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RESEARCH ARTICLE
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Enhancing Rumen Efficiency and Reducing Methane Emissions with South Sulawesi Seaweed as Feed Additives

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ABSTRACT

Livestock methane emissions contribute significantly to global warming, necessitating the exploration of sustainable feed additives to enhance rumen efficiency and reduce environmental impact. This study investigates the potential of South Sulawesi seaweeds as feed additives to improve rumen fermentation efficiency and reduce methane emissions in ruminants. Seven seaweed species—*Eucheuma cottoni*, *Eucheuma denticulatum*, *Caulerpa* sp. (cultivated and non-cultivated), *Gracilaria* sp., *Halimynea* sp., and *Sargassum* sp.—were evaluated at inclusion levels of 0, 5, 10, and 15% in complete feed formulations using a factorial in vitro design. Key parameters such as dry matter degradation (DMD), organic matter degradation (OMD), ammonia concentration (NH_3), volatile fatty acids (VFAs), gas production, and methane (CH_4) output were measured. The highest DMD and OMD were observed with *Sargassum* sp. and *Gracilaria* sp. at the 15% inclusion level, suggesting enhanced nutrient utilization. *Gracilaria* sp. and *Halimynea* sp. produced the highest VFAs, indicating efficient fermentation. Methane production was significantly lower with *Sargassum* sp. and *Eucheuma cottoni*, likely due to bioactive compounds such as tannins and saponins. Non-cultivated species exhibited higher bioactive compound levels than cultivated ones, potentially influenced by environmental factors. These findings demonstrate that seaweed inclusion at 15% optimizes rumen efficiency and reduces methane emissions, presenting a sustainable strategy for improving livestock productivity while mitigating climate change impacts.

Keywords: In vitro fermentation, Seaweed feed additives, Methane reduction, Rumen fermentation efficiency, Bioactive compounds.

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INTRODUCTION

Enteric fermentation allows ruminants to digest high-fiber feeds such as grasses and straw through microbial activities in the rumen, resulting in the production of volatile fatty acids (VFAs), a primary energy source for the animals (Knapp et al., 2014; Matthews et al., 2019). However, this process also generates methane (CH_4), a potent greenhouse gas that contributes significantly to global warming (Mar et al., 2022). Methane emissions from rumen fermentation account for approximately 44% of the greenhouse gases from the livestock sector, emphasizing the urgency of methane reduction to mitigate climate change (Gerber et al., 2013; IPCC, 2014; Tseten et al., 2022). Methane production also represents an energy loss for animals, with 2-12% of feed energy being

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converted into methane instead of used for growth or production (Patra, 2016; Cardoso-Gutierrez et al., 2021). Research into dietary adjustments, feed additives, and manipulation of rumen microbiota aims to optimize microbial efficiency, reducing emissions while improving livestock productivity and sustainability (Hristov et al., 2013; Knapp et al., 2014).

Reducing methane emissions from ruminants is essential for sustainable livestock practices, and seaweed has demonstrated significant potential as a natural feed additive. Seaweed contains bioactive compounds, such as bromoform and sulfated polysaccharides, inhibiting the rumen's methanogenic microbes. *Asparagopsis taxiformis* has been shown to reduce methane emissions by up to 99% in laboratory studies (Machado et al., 2014; Kinley et al., 2020; Roque et al., 2021). Other promising species include *Ascophyllum nodosum*, which contains polyphenols; *Gracilaria* spp., which has agar and phycobiliproteins; and *Chondrus crispus*, which contains carrageenan (Abbott et al., 2020; Vijn et al., 2020). Brown and green seaweeds, such as *Sargassum spp*., *Ecklonia radiata*, and *Ulva spp*., suppress methane with compounds like phlorotannins and fucoidan (Wright et al., 2022; McGurrin et al., 2023). Field trials have confirmed their efficacy without compromising feed intake or health, making seaweed a promising option for reducing the carbon footprint of livestock. Although challenges in scaling production exist, ongoing research provides potential solutions (Roque et al., 2021; De Bhowmick & Hayes, 2023).

In addition to reducing methane production, seaweed has shown potential for enhancing rumen fermentation efficiency by boosting volatile fatty acid (VFA) production and supporting fermentative microbial populations, particularly microbial protein synthesis (MPS) (Choi et al., 2021; Cheong et al., 2023). Higher VFA levels indicate more efficient fermentation, directly improving livestock performance (Cheong et al., 2023). A robust microbial population, particularly MPS, is critical for breaking down fiber and complex carbohydrates in the rumen (Nagarajan et al., 2021). Studies have demonstrated that increased rumen microbial populations can improve fiber digestion and livestock productivity (Hook et al., 2010; Kim et al., 2013). Therefore, incorporating seaweed into livestock diets can provide dual benefits: reducing methane emissions and creating a more productive and efficient rumen microbial environment (Kinley et al., 2016; Roque et al., 2019; Cowley et al., 2024).

