Research Article

Evaluation of Brown Seaweed (*Sargassum polycystum*) Flour Supplementation in Dairy Goat Diets on Estimated Methane Production and Nutrient Digestibility

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Abstract |This study evaluated how brown seaweed (*Sargassum polycystum*) flour supplementation in dairy goat diets affected estimated methane production and nutrient digestibility. Twenty-four Sapera dairy goats in their second to fourth lactation were allocated to six replicate groups in a randomized block design with four dietary treatments: T0 (basal diet without *S. polycystum* flour), T1 (basal diet + 2% *S. polycystum* flour), T2 (basal diet + 4% *S. polycystum* flour), and T3 (basal diet + 6% *S. polycystum* flour). Supplementation did not significantly affect (P>0.05) rumen pH, total volatile fatty acids, most individual VFA (except propionate), estimated methane production, microbial protein synthesis, or nutrient intake and digestibility. However, increasing inclusion levels significantly (P<0.05) increased ammonia (NH_3) and propionate concentrations and decreased the acetate:propionate ratio, indicating a shift in rumen fermentation toward a more energetically efficient pathway. Although the 6% level showed a slight numerical increase in estimated methane, no adverse effects on rumen function, nutrient utilization, or lactation performance were observed, and the 4% level appeared to provide a more stable response. *Sargassum polycystum* shows potential as a sustainable feed additive that enhances rumen efficiency without compromising animal performance, but direct methane measurements are needed to confirm its mitigation effects.

Keyword: Supplementation, *Sargassum polycystum*, Methane, Digestibility, Dairy Goat

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INTRODUCTION

Indonesia is a maritime nation with abundant marine resources, including seaweed. Among these resources, seaweed is a promising feed ingredient that remains underutilized. Seaweed has gained increasing attention as a sustainable addition feed additive because it does not compete with humans for land and freshwater resources. Moreover, several seaweed species contain high concentrations of minerals, macronutrients, and bioactive compounds that have been shown to significantly reduce enteric methane emissions.

Methane (CH_4) forms naturally during microbial fermentation of nutrients in the digestive tract of ruminants. Emissions of methane from the livestock sector contributes approximately 14.5–19% of global greenhouse gas emissions (Wanapat *et al*., 2024), of which

43% originate from ruminant livestock (Herrero *et al.*, 2016). In addition to its environmental impact, methane represents a loss of dietary energy, accounting for up to 12% of the gross energy intake of ruminants (Mayberry *et al.*, 2019). Therefore, reducing ruminal CH_4 production has the potential to improve feed efficiency and overall ruminant productivity (Kinley *et al.*, 2020).

Seaweed supplementation has been widely studied as a strategy to reduce enteric methane, with particular emphasis on tropical red seaweeds like *Asparagopsis* sp. (Kinley *et al.*, 2016). This species exhibits strong antimethanogenic activity, primarily due to its high content of halogenated compounds, notably bromoform (Machado *et al.*, 2016). However, bromoform is classified as a carcinogenic compound (DeMarini, 2020), and even at low incusion levels (67 g DM), it has been reported to cause rumenitis and result in detectable residues in urine and milk (Muizelaar *et al.*, 2021). These potential risks have increased interest in identifying more sustainable and safer seaweed resources that do not contain bromoform, such as brown seaweed.

Brown seaweeds of the genus *Sargassum* are widely distributed in tropical Indonesian waters. Several studies have evaluated *Sargassum sp.* supplementation in ruminant diets at various inclusion levels, but the results have been inconsistent. Prayitno *et al.* (2021) reported that supplementing with 2% *Sargassum sp.* reduced methane production by 83% and methanogenic bacteria by 75% in an in vitro study. Similarly, Park *et al.* (2022) demonstrated that inclusion of *Sargassum horneri* from Korea at 4% of the diet significantly reduced methane production. In contrast, Thorsteinsson *et al.* (2023) found that supplementing with 2% *Sargassum muticum* from Norway did not reduce CH_4 emissions, although no adverse effects on nutrient digestibility or milk production occurred during short-term trials. These inconsistent findings suggest that the antimethanogenic efficacy of *Sargassum* may depend on species, bioactive composition, dietary context, and adaptation of the rumen microbial ecosystem. Therefore, further in vivo studies are required to validate the effects of locally available *Sargassum* species under practical feeding conditions.

Table 1: Nutritional composition of treatment diets

Nutritional Composition (%)[a]	Treatments			
	T0	T1	T2	T3
DM	89.78	91.46	93.14	94.83
Ash	8.61	9.13	9.66	10.18
CP	15.52	15.92	16.32	16.72
EE	3.06	3.09	3.11	3.14
CF	20.69	21.17	21.65	22.13
NFE[b]	52.12	52.69	53.26	53.83
NDF	47.48	47.90	48.32	48.74
TDN[c]	65.28	66.15	67.02	67.89

[a] Dry Matter (DM) is expressed on an as-fed basis. Ash, crude protein (CP), ether extract (EE), crude fiber (CF), nitrogen-free extract (NFE), neutral detergent fiber (NDF), and total digestible nutrient (TDN) are expressed on a dry matter basis. DM, ash, CP, EE, CF were determined by proximate analysis. NDF was analyzed using the method of Van Soest.[b] NEF values were calculated according to the equation described by Budiman *et al.* (2006): NFE = 100 – (Ash + CP + EE + CF)[c] TDN values were calculated according to the equation described by Sutardi *et al.* (2001): TDN = 70.6 + (0.259 x CP) + (1.01 x EE) + (0.1 x NFE) – (0.76 x CF).

