Veterinary Integrative Sciences 2026; 24(3): e2026064-1-17. DOI: 10.12982/VIS.2026.064

Veterinary Integrative Sciences
ISSN; 2629-9968 (online)

Research article

Effects of commercial seaweed supplementation on *in vitro* methane production and milk performance in dairy

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Abstract

This study aimed to evaluate the effects of commercial seaweed supplementation on ruminal fermentation, methane production, and milk performance in dairy cows during prepartum and postpartum periods. The experiment consisted of two parts. Experiment 1 assessed the impact of seaweed on rumen digestion using the *in vitro* gas production technique with rumen fluid collected from two Holstein Friesian cows (with an average body weight of 427 ± 10 kg and an average age of 3 years). Total gas, methane, pH, ammonia-nitrogen (NH_3-N), volatile fatty acids (VFA), and microbial biomass yield were analyzed in a completely randomized design. Experiment 2 consisted of on-farm trials conducted at two commercial dairy farms. At Thongsak Farm, twenty prepartum cows (18.16 ± 1.06 kg/day milk yield; 420 ± 25 kg BW) were evaluated from 30 days before to 90 days after calving and assigned to either the control (TMR only) or treatment (TMR + 40 g seaweed/day) group. Feed intake was recorded daily, while blood samples were collected three times and milk samples seven times throughout the experimental period. At Somsak Farm, another twenty postpartum cows (16.5 ± 1.0 kg/day milk yield; 425 ± 25 kg BW; 100.05 ± 67.25 DIM) were evaluated for 30 days using the same treatment structure and sampling protocol as in the prepartum trial, with feed intake and milk production recorded concurrently. *In vitro* results showed that seaweed supplementation significantly enhanced gas production at 2 hours (7.27 vs 6.61 mL/200 mg DM) and reduced methane production (5.22 vs 2.30 mL/200 mg; $p < 0.05$). On-farm results showed a tendency toward higher milk yield in the seaweed group, along with increased protein, total solids, and solids-not-fat. Blood profiles remained within normal ranges, indicating no adverse effects. Seaweed supplementation improved rumen fermentation efficiency reduced methane emissions and positively influenced milk yield and composition in dairy cows without compromising animal health.

Keywords: Dairy cow, Methane, Milk production, Rumen fermentation, Seaweeds.

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Article history; *received manuscript: 19 September 2025,*
revised manuscript: 25 October 2025,
accepted manuscript: 26 December 2025,
published online: 14 January 2026,
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Livestock production has been estimated to contribute approximately 28–33% of global methane (CH_4) emissions; however, Goodland and Anhang (2009) suggested that the contribution from livestock could be as high as 51%. Methane is a potent greenhouse gas with a global warming potential 23 times greater than that of carbon dioxide. In ruminants, methane is primarily produced during ruminal fermentation, where methanogenic archaea utilize hydrogen and carbon dioxide generated during microbial digestion of organic matter to form methane, which is expelled via eructation (Owens et al., 1998; Janssen and Kirs, 2008). A lactating dairy cow typically emits 300–600 L of methane per day, highlighting the environmental significance of enteric methane emissions.

Among dietary mitigation strategies, seaweed supplementation has gained attention due to its potential to suppress methanogenesis while maintaining animal productivity. Red seaweeds, particularly *Asparagopsis* spp., contain bromoform, which inhibits methyl-coenzyme M reductase, the terminal enzyme in the methanogenic pathway (Machado et al., 2016; Kinley et al., 2020). Brown seaweeds provide phlorotannins and minerals that may modulate rumen microbial populations and fermentation patterns (Wang et al., 2021), whereas green seaweeds supply polysaccharides and bioactive compounds that may enhance nutrient utilization and immune function (Choi et al., 2021). Therefore, combining multiple seaweed species may offer complementary and synergistic effects on rumen fermentation and animal performance.

Previous studies have evaluated both individual and mixed seaweed species as dietary additives for dairy cattle. Nichols et al. (2019) reported that supplementation with *Saccharina latissima* and *Fucus serratus* reduced methane emissions by 6.1–13.9% and altered milk composition, whereas *Chondrus crispus* had no effect. In addition, low-dose inclusion of red seaweeds has been shown to markedly reduce methane emissions without adversely affecting feed intake, milk yield, or animal health (Kinley et al., 2020; Roque et al., 2021; Bhowmick et al., 2023).

Commercial blended seaweed supplements, containing red, green, and brown seaweeds, are increasingly available and offer practical advantages for smallholder dairy systems, particularly in tropical regions such as Thailand, due to their standardized composition and ease of application. However, information remains limited regarding the combined effects of such commercial products on rumen fermentation and lactational performance under tropical on-farm conditions.

Therefore, this study aimed to evaluate the effects of a commercial blended seaweed supplement on methane production using an *in vitro* gas production technique and to determine its impacts on milk yield, milk composition, and blood metabolites in dairy cows under practical smallholder farming conditions. We hypothesized that seaweed supplementation would reduce *in vitro* methane production and improve milk yield and composition without negatively affecting metabolic health.

MATERIALS AND METHODS

Experiment 1: Effect of seaweed supplementation on rumen degradation assessed by *in vitro* gas production

Seaweed information

A commercial pelleted seaweed supplement was used in this study. The product consisted of a blend of red (*Asparagopsis taxiformis* and *Gracilaria* spp.), green (*Ulva* spp.), and brown (*Ascophyllum nodosum* and *Laminaria digitata*) seaweeds. Seaweed was added to the substrate at the same proportional inclusion

rate 40 g/day in the *in vivo* supplementation level (Newton et al., 2021), equivalent to 0.17% of substrate dry matter *in vitro* supplementation level.