Geographic and environmental factors, such as temperature, salinity, and water quality, significantly affect the chemical composition of seaweed, highlighting the importance of studying seaweeds from specific regions, such as South Sulawesi (Mandalka et al., 2022; Basyuni et al., 2024). Renowned for its marine biodiversity, this region offers seaweeds rich in bioactive compounds, such as polysaccharides and phenolic compounds, which influence their fermentative properties and methane-reducing potential (Lomartire & Gonçalves, 2022; Bouzenad et al., 2024). This study selected seven types of seaweed from South Sulawesi waters to evaluate their ability to reduce methane emissions and enhance nutrient utilization during rumen fermentation. Environmental variables affect active compound concentration, impacting methane suppression and fermentation efficiency. This research assesses the effectiveness of these seaweeds in methane reduction and examines how their type and level influence other key fermentation parameters, contributing to sustainable livestock practices.

MATERIALS & METHODS

Collection and preparation of Seaweed samples

The seaweed samples used in this study included both cultivated and non-cultivated species. Cultivated seaweeds are deliberately grown and managed by farmers through aquaculture practices. These species are usually cultivated in controlled environments, such as silvofishery ponds or coastal waters, to ensure consistent quality and sustainable yields. Cultivated seaweeds used in this study include *Eucheuma cottonii, Eucheuma denticulatum, Gracilaria sp.*, and *Caulerpa sp.* Meanwhile, non-cultivated seaweeds grow naturally and are harvested from wild environments without human intervention in the growth process. They are usually collected from coastal areas or other natural habitats, and their availability depends on the season and environmental conditions. Non-cultivated seaweed samples used in this study included *Sargassum sp., Caulerpa sp.,* and *Halymenia sp*.

Dried *Gracilaria sp*. seaweed samples were obtained from the Silvofishery Pond Installation under the Fisheries Training and Extension Center, Ministry of Marine Affairs and Fisheries, in Maros. The *Eucheuma cottonii* and *Eucheuma denticulatum* varieties were collected from seaweed farmers in Suppa District, Pinrang Regency. Fresh *Caulerpa sp*. seaweed was obtained from farmers in Beba Village, North Galesong District, Takalar Regency. Samples of non-cultivated *Caulerpa sp*. seaweed were acquired from the fish auction market in Maros Regency. *Sargassum sp*. seaweed was harvested directly from the Bojo coastline in Barru Regency, while dried *Halymenia sp*. was procured from fishermen in Kambunong Village, Karossa District, Central Mamuju Regency, West Sulawesi. All fresh samples, including *Caulerpa sp*. (cultivated), *Caulerpa sp*. (non-cultivated), and *Sargassum sp*., were sun-dried for approximately 3-4 days. Cultivated and non-cultivated seaweed samples from farmers were identified based on morphological characters (Atmaja et al., 1996). The identification process was carried out through observation of morphological characteristics of macroalgae, including shape, color, and talus structure.

After collection, the dried seaweed samples were prepared for washing to eliminate dirt and salt crystals. The washing process followed the methodology outlined by Kusuma et al. (2013), where each type of seaweed was weighed before washing. Washing was conducted using fresh water at a ratio of 1kg of seaweed to 10L of water, with each cycle lasting around 10min. Every sample was washed five times and drained thoroughly to remove residual water. Subsequently, the samples were oven-dried at a temperature of 65°C until achieving complete dryness. Once fully dried, the seaweed samples were ground into a fine powder using a blender, making them ready for use in complete feed formulations. The seaweed samples were analyzed for proximate chemical composition using the AOAC (2019) method, while fiber composition was assessed following the method described by Goering & Van Soest (1970). The results of the analysis are presented in Table 1. On the basis of this analysis, formulate a complete feed was formulated.

Experimental Design and Preparation of complete feed

The experiment used a Completely Randomized Design (CRD) with a factorial pattern. The first factor consisted of seven types of seaweed, i.e., *Eucheuma cottoni*, *Eucheuma denticulatum*, *Caulerpa sp*. (cultivated), *Gracilaria sp*., *Halymenia sp*., *Sargassum sp*., and *Caulerpa sp*. (non-cultivated). The second factor was the level of Seaweed inclusion in the complete feed, which is 0, 5, 10, and 15%.

Other feedstuff used for complete feed formulation, such as corn meal, rice bran, coconut meal, tofu waste, elephant grass, rice straw, and minerals, was collected from locally available stuff. All materials were dried to 80-85% dry matter before grounding into little particles to ensure a homogenous mixture with seaweed. The complete feed's crude protein and total digestible nutrient (TDN) target was 12-13% for crude protein and 58-62% for TDN. Therefore, all feedstuff was analyzed for chemical components, i.e., dry matter (DM), organic matter (OM), crude protein, crude fiber, ether extract, and nitrogen-free extract. The composition of the experimental diets is presented in Table 2.