Norskov *et al.* (2021) reported that brown macroalgae of the genus *Sargassum* do not contain detectable levels of halomethanes. Instead, these seaweeds are characterized by the presence of phlorotannins, a class of natural phenolic compounds. Blanche *et al.* (2016) demonstrated that phlorotannins in brown seaweeds positively influence rumen function while reducing total gas production, including methane. In addition, brown seaweeds are rich in polysaccharides such as fucoidan, laminaran, and alginate (Wang and Cheong, 2023). Supplementation with these polysaccharides has been shown to increase the production of propionate (C_3) decreasing the proportion of acetate (C_2), thereby reducing hydrogen (H_2) availability for methanogenesis (Cheong *et al.*, 2023).

Despite the abundance of *Sargassum sp.* in Indonesian waters, particularly in the Java Sea, studies evaluating its use as a feed supplement in ruminant diets, especially in lactating dairy animals, remain limited. One of the most prevalent species in this region is *Sargassum polycystum* (AlgaeBase, 2022). To date, no in vivo studies have comprehensively evaluated this locally sourced brown seaweed effects on rumen fermentation, methane production, and nutrient utilization in lactating dairy ruminants. Therefore, this study aimed to evaluate the effects of brown seaweed (*Sargassum polycystum*) flour supplementation in dairy goat diets on estimated methane production and nutrient digestibility.

MATERIALS AND METHODS

Ethical approval

All procedures followed standard operating protocols. The experimental method was approved by the Research Ethics Committee of the Animal and Agricultural Sciences, Diponegoro University (No. 60-09/A-17/ KEP-FPP).

Experimental design, animal, and diet

Figure 1 shows a graphical summary of the experimental design and methods. The experiment was conducted using a randomized block design (RBD). Twenty-four Sapera dairy goats in their second to fourth lactation, with an

average milk yield of 1141.5 ± 286.5 g/day, were used as experimental units. Animals were blocked based on pre-experimental daily milk yield and randomly assigned to four dietary treatments (n = 6 per treatment): T0 (basal diet only without *S. polycystum* flour (SPF)), T1 (basal diet + 2% SPF), T2 (basal diet + 4% SPF), and T3 (basal diet + 6% SPF). The design consisted of six blocks, each comprising four goats with similar milk production. Within each block, animals were randomly allocated to T0, T1, T2, or T3 so that each treatment was represented once per block.

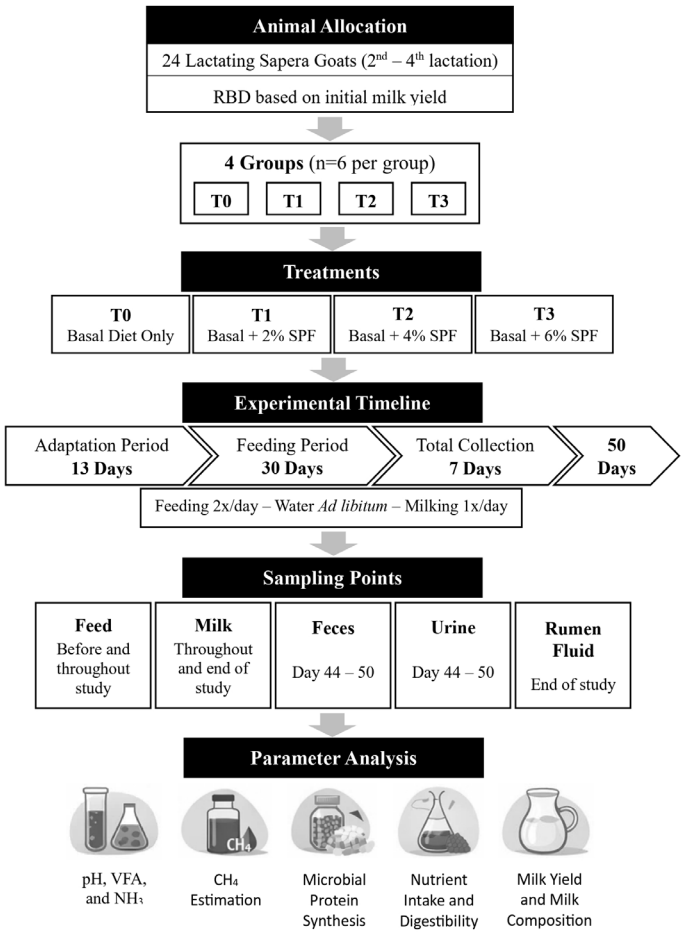

Figure 1: Graphical overview of the experimental materials and methods.

The study lasted 50 days, consisting of a 13-day adaptation period, a 30-day feeding period, and a 7-day total collection period. Goats were housed individually in stanchion-type pens with separate feeders and water containers. The basal diet consisted of field grass (30% of DM), tofu dregs (40% of DM), cassava dregs (10% of DM), and concentrate (20% of DM). Brown seaweed (*Sargassum polycystum*) flour was mixed into the basal diet at the designated inclusion levels before feeding. The nutritional composition of the experimental diets is presented in Table 1. Feed was offered twice daily (06:00 and 15:00). Fresh drinking water was available continuously, and milking occurred once daily at 08:00.

Brown seaweed (*Sargassum polycystum*) flour preparation

Fresh brown seaweed (*Sargassum polycystum*) was collected from the coastal area of Soko Beach, Sluke District, Rembang Regency, Central Java, Indonesia. Freshly harvested seaweed was washed thoroughly with running fresh water, soaked for six hours, then rinsed again to reduce salt content to below 10%. After washing, the seaweed was sun-dried until reaching a moisture content of approximately 10 – 12%. The dried seaweed was then ground into flour using a hammer mill equipped with a 2-mm screen. The seaweed flour was homogenized and stored in a cool, dry place until use.