Gas production technique

Rumen fluid was collected from two lactating Holstein Friesian dairy cows with an average body weight of 427 ± 10 kg and an average age of 3 years. The cows were offered about 25 kg of fresh corn forage as roughage and 6 kg of concentrate per cow daily. For the *in vitro* gas production assay, three replicates of 230 mg of dried TMR (Farm 1 formulation) were weighed into 100 mL calibrated glass syringes. Rumen fluid was collected using the stomach tube method and subsequently mixed with a buffered medium containing distilled water, buffer solution, macromineral solution, resazurin indicator, micromineral solution, and reducing agent. The buffered rumen fluid was incubated with two treatments in vitro: a control group and a seaweed-supplemented group containing TMR with seaweed at 0.39 mg per syringe (equivalent to 0.17% of substrate dry matter), corresponding to the *in vivo* supplementation rate of 40 g/day per cow. The incubation was carried out under anaerobic conditions at a constant temperature of approximately 39°C. Gas production was recorded at 2, 4, 6, 8, 10, 12, 24, 48, 72, and 96 hours following the method of Menke et al. (1979).The net gas production at 24 hours was determined using the following equation: GP (mL/200 mg DM, 24 h) = (V24 – V0 – GPo) x 200 x (Fh + Fc)/2W, where V0 = Gas production before incubating, V24 = Gas production at 24 hrs., GPo = Average gas production at 24 hrs., Fh = 44.16/(GPh – GPo); roughage correction factor, Fc = 62.6/(GPh – GPo); concentrate correction factor, and W = Weight of samples (mg). Kinetic of gas production was calculated using the Ørskov and McDonald (1979) model: $y = a + b(1 - \exp^{-ct})$, where y = Gas production at time t (mL), a = Gas production from the degradation of water-soluble components (mL), b = Gas production from the degradation of insoluble components (mL), c = Gas production rate constant (%/h), exp = Exponential function, and t = Time of measurement (h).

In vitro ruminal pH

The pH of the fermentation content (rumen fluid-based incubation medium) was measured immediately after 24 h of incubation using a portable pH meter (EcoTestr pH 1, Eutech Instruments), as described by Zebeli et al. (2008).

Ammonia determination

Ammonia-nitrogen (ammonia-N) concentration was determined from the supernatant using the colorimetric method of Weatherburn (1967).

Methane measurement

Gas samples for methane measurement were collected after 24 h of incubation in sealed glass bottles, and methane concentration was determined by gas chromatography (Hewlett–Packard model 6890) using a GC column (4.6 m × 0.318 cm × 2.1 mm) compatible 60/80 Carboxen-1000, model 1-2390, Supelco, Inc, Bellefonte, PA) and flame ionization detector (FID). The separation rate of the injection port (220 °C) was 100:1. Helium was used as the carrier gas with a flow of 40 mL/min as described by Wingard et al. (2018). The column was initially held at 130 °C for 10 min, then increased to 200 °C (slope of 80 °C/min) for 1 min, and the post-operation temperature was 120 °C. The detector temperature was 200 °C with hydrogen and air flows of 40 mL/min and 200 mL/min, respectively. Methane production is reported as the change in concentration over time.

Volatile fatty acids analysis

The rumen fluid inoculated at 24 h was prepared using a modified method of Fortina et al. (2022). Volatile fatty acid concentrations, including acetate (C2), propionate (C3), and butyrate (C4), were analyzed using a gas chromatography (GC). The 1 µL of samples were injected into a Shimadzu Nexis GC-2030 equipped with an automatic injector, Shimadzu AOC-20i Plus, and a column, Zebron ZB-

FAME (30 m length x 0.25 mm diameter (i.d) x 0.20 µm film thickness; Phenomenex, USA) in split mode at 160°C, using helium as the carrier gas at a constant flow of 1 mL/min. The oven temperature was programmed from 60°C to 115°C at 5°C/min, then to 130°C at 3°C/min, and to 230°C at 15°C/min for 3 minutes. The FID was maintained at 250°C. External standard used is VFA mixture (Supelco, USA serial no. CRM46975). VFA production was expressed as the change in concentration over each sampling interval.

Microbial biomass yield

The microbial biomass yield (MBY) was determined according to the method of Blümmel et al. (1997). A 500 mg sample was weighed and incubated with the rumen-medium mixture for 24 hours, following the *in vitro* degradability procedure. After incubation, the samples were filtered and dried in a 100°C oven overnight. The difference between the initial weight and the weight after filtration and incubation was considered the apparently degraded substrate. After drying, the filter papers were rinsed in beaker glasses with neutral detergent solution (NDS), following the NDF method of Van Soest et al. (1991). The difference between the initial weight and the weight after NDS washing was considered the truly degraded substrate. The microbial biomass yield was then calculated using the formula from Blümmel et al. (1997): MBY (mg/500 mg DM) = truly degraded substrate – apparently degraded substrate / truly degraded substrate.

Experiment 2: Effect of seaweed supplementation on milk yield and composition in dairy cows

Animal research approval

This experiment was conducted in strict accordance with the guidelines for the use of animals in scientific research. The use of animals in this study was approved by the Animal Care and Use Committee for Scientific Purposes (Agricultural Animals), Faculty of Agriculture, Chiang Mai University, under the approval document (approval number) AG01004/2566

Animals, experimental design, and feeding management

For the *in vivo* experiment, the supplementation rate was fixed at 40 g/day. This level was calculated based on an average DMI of 24 kg/day, corresponding to 0.17% of DMI.

This experiment was conducted at two commercial dairy farms in Lamphun Province, Thailand, using a total of forty Holstein Friesian cows. The cows were categorized into two physiological stages: (1) Twenty transition cows, monitored from 30 days before the expected calving date to 90 days postpartum were selected from Farm 1. Cows were blocked by body weight. (2) Twenty mid-lactation cows, averaging approximately 100 ± 67 days in milk (DIM) were selected from Farm 2. Cows were blocked by milk yield.