In vitro studies

The *in vitro* fermentation process was carried out following the method of Theodorou et al. (1994). A treatment ratio of 0.75g was placed in a 100mL infusion bottle, followed by the addition of 25mL of rumen fluid and 50mL of McDougall's solution. The infusion bottles were sealed with rubber stoppers and crimped to secure airtightness. The bottles were incubated in a water bath at 39°C, and gas production was measured at 2, 4, 6, 8, 10, 12, and 24 hours. Digestibility and fermentability tests were conducted after a 24-hour incubation period, during which the substrate was centrifuged in Corning tubes at 4000rpm for 10min. Centrifugation separated the substrate into supernatant and sediment. The supernatant was analyzed for the ammonia (NH_3) concentration and total Volatile Fatty Acids (VFA). At the same time, the sediment was used to determine the dry matter digestibility coefficient (DMD) and organic matter digestibility coefficient (OMD).

The ammonia (NH3) concentration was determined using the Conway microdiffusion technique (General Laboratory Procedure, 1966). The total VFA concentration was analyzed using the steam distillation method (General Laboratory Procedure, 1966)

Gas Production Measurement

Gas production was measured manually at 0, 2, 4, 6, 8, 12, and 24 hours. The gas volume was measured using a 50mL plastic syringe with a needle positioned perpendicularly. The needle was inserted through the rubber stopper of the infusion bottle. The gas volume displaced the syringe plunger, and the reading was taken manually from the syringe scale. After measurement, the syringe was removed. The data for gas production reported in this study was the total production for 24 hours.

CH_4 measurement

The gas collected during the gas production analysis (24 hours) was retained for further analysis and measurement of methane (CH_4) concentration. The gas was transferred into the vacuum bottle and sealed. The concentration of CH_4 was measured using the procedure of SNI 9224-1:2023 Part 1 (BSN, 2023), in which CH_4 concentration was measured using Shimadzu 8A GC with Flame Initiation Detector. The production of CH4 was calculated by multiplying the total gas production with CH_4 concentration.

Statistical Analysis

The data for bioactive compounds and phytochemical contents of the Seaweed samples were analyzed descriptively. The data for the in vitro studies were analyzed using factorial analysis of variance (ANOVA) with a 7 x 4 factorial design based on a completely randomized design (CRD), utilizing SPSS 26.0 statistical software. For data showing significant differences ($P<0.05$), further analysis was conducted using Duncan's Multiple Range Test (DMRT) to determine specific treatment differences.

Table 1: Chemical components of Seaweed samples

Parameters	*Euchema cottoni*	*Euchema denticulatum*	*Caulerpa sp* (Cultivated)	*Gracilaria sp*	*Halimynea sp*	*Sargassum sp*	*Caulerpa sp* (Non-cultivated)
Proximate Analysis							
Water content	15.41	15.39	11.38	13.77	14.26	15.87	10.04
Crude Protein	6.31	5.34	16.02	14.93	18.48	8.95	13.41
Lipid	0.09	0.19	1.66	0.28	0.44	0.57	1.55
Crude fiber	7.86	7.81	7.59	7.67	6.61	15.52	8.77
NFE	72.04	75.42	68.16	70.95	52.12	59.92	69.73
Ash	13.70	13.43	6.57	6.18	6.18	15.05	6.54
Van soest analysis							
ADF	13.10	10.54	19.26	9.52	10.46	40.31	21.29
NDF	34.56	23.52	74.33	30.11	12.08	42.38	63.79
Cellulose	2.45	2.19	16.68	9.02	1.28	27.76	18.59
Hemicellulose	21.46	12.98	55.07	20.59	1.62	2.07	42.50
Lignin	9.88	7.67	2.05	0.43	8.33	10.82	2.30
AIA	0.76	0.69	0.53	0.07	0.85	1.74	0.39

Source: Feed Chemical Laboratory, Faculty of Animal Science, Hasanuddin University, 2024.

Table 2: Feedstuff composition and chemical components of each complete feed containing different levels of seaweed