Sampling procedures and sample analysis

Feed samples were collected during the pre-experimental period. Daily feed intake was calculated as the difference between feed offered and refusals, recorded throughout the study. During the 7-day total collection period, feces were collected daily using fecal collection nets, treated with 10% H_2SO_4 to prevent nitrogen loss, weighed after 24 hours, and subsampled (10%). Subsamples were dried, pooled per animal, homogenized, ground, and analyzed for nutrient composition. Feed intake and fecal nutrient data were used to calculate apparent nutrient digestibility according to the equations described by Tillman *et al.* (1991), as follows:

$$\text{Feed Nutrient Digestibility (\%)} = \frac{\text{Feed Nutrient Consumption (g)} - \text{Fecal Nutrient (g)}}{\text{Feed Nutrient Consumption (g)}} \times 100\%$$

Feed Nutrient Consumption (g) = Feed Consumption (g) x % Feed Nutrient Levels

Rumen fluid for fermentation analysis was collected from three donor goats of similar physiological status fed the same basal diet as the experimental animals. Samples were collected three hours after morning feeding (peak fermentation activity) using a stomach tube with a suction pump. The rumen fluid was immediately filtered through two layers of cheesecloth and temporarily stored in a pre-warmed thermos at 39 °C to preserve microbial viability and rumen-like temperature. The samples were pooled prior to incubation to reduce individual microbial variation and to obtain a representative inoculum. For in vitro incubation, pooled rumen fluid was dispensed into bottles containing substrate samples from each treatment and mixed with pre-warmed McDougall's buffer (1:4 ratio). Bottles were flushed with CO_2, sealed with butyl rubber stoppers, and incubated anaerobically at 39 °C in a water bath for 3 hours. After incubation, samples were centrifuged and the supernatants were used to determine pH, NH_3, and VFA concentrations. Ruminal pH directly measured with a calibrated pH meter. NH_3 concentration was analyzed using the Conway microdiffusion method (Dept. Dairy Sci., 1996). VFAs concentration were analyzed by gas

chromatography. Methane production was estimated from the molar proportions of acetate, propionate, and butyrate according to the equations described by Ørskov (1992), as follows:

Methane (mM) = 0.5 (Acetate) – 0.25 (Propionate) + 0.5 (Butyrate)

Microbial protein synthesis was calculated from urinary purine derivatives. Urine was collected daily during the total collection period, acidified with 10% H_2SO_4 to maintain pH < 3, measured for total volume and weight after 24 hours, and subsampled (10%). Subsamples were pooled per animal and analyzed for purine deruvates (allantoin, xanthine, hypoxanthine, and uric acid) using spectrophotometry. Microbial protein synthesis was estimated using equations from Chen and Gomes (1995), based on absorbed purines (X) calculated from total urinary purine derivative excretion (Y):$Y = 0.84X + (0.15W^{0.75} e^{-0.25x})$

Microbial nitrogen supply was subsequently calculated according to the equation described by Chen and Gomes (1992), as follows:

$$Microbial\ Nitrogen\ (g\ N/d) = \frac{X \times 70}{0.116 \times 0.83 \times 1000} = 0.727X$$

Average daily milk yield was obtained from production records during the feeding and collection periods. Milk samples (100 mL per goat) were collected during milking on the final day of the experiment, stored frozen, and analyzed for milk composition (fat, protein, and lactose using a Lactoscan.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) at $\alpha = 0.05$ to evaluate treatment effects. Prior to analysis, all data were tested for homogeneity of variance using Levene's test. Outliers were evaluated using standardized Z-scores, and observations with Z values below –2.5 or above +2.5 were considered potential outliers. The statistical model used was:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

where Y_{ij} is the observed response, μ is the overall mean, τ_i is the fixed effect of treatment (i = 0,1,2,3), B_j is the random effect of block based on pre-experimental milk yield (j = 1,2,3,4,5,6), and ε_{ij} is the residual error. When significant differences were detected, mean comparisons were analyzed using Duncan's multiple range test (DMRT). Statistical analyses were conducted using SPSS Statistics version 27 (IBM Corp., Armonk, New York, USA).

RESULT AND DISCUSSION

Ruminal pH

Sargassum polycystum flour supplementation did not significantly affect ruminal pH ($P > 0.05$). Ruminal pH values ranged from 6.23 to 6.30 across treatments, which remained within the optimal range for rumen microbial activity. Zhang *et al*. (2023) reported that normal ruminal pH for effective feed fermentation and microbial growth ranges from 5.5 to 7.0. The stable ruminal pH in this study suggests SPF supplementation did not cause excessive acid accumulation or impair the rumen buffering system. This indicates a balance between the production and utilization of fermentation acids, thereby maintaining favorable rumen conditions that support stable microbial fermentation and metabolic activity.

Ammonia concentration (NH_3)

Supplementation with SPF significantly affected ruminal ammonia (NH_3) concentration ($P < 0.05$), with values increasing progressively from T0 (4.08 ± 1.13 mM/L) to T3 (6.72 ± 1.54 mM/L). Post-hoc analysis indicated that NH_3 concentration in T3 was significantly higher than in T0 and T1, whereas T2 showed intermediate values and did not differ significantly from any treatment (Table 2). Furthermore, no significant difference was observed between T0 and T1, indicating that low-level SPF supplementation did not substantially alter ruminal nitrogen availability. The intermediate response in T2 indicates a gradual increase in nitrogen degradation. The marked increase at T3 reflects enhanced deamination of dietary protein and amino acids by rumen microbes. This effect is likely associated with the higher organic nitrogen supply from SPF at greater inclusion levels. Min *et al*. (2021) reported that brown seaweeds contain substantial crude protein and non-protein nitrogen compounds, which increase nitrogen substrate availability for proteolytic microorganisms and stimulate ruminal NH_3 production.