The cows were assigned to one of two dietary treatments: a control group (CON) fed a total mixed ration (TMR) without seaweed, and a seaweed-supplemented group (SW) fed a TMR containing 40 g/cow/day of seaweed (Newton et al., 2021) top-dressed onto the TMR before the morning milking. The supplementation level corresponds to approximately 0.17% of DM on the *in vitro* experiment.

Experimental diet (Total Mixed Ration) for the transition cows and mid-lactation cows was formulated differently. The ingredients and chemical compositions of TMR for transition cows and mid-lactation cows were listed in Table 1. The TMR was fed to both cows *ad libitum* twice daily at 04:30 and 15:30 to meet the daily nutrient requirements. However, the rice straws from both farms have different chemical composition. The chemical composition of the rice straw and seaweed used in the experimental diets is presented in Table 2. The same batch of seaweed was used for both *in vivo* and the *in vitro* experiment to ensure

consistency in nutrient composition and bioactive content. The chemical composition of the total mixed rations (TMR) and seaweed, including dry matter, organic matter, crude protein, crude fiber, ether extract, ash, nitrogen-free extract (NFE), non-fiber carbohydrates (NFC), and total digestible nutrients (TDN), as well as fiber fractions (NDF, ADF, and ADL), was analyzed following AOAC (1990) for proximate composition and Goering and Van Soest (1970) for fiber analysis.

No additional drying-off was applied during the experiment, reflecting typical management practices in smallholder dairy farms in Thailand. Clean drinking water and commercial mineral blocks (Betagro®, Thailand) were freely available throughout the experimental period. Animals were housed in open-sided barns with concrete flooring, with each cow provided 12 m² of individual space, including a designated feeding and resting area.

Table 1 Feed ingredients and chemical composition of total mixed rations (TMR) used in the *in vitro* experiment (Experiment 1) and *in vivo* trial conducted at Farm 1 and Farm 2 (Experiment 2; %, DM).

Item (%DM basis)	Experimental diets (TMR)	
	Transition (Farm 1& *in vitro*)	Mid-lactation (Farm 2)
Feed ingredients (%)		
Sweet corn husk and cob / Corn cob	35.63	9.26
Rice straw	5.49	23.15
Soybean meal	16.03	13.89
DDGS	8.06	9.26
Ground corn	15.68	4.63
Broken rice	9.62	6.48
Starch	6.56	-
Cassava chip	-	9.26
Pineapple stem	-	18.52
Soy sauce by-product / Soy sauce residue	-	3.70
Mineral mix	2.28	1.85
Salt + baking soda	0.64	-
Chemical composition (%DM basis)		
Dry matter	39.00	42.00
Organic matter	93.46	89.49
Crude protein	12.45	14.33
Crude fiber	24.73	27.64
Ether extract	2.92	3.20
Ash	6.54	10.51
NFE	53.36	44.32
NFC	31.33	24.34
NDF	46.76	47.62
ADF	28.42	23.69
ADL	12.42	9.67
TDN	87.95	74.19

[1]Farm 1 and the *in vitro* experiment used the same TMR formulation.
[2]The TMR formulations differed between farms depending on local ingredient availability but were formulated to provide comparable nutrient levels.
[3]DDGS = Dried distillers grains with soluble; NFE = Nitrogen-free extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin; TDN (%) = 88.9 – (0.79 × ADF, %DM).

Table 2 Chemical composition of seaweed and rice straw used in the experimental diets (%, DM basis)

Chemical composition (%DM basis)	Seaweed (Farm 1& Farm 2)	Rice straw (Farm 1)	Rice straw (Farm 2)
Dry matter	87.15	94.63	93.50
Organic matter	98.88	84.01	83.04
Ash	1.12	15.99	16.96
Crude protein	6.62	3.14	3.42
Crude fiber	42.51	36.68	34.58
Ether extract	0.01	0.82	1.85
NFE	49.74	43.37	43.19
NFC	22.37	11.72	9.64
NDF	69.88	68.33	68.13
ADF	56.84	42.96	42.86
ADL	6.72	3.88	3.82

Values are expressed on a dry matter (DM) basis.
The same seaweed source was used in both farms and in the *in vitro* experiment.
Chemical analyses were performed according to the procedures of AOAC (1990) for proximate composition and Van Soest et al. (1991) for fiber fractions.

Sampling and measurements

Feed intake

In Experiment 2, feed intake was measured daily on two commercial dairy farms operating under the same experimental protocol. Diet formulations and chemical composition are presented in Table 1. Feed offered and refusals were recorded daily for each group, and dry matter intake (DMI) was determined using samples oven-dried at 60 °C. During the prepartum period, cows were group-fed within each farm; therefore, individual dry matter intake (DMI) could not be accurately measured. Individual DMI was recorded only during the mid-lactation phase, when cows were housed and fed individually.

Blood sampling and biochemical analysis

For transition cows only, approximately 10 mL of jugular blood was collected into plain vacuum tubes before the morning feeding on days 1, 3, and 7 postpartum. Serum was obtained by centrifugation (3,000 rpm, 20 min) and analyzed for Calcium (Ca), Phosphorus (P), Blood urea nitrogen (BUN), total protein, albumin, globulin, creatinine, Alanine aminotransferase (ALT), and Alkaline phosphatase (ALP) using an automated chemistry analyzer (DRI-CHEM NX700V).

Milk yield and composition

Milk yield was recorded twice daily at 04:45 and 15:45 throughout the experiment. Due to physiological differences between early- and mid-lactation cows, milk sampling schedules were adjusted accordingly. For transition cows, milk samples for compositional analysis were collected on days 15, 30, 45, 60, 75, and 90 postpartum to monitor responses during early lactation when metabolic adaptation is most dynamic. While, for mid-lactation cows, milk samples were collected on days 0, 10, 20, and 30 during the 30-day supplementation period to evaluate the effects in cows with established milk production.