Percentage	C	*E. cottoni*			*E. denticulatum*			*Caulerpa* (cultivated)			*Gracillari sp*			*Halimynea sp*			*Sargassum sp*			*Caulerpa sp* (non-cultivated)		
Seaweed	0	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15
Corn meal	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Copra meal	20	19	18	18	20	20	20	18	15	12	18	16	13	18	14	10	19	18	16	18	14	11
Rice bran	10	9	7	5	9	7	5	8	8	8	8	8	8	8	8	7	9	7	6	8	9	7
Tofu waste	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
E. grass	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Rice straw	20	17	15	12	16	13	10	19	17	15	19	16	14	19	18	18	17	15	13	19	17	17
Mineral	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Nutrient																						
DM	88.0	88.0	87.9	87.9	88.0	88.0	87.9	88.0	87.9	87.9	88.0	87.9	87.9	88.0	87.9	87.9	88.0	87.9	87.9	89.0	89.0	88.9
OM	90.4	90.8	91.1	91.6	91.0	91.4	91.9	90.6	90.7	90.9	90.6	90.9	91.1	90.6	90.6	90.5	90.8	91.1	91.4	90.6	90.7	90.7
CP	12.7	12.7	12.6	12.7	12.5	12.5	12.4	12.9	12.9	12.9	12.8	13.0	13.0	13.0	13.0	12.9	12.9	13.0	13.0	13.0	12.9	13.0
CF	24.5	25.1	25.9	26.6	25.1	25.8	26.6	25.3	25.8	26.3	25.3	25.8	26.3	25.3	25.8	26.5	25.1	25.9	26.5	25.3	25.6	26.5
EE	4.6	4.4	4.2	4.0	4.5	4.4	4.2	4.2	3.9	3.6	4.2	4.0	3.7	4.2	3.8	3.4	4.4	4.2	3.9	4.2	3.9	3.5
NFE	48.6	48.6	48.5	48.2	48.9	48.8	48.7	48.2	48.1	48.1	48.2	48.1	48.1	48.1	48.0	47.8	48.4	48.1	48.0	48.1	48.2	47.8
TDN	62.0	61.4	60.6	60.1	61.6	61.1	60.6	60.9	60.0	59.2	60.9	60.3	59.4	60.9	59.8	58.4	61.4	60.6	59.7	60.9	60.0	58.7

DM: dry matter, OM: organic matter, CP: crude protein, CF: Crude fiber, EE: ether extract, NFE: Nitrogen free extract, TDN: total digestible nutrient.

RESULTS

Screening of Bioactive compounds of the Seaweed samples

The results of laboratory tests in Table 3 below show the content of bioactive components and phytochemical screening results in seven types of seaweed, namely *Eucheuma cottonii*, *Eucheuma denticulatum*, *Caulerpa sp.* (cultivated*), Gracilaria sp., Halimynea sp., Sargassum sp.*, and *Caulerpa sp.* (non-cultivated). Analyses included tannin, flavonoid, and saponin (%), as well as the presence of bioactive compounds such as alkaloids, saponins, tannins, phenolics, flavonoids, glycosides, triterpenoids, and steroids based on the results of phytochemical screening.

Invitro Studies

The in vitro study evaluated parameters based on the 24-hour fermentation period, including dry matter degradability, organic matter degradability, N determination of dry matter Degradation (DMD), total gas production, and CH_4. The results of these parameters are presented in Tables 4 and 5.

Increasing seaweed levels in complete diets generally increases dry matter and organic matter degradation. However, the degradation response varied depending on the seaweed species used, with some species showing higher degradation ability than others. Average dry matter degradation increased significantly ($P<0.05$) as the level of seaweed in the complete diet increased, from 31.53% at the 0% level to 37.26% at the 15% level. Table 4 shows that among the seaweed species tested, *Eucheuma cottonii*, *Gracilaria sp*, and *Sargassum sp* showed the highest average dry matter degradation, which was significantly different ($P<0.05$) compared to *Caulerpa sp* (cultivated), which had the lowest average. Similarly, the average organic matter degradation increased from 42.82% at the 0% level to 48.41% at the 15% level. *Caulerpa sp* (cultivated) and *Halimynea sp* showed the lowest average organic matter degradation, which was statistically significantly different ($P<0.05$) compared to other species such as *Eucheuma cottonii*, *Eucheuma denticulatum, Gracilaria sp*, and *Sargassum sp*, which had higher average organic matter degradation.

Increasing the level of seaweed in the complete diet significantly affected various rumen fermentation parameters ($P<0.05$), with the impact varying depending on the type of seaweed used. NH_3 concentration increased at 5, 10, and 15% levels compared to the control (0%), with *Gracilaria sp*. producing significantly higher NH_3 concentrations than other species such as *Caulerpa sp.* (cultivated) and *Euchema denticulatum*. VFA production also increased significantly with seaweed addition, although there was no significant difference between the 5, 10 and 15% levels. *Gracilaria sp*. showed the highest VFA production among all species. Gas production increased as the seaweed level increased, with a peak at the 10% level. Species such as *Euchema cottonii* and *Euchema denticulatum* produced the highest gas production, reflecting better fermentation ability. In contrast, methane production decreased significantly at the 5, 10, and 15% levels compared to the control (0%), with the most significant decrease for *Euchema cottonii.*

DISCUSSION

Bioactive components of seaweed

A descriptive analysis of seven Seaweed samples from South Sulawesi highlights the variability in bioactive compound content and phytochemical profiles between cultivated and non-cultivated species. Non-cultivated species, such as *Sargassum sp* and *Halimynea sp*, demonstrate higher concentrations of tannin, flavonoid, and a full spectrum of phytochemicals, including steroids and triterpenoids. These species exhibit robust antioxidant, anti-inflammatory, and antimicrobial properties, aligning with findings from prior studies that emphasize the rich bioactive profiles of seaweed grown in natural habitats exposed to environmental stressors (McGurrin et al., 2023; Matin et al., 2024;). In contrast, cultivated species like *Euchema cottoni* and *Euchema denticulatum* have lower tannin and flavonoid levels but excel in saponin content, particularly *Euchema denticulatum* (1.19%), making them valuable for use in emulsifiers, natural soaps, and food additives, as highlighted in studies by Lomartire et al. (2021).