Ruminal NH_3 concentration is strongly influenced by the solubility and degradability of dietary protein (Hambakodu *et al*., 2019; Prayitno *et al*., 2018). Holik *et al*. (2019) similarly reported that higher dietary protein levels are associated with increased ruminal N–NH_3 concentrations in ruminants. Accordingly, the observed increase in NH_3 concentration in this study is likely associated with the gradual increase in crude protein content of the experimental diets, which rose from 15.52% in T0 to 16.72% in T3. Importantly, NH_3 concentrations across all treatments remained within the optimal range required to support rumen microbial growth and microbial protein synthesis, which has been reported to be between 3–9 mM/L (Hong *et al*., 2015). Furthermore, the increase in NH_3 concentration in this study was accompanied

Table 2: Rumen Fermentability Results Due to *Sargassum polycystum* Flour Supplementation

Parameters	Treatments			
	T0	T1	T2	T3
Ruminal pH	6.24 ± 0.08	6.24 ± 0.05	6.23 ± 0.04	6.30 ± 0.00
NH_3 (mM/L)	4.08^{b} ± 1.13	4.41^{b} ± 0.73	5.07^{ab} ± 1.23	6.72^{a} ± 1.54
Total VFA (mM)	151.16 ± 12.74	156.52 ± 20.04	158.26 ± 21.81	167.00 ± 7.49
Partial VFA (mM)				
Acetate	81.12 ± 9.67	82.46 ± 10.55	82.92 ± 11.73	87.54 ± 5.63
Propionate	39.02^{b} ± 2.54	44.12^{ab} ± 4.10	45.46^{a} ± 4.62	47.36^{a} ± 1.96
Isobutirate	3.78 ± 0.26	3.42 ± 0.69	3.48 ± 0.65	3.76 ± 0.34
Butirate	16.70 ± 0.89	16.58 ± 3.09	16.72 ± 3.32	17.74 ± 1.18
Isovalerate	6.76 ± 0.60	6.34 ± 1.33	6.32 ± 1.13	7.04 ± 0.46
Valerate	3.78 ± 0.35	3.60 ± 0.67	3.36 ± 0.61	3.62 ± 0.27
AP Ratio (mM)	2.083^{b} ± 0.29	1.864^{ab} ± 0.09	1.817^{a} ± 0.09	1.852^{ab} ± 0.15
CH_4 (mM)	39.16 ± 5.59	38.49 ± 5.84	38.46 ± 6.38	40.82 ± 3.20
(95% CI)	(39.16 ± 5.87)	(38.49 ± 6.13)	(38.46 ± 6.70)	(40.82 ± 3.36)
Microbial Protein Synthesis (g N/d)	8.79 ± 8.46	9.90 ± 2.99	11.76 ±7.66	11.98 ± 10.95
Allantoin	1.87 ± 0.18	1.88 ± 0.14	1.93 ± 0.05	1.87 ± 0.21
Uric Acid	0.16 ± 0.03	0.17 ± 0.02	0.17 ± 0.03	0.15 ± 0.02

Values are presented as mean ± SD (n = 6 samples per treatment).
^{ab}: Different superscripts within the same row indicate significant differences (P < 0.05).

by higher microbial protein synthesis and total VFA production. This suggests that the significant differences among treatments reflect an enhancement in ruminal nitrogen metabolism and microbial activity, rather than an inefficiency in nitrogen utilization.

Volatile fatty acid (VFA) concentration

Total VFA concentrations ranged from 151.16 ± 12.74 mM (T0) to 167.00 ± 7.49 mM (T3), showing a numerical increase with higher SPF levels that was not statistically significant (P > 0.05). Total ruminal VFA concentration is strongly influenced by dietary chemical composition, particularly carbohydrate availability (Riswandi *et al.*, 2017), and the lack of significant differences in this study may be attributed to the similar carbohydrate composition among treatments, as reflected by comparable crude fiber and nitrogen-free extract levels. This is supported by similar DM and OM digestibility across diets. Syarifudin *et al.* (2019) reported that increases in carbohydrate fermentation products, expressed as VFA, are closely associated with increases in dietary carbohydrate supply and show a parallel relationship with DM and OM digestibility. Nevertheless, the numerical increase in total VFA concentration with higher SPF levels suggests that SPF did not impair ruminal fermentation activity. This pattern should be interpreted cautiously, as it may reflect normal biological variability rather than a definitive treatment effect. However, considering the complex polysaccharide composition of SPF, which can serve as fermentable substrates for rumen microorganisms (Wang and Cheong, 2023), it is plausible that SPF provided additional fermentable carbohydrates without negatively affecting microbial metabolism. The inclusion of such substrates has been reported to stimulate microbial activity, growth rate, and substrate degradation in the rumen, potentially influencing VFA production under certain conditions (Nuswantara *et al.*, 2021). Therefore, while no statistically detectable enhancement of total VFA was observed in the present study, the data suggest that SPF supplementation maintained normal ruminal fermentation dynamics.

Acetate (C2) concentration in this study tended to increase with increasing levels of SPF supplementation, although the effect was not statistically significant (P > 0.05). Suwandyastuti (2013). noted that minimal differences in dietary crude fiber content do not result in significant changes in ruminal fermentation end products, which is consistent with the relatively similar crude fiber levels among the experimental diets. Acetate concentrations ranged from 81.12 ± 9.67 mM in T0 to 87.54 ± 5.63 mM in T3. These relatively high and increasing values indicate that fiber degradation in the rumen remained active under SPF supplementation, which is consistent with the generally high crude fiber content of the experimental diets known to favor acetate production during ruminal fermentation (Li *et al.*, 2019). In addition, the polysaccharide fraction of SPF may have contributed additional structural carbohydrates, thereby supporting acetate formation in the rumen (Wanapat *et al.*, 2014).