All milk samples (30 mL in triplicate) were analyzed for fat, protein, lactose, solids-not-fat (SNF), and total solids (TS) using an automated analyzer (MilkoScan FT2, FOSS, Denmark). Fat-corrected milk (3.5% FCM) and energy-corrected milk (ECM) yields were calculated using equations described by Tyrrell and Reid (1965) and Sjaunja et al. (1998), respectively, where 3.5% FCM (kg) = (0.432 × milk yield, kg) + (16.23 × milk fat yield, kg), and ECM (kg) = milk yield × [(0.38 × milk fat, %) + (0.24 × milk protein, %) + 0.17] / 3.14. Fat and protein yields were determined based on the corresponding concentrations and daily milk yield.

Statistical analysis

All data were analyzed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA). The *in vitro* experiment conducted as a completely randomized design and analyzed using according to the following model $Yij=\mu+\alpha i+\varepsilon ij$, where Yij is the observed value, μ is the overall mean, αi is the fixed effect of the treatment (i= 1,2), and εij is the random error assumed to be normally distributed with mean zero and constant variance. Mean differences among treatments were determined using Tukey's HSD test.

The on-farm lactation performance trial was analyzed using analysis of covariance (ANCOVA) in a randomized complete block design (RCBD), with days in milk (DIM) at the beginning of the trial included as a blocking factor. The model was: $Yij=\mu+\tau i+\beta(Xij-\bar{X})+\rho j+\varepsilon ij$ where Yij is the dependent variable for cow j in group i, μ is the overall mean, τi is the treatment effect, β is the regression coefficient for the covariate, Xij is the covariate value, $\bar{X}$ is the overall mean of the covariate, ρj is the effect of block j (DIM), and εij is the random error term assumed to be normally distributed with mean zero and constant variance. Treatment means were compared using Bonferroni's test.

All data are presented as mean ± standard error of the mean (SEM) with statistical significance set at $P < 0.05$.

RESULTS

Effect of seaweed supplementation on rumen degradation assessed by *in vitro* gas production

The effect of commercial seaweed supplementation on *in vitro* gas production is shown in Table 3. At 2 hours of incubation, gas production of the treatment group (7.27 mL/200 mg DM) was significantly higher ($p < 0.05$) than that in the control group (6.61 mL/200 mg DM). However, no significant differences ($p > 0.05$) were observed between groups at later incubation times (4, 6, 8, 10, 12, 24, 48, 72, and 96 hours). Regarding gas production kinetics, no significant differences ($p > 0.05$) were detected between the control and treatment groups in the parameters a (soluble fraction), b (insoluble fraction), c (rate constant), or the sum of a + b.

Table 3 Effect of Commercial Seaweed supplementation on *in vitro* gas production

Time (hr.)	CON	SW	SEM	P-value
Gas accumulation (mL / 200 mg DM)				
2	6.61[b]	7.27[a]	0.330	0.033
4	16.43	15.54	0.445	0.610
6	28.73	25.86	1.435	0.514
8	38.22	34.81	1.705	0.896
10	41.45	39.12	1.165	0.592
12	44.91	42.67	1.120	0.458
24	53.34	49.02	2.160	0.203
48	75.06	74.68	0.190	0.405
72	79.02	78.75	0.135	0.392
96	81.00	80.44	0.280	0.392
Kinetics of gas production				
a (mL)	0.86	3.39	0.025	0.328
b (mL / 200 mg DM)	77.85	76.26	0.071	0.618
c (/hr.)	0.07	0.05	0.159	0.987
|a|+b	78.71	79.65	0.097	0.657

a, b Means along row among treatments with different superscripts are significantly different at $p < 0.05$. CON = control diet; SW = seaweed-supplemented diet; b = the actual insoluble fraction gas production; c = the insoluble fraction gas production rate

Seaweed supplementation significantly reduced *in vitro* methane production (2.30 vs. 5.22 mL/200 mg DM; p <0.001) and increased true dry matter degradability (67.08% vs. 57.77%; p = 0.033) compared to the control (Table 4). No significant differences were observed in rumen pH, ammonia concentration, total volatile fatty acids, individual VFA profiles, acetate to propionate ratio and microbial biomass yield between treatments (p > 0.05).

Table 4. Effect of seaweeds supplementation on *in vitro* rumen fermentation product and methane emission

Items	T1	T2	SEM	P-value
CH_4 (mL/200mg DM)	5.22[a]	2.30[b]	0.186	<0.001
pH	7.18	7.11	0.045	0.325
NH_3 (mg/mL)	29.95	27.63	1.160	0.310
% True degradability	57.77[b]	67.08[a]	1.470	0.033
Ruminal VFA (mmol)				
Total VFA	40.99	41.17	0.857	0.840
Acetate	21.27	21.28	0.427	0.914
Propionate	12.36	12.42	0.137	0.831
Butyrate	7.40	7.75	0.137	0.235
A:P	1.72	1.70	0.011	0.725
MBY				
(mg/500 mg DM)	4.33	7.99	1.060	0.160
(% of true degradable substrate)	7.53	11.99	1.714	0.263

[1] T1 = control group; T2 = seaweeds supplementation group. A:P = acetate: propionate ratio. MBY = Microbial Biomass Yield. [a, b] Means along row among treatments with different superscripts are significantly different at p < 0.05.

Effect of seaweed supplementation on blood profiles, milk composition and yield in dairy cows

Blood biochemical parameters

Seaweed supplementation had limited effects on blood biochemical parameters (Table 5). Serum calcium concentration was significantly lower in the treatment group on day 3 (8.26 vs. 9.07 mg/dL; p = 0.047), whereas no differences were observed on days 1 and 7 (p > 0.05). For other parameters, including phosphorus, blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, and globulin, no significant differences were detected between the control and treatment groups at any time point (p > 0.05).