Table 3: Bioactive components and phytochemical screening of Seaweed sample

Parameters	*Euchema cottoni*	*Euchema denticulatum*	*Caulerpa sp* (cultivated)	*Gracilaria sp*	*Halimynea sp*	*Sargassum sp*	*Caulerpa sp* (Non-cultivated)
Bioactive components (%)							
Tannin	0.16	0.07	0.21	0.24	0.63	0.74	0.20
Flavonoid	0.03	0.03	0.11	0.10	0.13	0.28	0.23
Saponin	0.99	1.19	0.81	0.78	1.04	0.89	0.89
Phytochmeical screening							
Alkaloid	+	+	+	+	+	+	+
Saponin	+	+	+	+	+	+	+
Tanin	+	+	+	+	+	+	+
Fenolik	+	+	+	+	+	+	+
Falavonoid	+	+	+	+	+	+	+
Glikosida	+	+	+	+	+	+	+
Triterpenoid	+	+	+	+	+	+	+
Steroid	-	-	+	-	+	+	+

Source: Laboratory of the Indonesian Instruments Standardization Testing Center for Spices, Medicinal Plants, and Aromatics.

Table 4: Mean of in vitro dry matter degradation (DMD) and organic matter degradation (OMD) according to different types of seaweed with varying levels of inclusion in the complete feed.

Parameters	Level (%) in complete feed				Mean
	0	5	10	15	
DM Degradation (%)					
Halimynea sp	31.535	32.935	36.800	33.215	33.621b
Sargassum sp	31.535	37.515	39.550	38.900	36.850d
Caulerpa sp (cultivated)	31.535	32.700	32.760	32.210	32.301a
Caulerpa sp (non-cultivated)	31.535	37.725	36.795	38.315	36.092cd
Euchema cottoni	31.535	37.225	37.335	37.235	35.832cd
Euchema denticulatum	31.535	37.110	38.360	34.890	35.473c
Gracilaria sp	31.535	38.715	39.315	38.605	37.042d
Mean	31.535a	36.275b	37.259c	36.195b	
OM Degradation (%)					
Halimynea sp	42.825	43.720	46.180	42.575	43.825a
Sargassum sp	42.825	47.845	50.300	49.940	47.626b
Caulerpa sp (cultivated)	42.825	44.245	43.955	45.040	44.016a
Caulerpa sp (non-cultivated)	42.825	48.940	47.785	50.035	47.396b
Euchema cottoni	42.825	47.895	49.400	49.030	47.287b
Euchema denticulatum	42.825	49.080	52.180	46.340	47.606b
Gracilaria sp	42.825	48.835	49.080	48.275	47.253b
Mean	42.825a	47.222b	48.411c	47.261b	

Means sharing different letters in the same row or in the same column for each fermentation profile differed significantly ($P<0.05$).

Table 5: In vitro fermentation profile according to the treatment of seaweed at different levels of inclusion