Propionate (C3) concentration was significantly affected by SPF supplementation ($P < 0.05$), showing a gradual and significant increase with increasing supplementation levels, from 39.02 ± 2.54 mM in T0 to 47.30 ± 1.96 mM in T3 (Table 2). The lowest propionate concentration observed in T0, which differed significantly from T2 and T3, reflects a ruminal fermentation pattern predominantly oriented toward the acetogenic pathway. This is further supported by the acetate-to-propionate ratio, which was highest in T0. The numerical increase in propionate concentration in T1 suggests that SPF supplementation began to shift the fermentation pattern toward propionate production; however, the effect was not sufficiently strong to produce a statistically significant difference relative to T0, T2, or T3. In contrast, the significant increases in propionate concentration observed in T2 and T3 compared with T0 indicate that SPF supplementation at levels of 4–6% was sufficient to markedly redirect ruminal fermentation toward the propionate pathway. This shift is likely associated with the presence of bioactive polysaccharides in *Sargassum* sp., which have been reported to stimulate populations of propionate-producing bacteria while simultaneously suppressing methanogenic microorganisms through competition for H_2 utilization (Belanche *et al.*, 2016; Cheong *et al.*, 2023). This increase is consistent with the trend observed for total VFA production, indicating enhanced ruminal microbial fermentation activity.

Butyrate (C4) concentration in this study remained relatively stable and was not significantly affected by SPF supplementation ($P > 0.05$). This lack of a significant effect may be attributed to the relatively similar levels of crude fiber and nitrogen-free extract matter among the experimental diets. Although no statistical differences were detected, butyrate concentrations showed a slight numerical increase from 16.70 ± 0.89 mM in T0 to 17.74 ± 1.18 mM in T3. This modest upward trend may reflect a well-balanced ruminal microbial ecosystem supported by the polysaccharide content of SPF, which can enhance the activity of butyrate-producing bacteria. Wang *et al.* (2022) reported that butyrate production primarily results from the degradation of slowly fermentable fibrous carbohydrates and starch, supporting the observed stability of C4 concentrations across treatments.

The concentrations of minor volatile fatty acids, including isobutyrate, isovalerate, and valerate, were also not significantly affected by SPF supplementation ($P > 0.05$). Puastuti *et al.* (2024) reported that branched-chain VFAs composition is influenced by the protein content of the diet, particularly through the degradation of branched-chain amino acids. The lack of significant differences in iso-VFA concentrations suggests that the modest increase in dietary protein with SPF supplementation was insufficient to substantially alter branched-chain amino acid fermentation. The observed ranges were 3.42–3.78 mM for isobutyrate, 6.32–7.04 mM for isovalerate, and 3.36–3.78 mM for valerate. The stability of iso-VFA concentrations, despite increased NH_3 levels, indicates that protein deamination occurred within normal physiological limits and that nitrogen utilization remained efficient to support microbial protein synthesis.

Acetate propionate ratio (A:P ratio)

Supplementation with SPF significantly affected the acetate-to-propionate (A:P) ratio ($P < 0.05$), resulting in a decrease in the A:P ratio in the supplemented groups (Table 2). The highest A:P ratio occurred in the control group (T0: 2.083 ± 0.25), while SPF-supplemented groups showed lower, relatively similar values: T1 (1.864 ± 0.09), T2 (1.817 ± 0.09), and T3 (1.855 ± 0.15). The significantly higher A:P ratio in T0 compared with T2 reflects the predominance of the acetogenic fermentation pathway in the control diet, which generates greater amounts of H_2 relative to the supplemented treatments. Treatments T1 and T3 exhibited intermediate A:P ratios and did not differ significantly from either T0 or T2, although both showed a numerical reduction relative to the control. The lowest A:P ratio was observed in T2, which differed significantly from T0, indicating that SPF supplementation at 4% effectively shifted ruminal fermentation toward the propionate-producing pathway. A lower A:P ratio reflects a greater contribution of propionate to total VFA production. Choi *et al.* (2020) reported that an increased proportion of propionate reduces energy loss as methane, as propionate formation competes with methanogenesis by consuming H_2, a key substrate for methane synthesis.

The significant decrease in the A:P ratio observed in this study is consistent with the significant increase in propionate concentration and the absence of a significant effect on acetate concentration. This shift in fermentation from acetate toward propionate has important implications for ruminal energy efficiency, as propionate formation utilizes H_2 as a reductant, whereas acetate formation releases free H_2, thereby supporting methanogenic activity (Martin *et al.*, 2007). Furthermore, the reduction in the A:P ratio, accompanied by a gradual increase in total VFA concentration, indicates that ruminal microbial fermentation activity remained optimal. These results suggest that SPF supplementation did not impair the capacity of rumen microbes to digest fiber and carbohydrates, but rather redirected fermentation toward a more energetically efficient pathway with lower methane production potential. In agreement with these findings, Belanche *et al.* (2016) reported that polyphenolic compounds in brown seaweeds selectively suppress methanogenic activity without inhibiting fibrolytic

bacterial populations responsible for fiber degradation.

Estimated methane gas production (CH_4)

Supplementation with SPF did not significantly affect estimated methane production ($P > 0.05$). Estimated CH_4 production remained relatively stable from T0 to T2 (39.16 ± 5.59, 38.49 ± 5.84, and 38.46 ± 6.38 mM, respectively) and showed a slight numerical increase at T3 (40.82 ± 3.20 mM). Although these differences were not statistically significant, SPF supplementation at 2–4% (T1–T2) tended to reduce methane production compared with the control treatment (T0). This pattern corresponds with the gradual reduction in A:P ratio observed from T0 to T2, indicating a modest shift in ruminal fermentation toward the propionate pathway. Propionate production by H_2-utilizing rumen microbes can reduce methanogenesis substrate supply, thereby lowering CH_4 production (Janssen, 2010).