Feed intake

The average daily dry matter intake (DMI) was 23.67 kg/day in the control group and 23.72 kg/day in the seaweed-supplemented group, with no significant difference between treatments (p = 0.228).

Milk yield and composition

Seaweed supplementation tended to enhance milk yield throughout the experimental period. Although the differences were not statistically significant, cows receiving seaweed showed numerical increases in milk yield compared with the control group at several time points.

Table 5. Effect of commercial seaweed supplementation on blood chemical measurement

Treatment	Day	CON	SW	SEM	p-value
Calcium, mg/dL	1	8.68	8.23	0.107	0.271
	3	9.07[a]	8.26[b]	0.093	0.047
	7	8.60	8.83	0.030	0.527
Phosphorus, mg/dL	1	6.18	5.66	0.114	0.536
	3	6.25	6.01	0.100	0.729
	7	6.09	6.23	0.161	0.832
Blood Urea Nitrogen, mg/dL	1	9.03	11.32	0.261	0.307
	3	8.61	7.53	0.024	0.585
	7	8.32	8.90	0.415	0.710
Creatinine, mg/dL	1	1.45	1.42	0.011	0.833
	3	1.47	1.29	0.013	0.261
	7	1.36	1.33	0.011	0.759
Alanine aminotransferase, U/L	1	14.00	16.40	0.317	0.218
	3	11.33	13.33	0.036	0.155
	7	13.56	13.67	0.202	0.960
Alkaline phosphatase, U/L	1	55.77	69.40	0.480	0.221
	3	54.67	61.17	0.676	0.551
	7	38.56	43.50	0.180	0.516
Total protein, g/dL	1	7.71	8.05	0.077	0.549
	3	7.93	7.82	0.061	0.877
	7	8.13	8.07	0.019	0.885
Albumin, g/dL	1	3.98	3.80	0.015	0.231
	3	4.08	4.00	0.014	0.671
	7	3.93	3.72	0.013	0.216
Globulin, g/dL	1	3.73	4.25	0.047	0.370
	3	3.90	4.20	0.006	0.670
	7	4.20	4.34	0.028	0.739

Values are presented as mean ± SEM. CON = control diet; SW = seaweed-supplemented diet; SEM = standard error of the mean. Means within a row with different superscripts differ significantly ($p < 0.05$).

Transition cows fed seaweed produced significantly higher 3.5% fat-corrected milk (FCM) on day 15 (19.91 vs 14.20 kg/d, $p = 0.009$), with tendencies toward higher FCM on days 45 (18.75 vs 15.79 kg/d, $p = 0.096$) and 75 (18.82 vs 15.47 kg/d, $p = 0.086$). Similarly, energy-corrected milk (ECM) was significantly greater in the treatment group on day 15 (17.56 vs 13.55 kg/d, $p = 0.040$) (Figure 1). For milk composition, seaweed supplementation had no significant effects on milk fat, protein, lactose, total solids (TS), or solids-not-fat (SNF) contents throughout the trial ($p > 0.05$). However, cows in the treatment group tended to have higher milk fat percentages on days 15 (3.39 vs 2.43 %, $p = 0.206$) and 75 (3.36 vs 2.69 %, $p=0.161$) and greater total solids on day 75 (12.08 vs. 11.07%; $p = 0.103$). Lactose concentrations were also slightly higher in the treatment group on days 30 and 60 (4.64 vs 4.42 %, $p = 0.165$ and 4.63 vs 4.24 %, $p=0.116$, respectively) (Figure 2).

Cow fed seaweed produced significantly on day 30 in mid-lactation (19.78 vs 15.91 kg/d, $p = 0.024$), while a tendency was also observed on day 20 (19.62 vs 16.55 kg/d, $p = 0.059$) (Figure 3). These results indicate that seaweed supplementation improved milk energy output. Notably, TS and SNF consistently tended to be higher in the treatment group, with p-values approaching significance on days 10–30 (TS: $p = 0.104$–0.141; SNF: $p = 0.073$–0.074) (Figure 4).

Overall, these findings suggest that seaweed supplementation may enhance milk energy-corrected yield and improve milk quality in dairy cows without adversely affecting overall production performance.