Parameters	Level (%) in complete feed				Mean
	0	5	10	15	
NH_3 (mmol)					
Halimynea sp	7.12	9.51	8.97	8.42	8.50bc
Sargassum sp	7.12	8.44	8.34	8.94	8.21b
Caulerpa sp (cultivated)	7.12	8.17	7.77	7.65	7.68a
Caulerpa sp (non-cultivated)	7.12	9.01	9.06	9.23	8.60bc
Euchema cottoni	7.12	9.06	9.39	9.07	8.66bc
Euchema denticulatum	7.12	7.15	7.43	7.81	7.37a
Gracilaria sp	7.12	9.24	9.63	9.46	8.86c
Mean	7.12[a]	8.65b	8.65b	8.65b	
VFA (mMol)					
Halimynea sp	70.62	127.32	128.84	129.94	114.18
Sargassum sp	70.62	115.91	118.75	121.82	106.78
Caulerpa sp (cultivated)	70.62	127.72	131.91	109.27	109.88
Caulerpa sp (non-cultivated)	70.52	129.29	120.26	128.19	112.09
Euchema cottoni	70.62	129.34	111.46	109.70	105.28
Euchema denticulatum	70.62	101.68	112.75	109.44	98.62
Gracilaria sp	70.62	133.76	128.69	125.97	114.76
Mean	70.62a	123.57b	121.81b	119.19b	
Gas Production (mL/g DM)					
Halimynea sp	117.90	132.10	133.75	136.20	129.99c
Sargassum sp	117.90	120.55	120.25	124.30	120.75a
Caulerpa sp (cultivated)	117.90	135.20	134.90	134.90	130.73cd
Caulerpa sp (non-cultivated)	117.90	140.45	140.30	127.00	131.41d
Euchema cottoni	117.90	140.95	142.10	138.40	134.84e
Euchema denticulatum	117.90	140.40	141.65	137.40	134.34e
Gracilaria sp	117.90	127.15	131.10	131.30	126.86b
Mean	117.90a	133.82c	134.86d	132.78b	
CH_4 (ppm)					
Halimynea sp	53,170.07	38,962.84	48,062.95	19,082.27	39,819.53
Sargassum sp	53,170.07	26,809.97	56,997.94	38,480.11	43,864.52
Caulerpa sp (cultivated)	53,170.07	53,085.38	34,545.00	28,145.82	42,236.57
Caulerpa sp (non-cultivated)	53,170.07	50,019.27	43,950.25	45,302.25	48,110.46
Euchema cottoni	53,170.07	24,814.15	28,322.57	19,469.43	31,444.05
Euchema denticulatum	53,170.07	37,212.40	28,776.57	25,413.49	36,143.13
Gracilaria sp	53,170.07	36,964.07	37,410.06	51,256.67	44,700.19
Mean	53,170.07b	38,266.870a	39,723.62a	32,449.99a	

Means sharing different letters in the same row or in the same column for each fermentation profile differed significantly ($P<0.05$).

Non-cultivated seaweed generally outperforms their cultivated counterparts in phytochemical richness due to stress-induced metabolite production, as suggested by previous research on secondary metabolite biosynthesis in marine algae (Bouzenad et al., 2024). Cultivated seaweed, while limited in diversity, holds significant promise for specific applications like the food and cosmetic industries. This study supports existing research advocating innovative cultivation methods, such as stress mimicry, to enhance bioactive compound yields (Mendes et al., 2024). These findings position South Sulawesi's Seaweed as a valuable resource for developing sustainable, bioactive-rich products for health and industrial applications.

Due to their rich bioactive compound content and nutritional profiles, Seaweed from South Sulawesi exhibits significant potential as feed additives for ruminant diets. Non-cultivated species like *Sargassum sp* and *Halimynea sp*, with their high tannin and flavonoid levels, can serve as natural alternatives to synthetic feed additives, offering antioxidant and antimicrobial properties that enhance ruminant health and digestion (Wang et al., 2009). Tannins, in particular, are known to reduce methane emissions in ruminants by inhibiting methanogenic archaea in the rumen, thereby contributing to more sustainable livestock production (Orzuna-Orzuna et al., 2021). Additionally, the saponin-rich content in cultivated species like *Eucheuma denticulatum* can improve protein utilization by reducing ammonia production in the rumen, as highlighted by recent studies on saponin effects in ruminant diets (Kholif, 2023). Flavonoid-rich seaweeds have been shown to reduce methane emissions in ruminants significantly. Flavonoids modulate the rumen microbial population by increasing beneficial bacteria such as Fibrobacter succinogenes, while suppressing methanogenic archaea and other less beneficial microbes (Kim et al., 2015; Choi et al., 2021). In addition, flavonoids can increase the digestibility of nutrients such as dry matter, organic matter, and fiber, improving feed efficiency and nutrient absorption (Lucio-Ruíz et al., 2024). The balanced presence of phenolics and glycosides across all Seaweed species further supports their potential as digestibility enhancers and gut health modulators, making them valuable components for formulating cost-effective and environmentally friendly ruminant feeds. This aligns with the growing interest in utilizing marine resources to address the dual challenges of livestock nutrition and environmental sustainability.

Dry Matter Degradation (DMD)

The data indicated that dry matter degradation (DMD) is influenced by the type of seaweed and its inclusion level in the complete feed. The highest average DMD was observed at the 15% inclusion level, with *Gracilaria sp* and *Sargassum sp* showing the highest DMD values at this level, at 37.04% and 36.85%, respectively. This suggests that *Sargassum sp* and *Gracilaria sp* can function effectively as feed components, optimizing dry matter degradation in the rumen. Supplementation of *Gracilaria sp*. seaweed as much as 4-8% in sheep feed can increase the digestibility of dry matter and organic matter (Prayitno et al., 2019). Previous studies have revealed that the polysaccharide content in seaweed can influence DMD by enhancing digestive enzyme activity and supporting microbial growth in the rumen (Cheong et al., 2023; Sofyan et al., 2022). On the other hand, cultivated *Caulerpa sp* exhibited the lowest DMD compared to other seaweed species, potentially due to its higher fiber content and antinutritional compounds that inhibit degradation. Wang et al. (2008) also highlighted that certain seaweed species with antioxidant and polyphenolic compounds may reduce DMD levels due to their antimicrobial effects, inhibiting the growth of rumen's fermentative microbes. This finding aligns with those who noted that different seaweed species can impact DMD variably, depending on their nutritional content (Gaillard et al., 2018). Some studies report that high inclusions of seaweed can reduce digestibility, while moderate supplementation often has no negative effects and can even improve nutrient utilization and feed efficiency (Hong et al., 2015; Terry et al., 2023). Overall, including *Sargassum sp* and *Gracilaria sp* at a 15% level appears to provide optimal benefits for dry matter degradation, supporting feed efficiency in livestock.