The slight numerical increase observed at T3 was remained within the normal range of biological variation. Moreover, the absence of a consistent dose–response pattern across treatments suggests that this change was likely influenced by individual animal variability rather than a direct treatment effect. However, the possibility of microbial adaptation to higher SPF doses cannot be excluded. Previous studies on seaweed supplementation have reported similar responses. Belanche *et al*. (2016) reported that inclusion of brown seaweed at 5% of dietary dry matter had minimal effects on the overall richness, diversity, and composition of ruminal bacterial and archaeal communities, but induced shifts in carbohydrate metabolism, including increased degradation of xylan and carboxymethylcellulose. This response is consistent with the higher VFA concentrations observed in this study at the 6% SPF supplementation level. Shannon and Abu-Ghannam (2016) reported that *Sargassum* sp. contains phlorotannins that are thought to suppress methanogen growth by binding to bacterial proteins such as enzymes and cell membranes, leading to cell lysis. Furthermore, they suggested that increasing levels of *Sargassum* sp. may reduce the antimethanogenic efficacy of phlorotannins, potentially due to a decrease in fibrolytic microbial populations that do not produce H_2, which may indirectly favor methanogenic activity. These findings suggest an optimal SPF inclusion level exists, beyond which methane mitigation efficiency may decline.

Although the 6% inclusion level (T3) showed a slight numerical increase in estimated methane production and a modest reduction in NDF digestibility compared with the 4% treatment (T2), these differences were not statistically significant and were not accompanied by reductions in overall nutrient digestibility or other aspects. Therefore, the numerical response observed at 6% does not provide statistical or biological evidence of a detrimental threshold effect under the conditions of this study. Rather, the data suggest an attenuation of antimethanogenic efficacy at higher inclusion levels, potentially related to broader antimicrobial activity of phlorotannins or microbial adaptation phenomena. Importantly, the absence of negative effects on rumen fermentation stability and productive performance indicates that 6% inclusion remains physiologically safe. However, based on the higher reduction in the A:P ratio and the absence of numerical attenuation in digestibility parameters at 4%, this level may represent a more efficient inclusion rate for fermentation modulation. Therefore, the 4% supplementation level may be considered a practical and efficient inclusion rate, as it achieved similar responses while using a lower dietary inclusion level.

In the present study, propionate concentration increased and the A:P ratio decreased significantly with increasing levels of SPF supplementation, indicating a shift in fermentation toward a more glucogenic pathway that theoretically competes with methanogenesis for H_2 utilization. However, the absence of a significant reduction in estimated methane production suggests that changes in VFA proportions are not always accompanied by measurable changes in overall ruminal H_2 utilization. This may be related to the methane values in this study being derived from stoichiometric estimation based on VFA profiles using the empirical equation developed by Ørskov (1992), rather than from direct gas measurements. This approach assumes a fixed relationship between fermentation end products and CH_4 formation and does not account for microbial population dynamics or alternative H_2 sinks. Therefore, although it is theoretically possible that part of the redirected reducing equivalents was utilized in pathways such as reductive acetogenesis, sulfate reduction, or incorporation into microbial biomass, these mechanisms were not directly evaluated in the present study and remain speculative. Consequently, a more parsimonious interpretation is that the VFA shift did not translate into a measurable change in methane under the conditions of this study, and that the indirect estimation method may have limited sensitivity to detect subtle shifts in H_2 partitioning.

The lack of a significant response in CH_4 production in the present study may also be partly explained by acetate and butyrate concentrations, which did not differ significantly among treatments, although both tended to increase. The increase in acetate and butyrate, together with the tendency for propionate production to increase, may mathematically elevate the calculated estimates of CH_4 production. Choi *et al*. (2020) suggested that inconsistencies in experimental methane emissions may be related to increased acetate and

butyrate concentrations, as both are positively correlated with CH_4 formation.

Overall, *S. polycystum* appears to modulate fermentation patterns by directing them toward the propionate pathway without substantially disrupting hydrogen balance or methanogen activity. This may explain why fermentation shifts did not translate into estimated methane reductions. Future studies should integrate molecular microbiological approaches and direct gas measurements to clarify ruminal hydrogen dynamics. Metagenomic analysis could evaluate methanogen population responses, and quantifying propionate-producing bacteria would show whether community structure changes accompanied the fermentation shift. Targeted molecular analysis alternative H_2-utilizing microbial groups would help explain the possible diversion of reducing equivalents beyond methanogenesis. In addition, the present study did not quantify halogenated compounds or phlorotannin concentrations in the tested *S. polycystum*. Therefore, the proposed antimethanogenic mechanisms and safety considerations remain literature-based and should be confirmed through detailed chemical and toxicological analyses in future studies.

Microbial protein synthesis

SPF supplementation did not significantly affect microbial protein synthesis ($P > 0.05$). Nevertheless, a gradual numerical increase was observed with increasing supplementation levels, from 8.79 ± 8.46 g N/d at T0 to 11.98 ± 10.96 g N/d at T3. This non-significant response is consistent with the pattern observed for total VFA concentration, which also did not differ statistically among treatments. The parallel trend between VFA production and microbial protein synthesis suggests that carbohydrate fermentation supplied sufficient fermentable energy to support microbial growth and nitrogen incorporation. This is in line with the theory of Chen and Gomes (1995) that microbial protein synthesis increases if the supply of NH_3 and fermented energy (VFA) is available in balance.