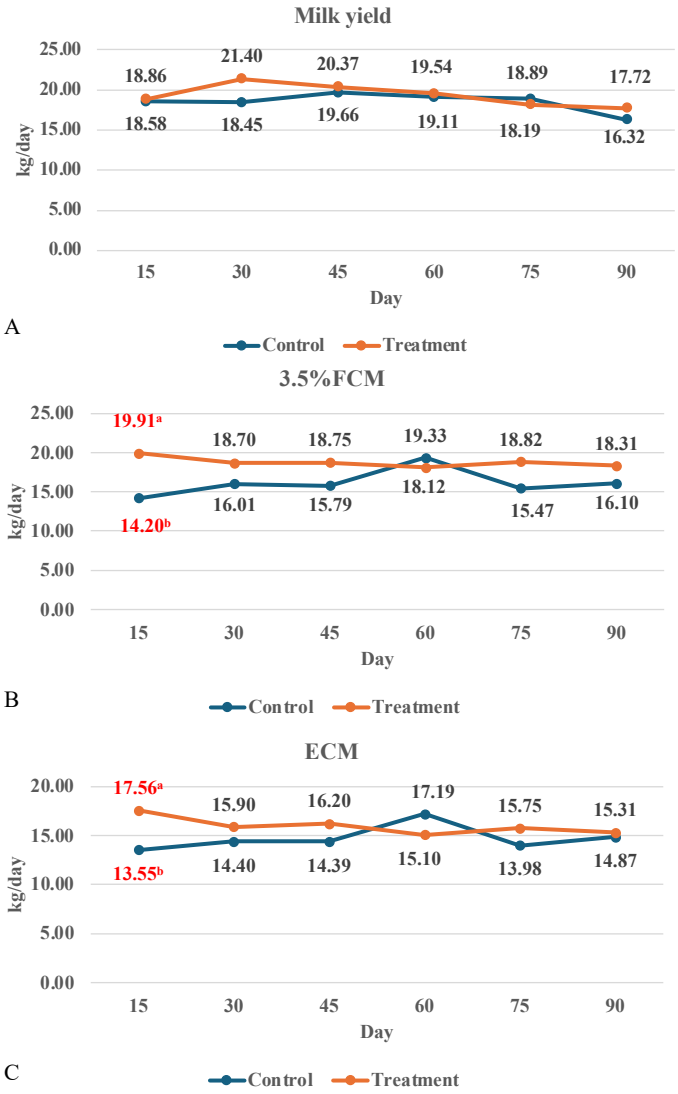

Figure 1 Effect of seaweed supplementation on (A) Milk yield, (B) 3.5% Fat-Corrected Milk (FCM), and (C) Energy-Corrected Milk (ECM) in dairy cows during the transition cows (Farm 1). Values are expressed as means ± SEM (n = 10). Different superscripts (a, b) indicate significant differences ($p < 0.05$).

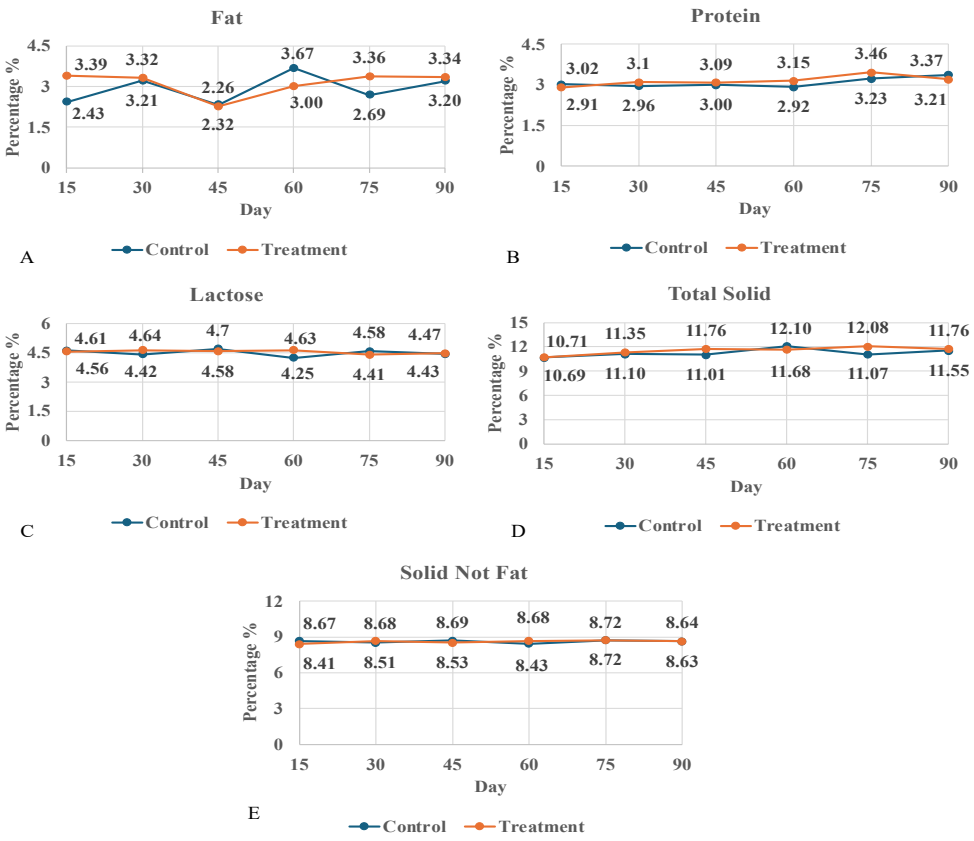

Figure 2 Milk composition of dairy cows fed control and seaweed-supplemented diets during transition cow. (A) Milk fat, (B) Protein, (C) Lactose, (D) Total solids, and (E) Solids-not-fat (SNF). Values are expressed as means ± SEM (n = 10). No significant differences were observed between treatments ($p > 0.05$).

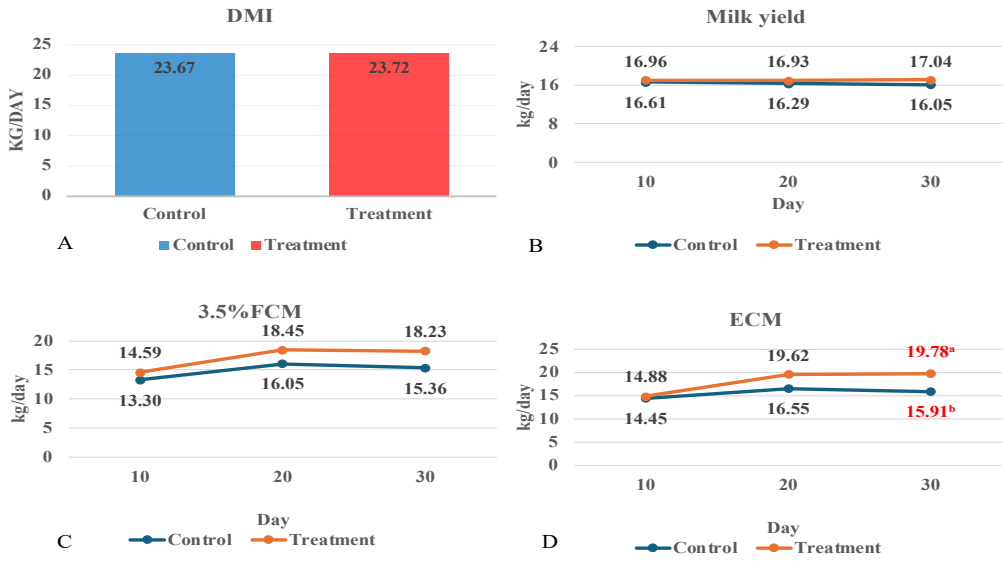

Figure 3 (A) Dry matter intake (DMI), (B) Milk yield, (C) 3.5% Fat-Corrected Milk (FCM), and (D) Energy-Corrected Milk (ECM) of dairy cows fed control and seaweed-supplemented diets during the 30-day mid-lactation (Farm 2). Values are expressed as means ± SEM (n = 10). Different superscript letters within the same parameter indicate significant differences ($p < 0.05$).

