regal AL, Alves V, Gomes R, Matos J, Bandarra NM, Afonso C, Cardoso C. 2020. Drying process, storage conditions, and time alter the biochemical composition and bioactivity of the anti-greenhouse seaweed *Asparagopsis taxiformis*. Eur Food Res Technol 246: 781-793. DOI: 10.1007/s00217-020-03445-8.

Roque BM, Salwen JK, Kinley R, Kebreab E. 2019. Inclusion of *Asparagopsis armata* in lactating dairy cows diet reduces enteric methane emission by over 50 percent. J Clean Prod 234: 132-138. DOI: 10.1016/j.jclepro.2019.06.193.

Ullah MR, Akhter M, Khan ABS, Yasmin F, Hasan MM, Bosu A, Haque MA, Islam M, Mahmud Y. 2023. Comparative estimation of nutritionally important chemical constituents of red seaweed, *Gracilariopsis longissima*, affected by different drying methods. J Food Qual 2023: 6623247. DOI: 10.1155/2023/6623247.

Uribe E, Vega-Gálvez A, García V, Pastén A, López J, Goñi G. 2018. Effect of different drying methods on phytochemical content and amino acid and fatty acid profiles of the green seaweed, *Ulva* spp. J Appl Phycol 31: 1967-1979. DOI: 10.1007/s10811-018-1686-9.

van der Heijden PG, Lansbergen R, Axmann H, Soethoudt H, Tacken G, van den Puttelaar J, Rukminasari N. 2022. Seaweed in Indonesia: Farming, Utilization and Research. Wageningen Centre for Development Innovation, Wageningen. DOI: 10.18174/578007.

Waters TJ, Lionata H, Prasetyo Wibowo T, Jones R, Theuerkauf S, Usman S, Amin I, Ilman M. 2019. Coastal Conservation and Sustainable Livelihoods through Seaweed Aquaculture In Indonesia: A Guide for Buyers, Conservation Practitioners, and Farmers, Version 1. The Nature Conservancy, Arlington VA and Jakarta.