The increase in the value of microbial protein synthesis is also in line with the pattern of changes in NH_3 concentration, which in this study increased gradually as the level of SPF supplementation increased. Hong *et al.* (2015) said that the concentration of N-NH_3 within the normal physiological range is able to support optimal rumen microbial growth and protein synthesis. The upward trend in microbial protein synthesis may also be associated with the slightly higher crude protein content of the treatment diets as SPF supplementation increased. Alves *et al.* (2014) stated that increasing protein levels in feed increases the availability of amino acids and peptides for rumen microbes, which can support microbial protein synthesis. Increased microbial protein synthesis indicates a shift towards more efficient rumen fermentation, which positively influences energy utilization by rumen microbes and supports the biological mechanism of methane reduction through increased H_2 utilization by non-methanogenic microbes.

Nutrient intake and digestibility

Dietary supplementation with SPF did not significantly affect nutrient intake ($P > 0.05$). Dry matter (DM), organic matter (OM), crude protein (CP), and neutral detergent fiber (NDF) intakes ranged from 2.242–2.314 kg, 2.037–2.078 kg, 0.348–0.387 kg, and 1.066–1.128 kg, respectively (Table 3). The absence of significant differences in DM intake is consistent with the similar DM content of the experimental diets and the lack of significant differences in DM digestibility among treatments. In addition, animal-related factors were relatively uniform across treatment groups, contributing to comparable intake levels. Lubis (1992) reported that DM intake is influenced by both dietary factors, such as digestibility and palatability, and animal-related factors, including breed, sex, age, body weight, and health status. Similarly, OM, CP, and NDF intakes were not significantly affected by SPF supplementation, reflecting their close association with DM intake. Zulbadri *et al.* (1995) stated that changes in DM intake are generally followed by proportional changes in the intake of other nutrients. Therefore, the stable intake of DM across treatments explains the lack of significant variation in OM, CP, and NDF consumption observed in this study.

Dry matter digestibility (DMD) in this study was not significantly affected by SPF supplementation ($P > 0.05$). Digestibility is strongly influenced by feed intake, the chemical composition of feed ingredients, and rumen microbial activity (Tillman *et al.*, 1998; McDonald *et al.*, 2010). This relationship is evident in the present study, where DMD values were consistent with the relatively similar DM intake observed across treatments. The relatively low inclusion level of SPF (2–6% DM) was insufficient to alter the overall nutrient balance of the ration, resulting in no significant change in total nutrient digestibility, including DMD. DMD remained high and stable, ranging from 78.00 ± 3.05% (T3) to 79.74 ± 2.24% (T1), indicating that SPF supplementation up to 6% DM did not disrupt rumen fermentation or fibrolytic microbial activity. These high and stable values suggest efficient nutrient degradation and energy supply to support productivity, consistent with the stable and relatively high total VFA concentrations observed. Hambakodu *et al.* (2019) noted that VFA serve as carbon skeletons for microbial growth, enhancing feed degradation efficiency and supporting stable digestibility values, particularly DMD and OMD.

Table 3: Nutrient Intake and Digestibility Results Due to *Sargassum polycystum* Flour Supplementation.

Parameters	Treatments			
	T0	T1	T2	T3
Nutrient Consumption (kg/day)				
Dry Matter	2.244 ± 0.07	2.242 ± 0.11	2.278 ± 0.11	2.314 ± 0.12
Organic Matter	2.051 ± 0.06	2.037 ± 0.09	2.058 ± 0.10	2.078 ± 0.10
Crude Protein	0.348 ± 0.01	0.357 ± 0.01	0.372 ± 0.01	0.387 ± 0.01
Neutral Detergent Fiber	1.066 ± 0.03	1.074 ± 0.04	1.101 ± 0.05	1.128 ± 0.05
Nutrient Digestibility (%)				
Dry Matter	78.60 ± 4.33	79.74 ± 2.24	78.14 ± 4.09	78.00 ± 3.05
Organic Matter	86.31 ± 2.44	85.74 ± 2.19	85.86 ± 2.98	86.22 ± 2.55
Crude Protein	84.95 ± 2.86	85.19 ± 2.23	85.21 ± 2.67	85.69 ± 2.05
Neutral Detergent Fiber	75.18 ± 5.81	74.15 ± 2.76	72.65 ± 4.17	73.67 ± 4.30

Values are presented as mean ± SD (n = 6 goats per treatment).
No significant differences were observed among treatments (P > 0.05).

Organic matter digestibility (OMD) in this study was not significantly affected by SPF supplementation (P > 0.05). It was consistent with similar torganic matter (OM) intake and dietary OM content across treatments. The OMD values obtained were relatively high and stable, ranging from 85.86 ± 2.98% (T2) to 86.31 ± 2.44% (T0). These stable values indicate that SPF supplementation up to 6% DM did not impair the capacity of rumen microorganisms to digest the organic fraction of the diet. Cheong *et al.* (2023) reported that polysaccharides present in brown algae can enhance rumen fermentation by stimulating the growth and activity of fermentative bacteria, thereby improving the utilization of organic matter. The high OMD values observed in this study further indicate that a large proportion of organic nutrients was effectively utilized by the animals. Wajizah *et al.* (2015) also reported that high DMD and OMD values are associated with a balanced supply of nutrients that supports optimal rumen microbial growth and activity, leading to efficient feed digestion.