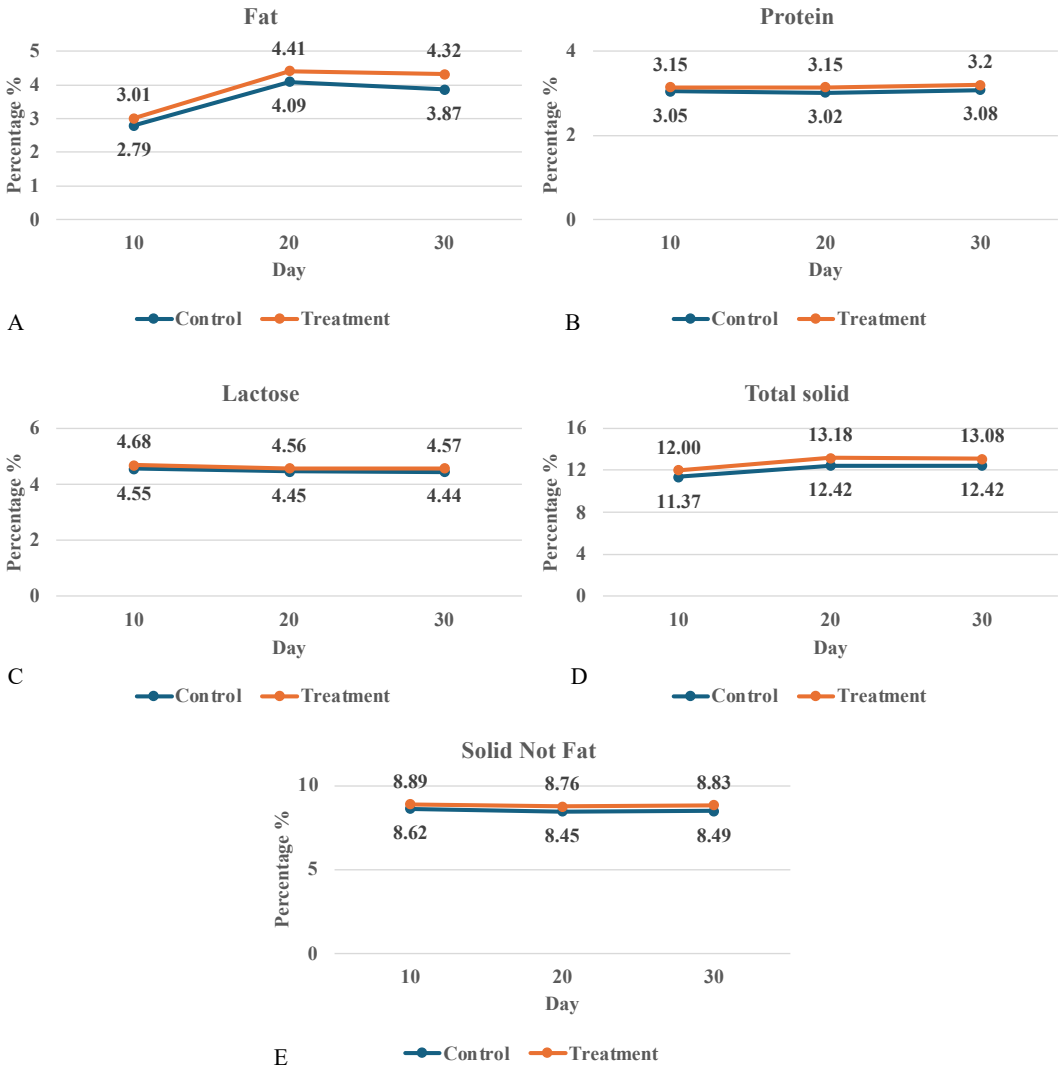

Figure 4 Milk composition, including (A) Milk fat, (B) Protein, (C) Lactose, (D) Total solids, and (E) Solids-not-fat (SNF) of dairy cows fed control and seaweed-supplemented diets during the 30-day postpartum period (Farm 2). Values are expressed as means ± SEM (n = 10). No significant differences were observed between treatments ($p > 0.05$), but a tendency toward higher TS and SNF contents was noted in the seaweed-supplemented group ($0.05 \leq p < 0.10$)

DISCUSSION

Experiment 1: Effect of seaweed supplementation on rumen degradation by *in vitro* gas production

Rumen fermentation and microbial activity

The addition of seaweed to the diet significantly increased gas production during the first 2 hours of fermentation. This initial increase in gas production can be attributed to the rapid fermentation of seaweed-derived polysaccharides such as alginate, laminarin, and fucoidan, which are highly fermentable by rumen microbes (Blümmel et al., 1997; He et al., 2022). These findings suggest enhanced early fermentation efficiency and microbial activity in the rumen (Erwin et al., 1961).

The ruminal pH remained stable within the optimal physiological range of 6.0–7.0 throughout the experiment, which is crucial for maintaining microbial balance and fermentation efficiency (Weatherburn, 1967; Janssen and Kirs, 2008). No significant differences were observed in ammonia nitrogen (NH_3-N) concentrations, indicating that nitrogen metabolism and protein degradation processes were not disrupted by seaweed supplementation (Menke et al., 1979; Blümmel et al., 1997).