Organic Matter Degradation (OMD)

Organic matter degradation (OMD) results also show significant effects of seaweed type and inclusion level in the feed. The highest average OMD was observed at the 15% inclusion level, with *Sargassum sp* and *Euchema denticulatum* showing the highest OMD values at this level, at 47.62% and 47.60%, respectively. These findings suggest that at a 15% inclusion level, the organic content of seaweed can be optimally utilized in the rumen, effectively supporting organic matter digestion. Several studies have reported that bioactive compounds in seaweed, including polysaccharides and minerals, enhance OMD by facilitating fermentative microbial activity in the rumen (Maia et al., 2019; Liu et al., 2024). Meanwhile, cultivated *Caulerpa sp* exhibited relatively lower OMD, likely due to its high fiber content and less degradable compounds. These findings are consistent with a study by Pandey et al. (2022), which noted that the bioactive compounds in seaweed support dry and organic matter degradation, particularly at higher inclusion levels. Furthermore, Canul-Ku et al. (2023) revealed that differences in seaweed species influence the effectiveness of organic matter degradation in the rumen, with *Sargassum sp* and non-cultivated *Caulerpa sp* showing higher efficiency. This study indicates that including seaweed at a 15% level positively impacts organic matter degradation, supporting feed utilization efficiency in ruminants.

NH_3 (Ammonia)

In vitro fermentation analysis revealed that NH_3 levels increased with higher seaweed inclusion levels for most species, with *Gracilaria sp* recording the highest average (8.86mM) and *Eucheuma denticulatum* the lowest (7.37mM). Elevated NH_3 levels in the rumen are crucial as they serve as a nitrogen source for microbial protein synthesis, which improves feed efficiency in ruminants

(Stefenoni et al., 2021; Orzuna-Orzuna et al., 2024). However, excessively high NH_3 levels can lead to nitrogen imbalances and energy losses, as surplus NH_3 requires excretion through urea metabolism, burdening livestock metabolism (Choi et al., 2021). Lower NH_3 levels in *Eucheuma denticulatum* and *Caulerpa sp* suggest better nitrogen utilization by rumen microbes. Other studies corroborate that seaweed protein sources can moderate NH_3 production by providing a stable nitrogen supply and reducing excessive NH_3 formation (Maia et al., 2019; Stefenoni et al., 2021). Thus, selecting species like *Eucheuma denticulatum* could help maintain nitrogen balance in the rumen without compromising microbial activity.

Volatile Fatty Acids (VFA)

Increased VFA production in rumen fermentation is desirable, as VFAs are the primary energy source for ruminants. The data showed that 5-15% seaweed inclusion levels increased VFA levels, with *Gracilaria sp* and *Halimynea sp* reaching the highest averages (114.76 and 114.18mM, respectively). Research indicates that higher VFA concentrations correlate with more efficient fermentation, providing more energy for livestock metabolism (Machado et al., 2014; Mhatre et al., 2019). This aligns with findings that seaweeds are rich in complex polysaccharides, which serve as substrates for optimal VFA fermentation by rumen microbes (Liu et al., 2022). The significant VFA increases in *Gracilaria sp* and *Halimynea sp* are supported by their high carbohydrate content, which breaks down into acetic, propionic, and butyric acids as the main rumen fermentation products. Previous studies also show that seaweed supplementation can increase propionate proportions, reducing methane production and making the process more environmentally friendly (Cardoso-Gutierrez et al., 2021; Min et al., 2021). Therefore, including seaweed, particularly *Gracilaria sp* and *Halimynea sp*, effectively enhances VFA production, supporting ruminant metabolic energy.