Crude protein digestibility (CPD) in this study was not significantly affected (P > 0.05) by SPF supplementation. Zahera *et al.* (2020) stated that CPD is influenced by the balance between protein availability in the diet and rumen microbial activity in degrading protein into ammonia and amino acids. The lack of a significant effect on CPD is consistent with similar crude protein intake and minimal variation in dietary CP levels among treatments. The CPD values obtained were relatively high and uniform, ranging from 84.95 ± 2.86% (T0) to 85.69 ± 2.06% (T3). These high CPD values indicate efficient nitrogen utilization, both through microbial protein degradation in the rumen and the absorption of amino acids derived from protein digestion in the small intestine (Tillman *et al.*, 1998). The uniformity of CPD across treatments suggests that SPF supplementation up to 6% DM did not disrupt proteolytic microbial activity or protein fermentation dynamics in the rumen.

Neutral detergent fiber digestibility (NDF-D) in this study was not significantly affected (P > 0.05) by SPF supplementation. This likely reflects the similar dietary NDF content and intake across treatments. Hambakodu *et al.*, (2019) noted that NDF digestibility is strongly influenced by the amount and composition of structural carbohydrates entering the rumen; thus, minimal variation in dietary NDF generally leads to comparable fiber degradation. This is supported by the rumen fermentation profile, where acetate and butyrate—key end main products of fiber fermentation—also showed no significant differences, indicating stable fibrolytic microbial activity across all treatment groups. NDF-D ranged from 72.65 ± 4.17% (T2) to 75.18 ± 5.81% (T0), showing a slight numerical decrease in SPF-supplemented treatments although it was not statistically significant. This minor reduction may be related to polyphenolic compounds, particularly phlorotannins in *Sargassum* spp., which can moderately inhibit fibrolytic microbes at higher inclusion levels (Shannon and Abu-Ghannam, 2016). However, the stable VFA profile, especially acetate and butyrate concentrations, suggests that fiber fermentation was not biologically compromised. It is likely because rumen microbes can utilize brown algae polysaccharides such as laminaran and alginate without suppressing degradation of other fiber fractions (Wang and Cheong, 2023). Consequently, rumen fermentation and fiber digestibility remained within a normal and efficient physiological range.

In addition to rumen fermentation and nutrient utilization parameters, productive performance indicators were evaluated to determine whether SPF supplementation affected the metabolic status of lactating goats (Table 4).

Table 4: Milk Yield and Milk Composition Results Due to *Sargassum polycystum* Flour Supplementation.

Parameters	Treatments			
	T0	T1	T2	T3
Milk Yield (kg/d)	1.072 ± 0.30	1.193 ± 0.23	1.190 ± 0.30	1.118 ± 0.30
Milk Composition (%)				
Fat	5.71 ± 0.68	5.26 ± 1.58	5.12 ± 0.49	5.14 ± 1.20
Protein	3.92 ± 0.13	3.70 ± 0.20	3.96 ± 0.17	3.76 ± 0.38
Lactose	3.71 ± 0.12	3.60 ± 0.09	3.75 ± 0.15	3.68 ± 0.28

Values are presented as mean ± SD (n = 6 goats per treatment).
No significant differences were observed among treatments (P > 0.05).

Supplementation of SPF at 2–6% DM did not significantly affect (P > 0.05) daily milk yield or milk composition (fat, protein, and lactose). Milk production ranged from 1.072 ± 0.30 kg/day (T0) to 1.193 ± 0.23 kg/day (T1). Similarly, milk fat (5.12–5.71%), protein (3.70–3.96%), and lactose (3.60–3.75%) contents remained stable across treatments and were within acceptable quality standards for fresh goat milk. The absence of significant differences in milk yield and composition is consistent with the unchanged nutrient intake and digestibility observed in this study, indicating that SPF supplementation did not impair nutrient availability for mammary synthesis. It was consistent with Thorsteinsson *et al.* (2023), who reported that several brown seaweeds improved rumen fermentation without significantly affecting digestibility, milk yield, or milk composition in dairy cows. Since milk production in ruminants depends on the supply of metabolizable energy, glucose precursors, and absorbable amino acids, the stable milk output supports the conclusion that metabolic performance was not adversely affected.

Although SPF supplementation significantly increased propionate concentration and reduced the acetate-to-propionate ratio, this fermentation shift did not translate into measurable improvements in milk production under the experimental conditions. The increase in propionate theoretically enhances hepatic gluconeogenesis and glucose availability (Cheong *et al.*, 2023), which may support lactose synthesis and milk volume (Muktiani, 2002; Adriani, 2021). However, the magnitude of this shift was insufficient to induce statistically detectable production responses. Therefore, the observed fermentation modulation should be interpreted as a metabolic adjustment rather than a confirmed productivity enhancement.

CONCLUSIONS

Supplementing with 2-6% *Sargassum polycystum* flour did not negatively affect nutrient intake, digestibility, rumen fermentation, microbial protein synthesis, milk yield, or milk composition in lactating dairy goats, indicating safety under these experimental conditions. Increasing inclusion levels enhanced ruminal propionate and improved the fermentation profile; however, these were not associated with significant reductions in estimated methane production. The slight numerical increase at the 6% level did not indicate a negative threshold effect, although 4% appeared to provide a more stable fermentation response. *Sargassum polycystum* shows potential as a sustainable feed additive that enhances rumen efficiency without compromising animal performance, but direct methane measurements are needed to confirm its mitigation effects.

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NOVELTY STATEMENT

This study provides the first in vivo evaluation of locally sourced Sargassum polycystum in lactating dairy goats, demonstrating its ability to shift rumen fermentation toward a more energetically efficient pathway without compromising nutrient utilization or animal performance.

AUTHOR'S CONTRIBUTION

Intan Claudia Singgi was responsible for the research conception, supervision, and writing of the original draft and the manuscript. Limbang Kustiawan Nuswantara and Anis Muktiani contributed to manuscript validation, with Limbang Kustiawan Nuswantara also involved in research conception. Nirwani Soenardjo contributed to project administration. All authors reviewed and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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