Gas Production

According to the data (Table 5), the highest gas production occurred at a 15% seaweed inclusion level, especially for *Eucheuma cottoni* and *Eucheuma denticulatum* (134.84 and 134.34mL/g DM, respectively). High gas production indicates effective fermentation of organic matter, though it may also signal by-product gas generation (Rey-Crespo et al., 2014). Excessive gas production can be detrimental, as a portion consists of methane, which harms the environment and reduces livestock energy efficiency (Maia et al., 2019). Seaweeds like *Gracilaria sp* and *Halimynea sp* showed relatively lower gas production at the 15% level, indicating more efficient fermentation with potentially lower methane output (Min et al., 2021; Y. Liu et al., 2022)

Methane (CH_4) Production

The data (Table 5) show that *Sargassum sp* and *Eucheuma cottoni* produced the lowest methane levels at a 15% inclusion rate, at 43,864.52 and 31,444.05ppm, respectively. Studies indicate that certain seaweeds can reduce methane production due to their bioactive compounds, such as tannins and saponins, inhibiting the rumen's methanogenic microbial activity (Kim et al., 2013; Stefenoni et al., 2021). This aligns with findings by Narvaez-Izquiedo et al. (2024), which suggest that seaweeds with anti-methanogenic compounds can effectively moderate methane production in rumen fermentation systems. Lower methane output also benefits livestock metabolic energy, as the energy saved from reduced methane production can be redirected toward growth. Furthermore, Glasson et al. (2022) support that incorporating seaweed as a feed additive improves feed efficiency and reduces greenhouse gas emissions. Thus, including *Sargassum sp* and *Euchema cottoni* at the 15% level offers a promising alternative for reducing methane emissions without compromising fermentation quality.

Indicators of Fermentation Efficiency and Sustainability

Dry matter degradation (DMD) and organic matter degradation (OMD) are key measures of feed utilization efficiency in the rumen, reflecting the effective microbial breakdown of feed components into essential nutrients like microbial protein and energy (Machado et al., 2014; Choi et al., 2021). Seaweeds, rich in complex polysaccharides, promote fermentative microbes that enhance DMD and OMD by breaking down fibers into volatile fatty acids (VFA) such as acetate, propionate, and butyrate, which are primary energy sources for ruminants (Liu et al., 2022). This process also supports microbial protein synthesis (MPS), balancing nitrogen use and minimizing losses (Bhatta et al., 2009; Choi et al., 2021).

NH_3, VFAs, gas production, and methane emissions further indicate fermentation quality. Optimal NH_3 levels support microbial growth and protein synthesis, while VFAs provide direct energy for livestock. Seaweeds like *Gracilaria sp*. and *Halimynea sp*. yield higher VFAs, particularly propionate, which reduces methane emissions by acting as a hydrogen sink (Bhatta et al., 2009; Orzuna-Orzuna et al., 2024). Balanced NH_3 levels mark efficient fermentation, high VFA production, and minimal energy losses through urea or gas (Wang et al., 2008; Gülzari et al., 2019).

Methane and gas production are critical indicators of energy efficiency and environmental sustainability. Methane represents energy loss, but seaweeds like *Sargassum sp.* and *Eucheuma cottoni* reduce emissions through bioactive compounds like tannins and saponins that inhibit methanogenic microbes (Kinley et al., 2020; Kholif, 2023). Lower methane output allows conserved energy to support growth, enhancing livestock productivity (Roque et al., 2021). Moderate gas production indicates efficient feed fermentation with minimal waste (Wanapat et al., 2024). These benefits position seaweed supplementation as a promising strategy for boosting productivity and reducing the carbon footprint of ruminant farming systems (Min et al., 2021; Ahmed et al., 2023; De Bhowmick & Hayes, 2023).

Conclusion

The study highlights the potential of incorporating seaweed into ruminant diets to enhance fermentation efficiency and reduce methane emissions. Seaweed inclusion at a 15% level demonstrated optimal benefits, with *Sargassum sp*, *Gracilaria sp*, and *Halimynea sp* standing out due to their ability to enhance dry matter degradation (DMD), organic matter degradation (OMD), and volatile fatty acid (VFA) production. These species supported efficient microbial fermentation in the rumen, crucial for improving energy availability and livestock productivity.

Additionally, *Sargassum sp* and *Eucheuma cottoni* showed the lowest methane emissions due to their bioactive compounds like tannins and saponins inhibiting methanogenic microbes. While high gas production was observed in species like *Eucheuma cottoni*, lower methane output and improved fermentation quality in other species suggest their suitability for sustainable livestock feed. The findings underscore the importance of selecting the right seaweed species and inclusion levels to balance nutrient utilization, fermentation efficiency, and environmental sustainability. These results pave the way for seaweeds to become eco-friendly feed additives, enhancing ruminant productivity while mitigating greenhouse gas emissions.

Recommendations

1. Optimal Seaweed Selection: Prioritize the use of *Sargassum sp*, *Gracilaria sp*, and *Halimynea sp* in ruminant diets due to their superior fermentation characteristics, such as high DMD, OMD, and VFA production, along with their ability to reduce methane emissions.
2. Further Research: Expand studies on the long-term effects of seaweed inclusion on livestock health, productivity, and environmental impact. Investigate the interaction between seaweed-based diets and other feed components to maximize overall feed efficiency.

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