Methane production and environmental impact

Importantly, methane production was significantly reduced in the seaweed-supplemented group. This result aligns with previous studies indicating that bioactive compounds in seaweed, particularly bromoform, inhibit methanogenic archaea in the rumen (Kinley et al., 2020; Roque et al., 2021). The inhibition likely occurs through disruption of the methyl-coenzyme M reductase pathway, a key enzyme in methane synthesis. Seaweed supplementation may also encourage alternative hydrogen utilization pathways, such as propionate formation, which further reduces methane emissions. These findings suggest that seaweed supplementation has potential environmental benefits by reducing enteric methane emissions and promoting sustainable ruminant production.

Moreover, the observed reduction in methane (–56%; $p < 0.001$) was accompanied by an improvement in true degradability, suggesting that seaweed supplementation enhanced rumen fermentation efficiency. Improved degradability likely reflects better feed utilization, which may reduce energy losses as methane, as reported by DiLorenzo et al. (2025), and support more efficient microbial growth by redirecting energy toward microbial protein synthesis, consistent with findings of Lu et al. (2019) and reviews on rumen microbiome energy efficiency (Badhan et al., 2025).

Volatile fatty acids and microbial biomass yield

Seaweed supplementation did not significantly alter total VFA concentrations or the molar proportions of individual VFAs, including acetate, propionate, and butyrate. Similarly, the acetate-to-propionate ratio (A:P) remained unchanged, suggesting that while seaweed supplementation may influence other aspects of rumen fermentation (e.g., methane reduction), it does not markedly affect the primary fermentation pathways leading to VFA production.

The microbial biomass yield (MBY), calculated as the proportion of truly degraded substrate, also showed no significant differences between the seaweed-supplemented group and the control group. These results indicate that seaweed supplementation did not adversely affect microbial growth or the utilization of fermentable substrates in the rumen. These findings are consistent with previous studies that reported no significant changes in microbial fermentation efficiency following dietary interventions (Menke et al., 1979; Blümmel et al., 1997).

Although microbial biomass yield (MBY) values in this study were relatively low (4.33 ± 1.06 mg/500 mg DM for the control and 7.99 ± 1.06 mg/500 mg DM for the seaweed-supplemented group), these results are consistent with previous reports using high-fiber substrates in *in vitro* fermentation systems (Menke et al., 1979; Blümmel et al., 1997). The low MBY may be attributed to the high fiber content of the substrate, which limits microbial growth, and to the presence of bioactive compounds in seaweed, such as laminarin and fucoidan, that can selectively modulate microbial populations. Moreover, the 24-hour incubation period may not fully capture maximal microbial biomass synthesis, particularly when using slowly degradable fiber-rich feeds. Despite the modest MBY, true dry matter degradability was significantly increased in the seaweed-supplemented group, suggesting that microbial utilization of fermentable substrates was efficient. This improvement in degradability likely contributed to the enhanced energy-corrected milk (ECM) observed in the treatment group, indicating that seaweed supplementation improved ruminal fermentation efficiency and nutrient utilization without adversely affecting microbial growth. These findings highlight that even moderate microbial

biomass production can be sufficient to support improvements in feed utilization and milk energy output in dairy cows.

Experiment 2: Effects of seaweed supplementation on milk yield and composition in dairy cows

Seaweed supplementation had minimal effects on blood biochemical parameters during the early postpartum period. A transient decrease in blood calcium levels was observed on day 3, likely due to the sudden increase in calcium demand for milk production in early lactation. This temporary hypocalcemia may occur because homeostatic mechanisms, including parathyroid hormone-mediated calcium mobilization from bone and enhanced intestinal absorption, cannot fully compensate for the rapid calcium outflow into milk (Stefenoni et al., 2021). Nevertheless, calcium concentrations remained within the normal range, and no clinical hypocalcemia was observed, indicating that seaweed supplementation did not increase the risk of milk fever. Similarly, phosphorus, blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels remained stable, with no significant differences between treatment and control groups. The results indicate that seaweed supplementation did not negatively affect mineral metabolism, renal function, or hepatic activity, supporting its safety during the early postpartum period (Goff, 2014; Jorjani et al., 2019).

Dry matter intake

Seaweed supplementation did not significantly affect dry matter intake (DMI), which is consistent with previous studies showing that moderate seaweed inclusion does not reduce feed intake in dairy cows (Wang et al., 2021; He et al., 2022). This suggests that the observed changes in milk yield and methane production were not influenced by differences in feed consumption.

Milk yield and composition

Seaweed supplementation tended to improve milk yield and energy-corrected milk (ECM) throughout the experimental period, suggesting an enhancement in the energy value of milk, particularly during transition cows. This improvement may be related to enhanced rumen fermentation efficiency and nutrient utilization, potentially stimulated by bioactive compounds in seaweed, such as laminarin and fucoidan (Weatherburn, 1967; He et al., 2022; Zhou et al., 2023). Although changes in milk fat, protein, lactose, total solids (TS), and solids-not-fat (SNF) were not statistically significant, numerical increases in milk fat and TS support the potential benefit of seaweed supplementation on milk composition.

CONCLUSIONS

Seaweed supplementation 40 g/day (0.17% of DMI) improved rumen fermentation and significantly reduced *in vitro* methane production using dairy cow rumen fluid, indicating a potential role in mitigating enteric methane emissions. Blood parameters remained within normal ranges, with temporary changes in calcium and phosphorus that possibly do not affect milk fever. Milk yield and quality showed positive but non-significant trends. Overall, seaweed appears safe and potentially beneficial for dairy cows in early lactation. Further studies with larger sample sizes and longer durations are recommended.

ACKNOWLEDGEMENTS

The research team would like to express our gratitude to Nutri-San Co., Ltd. for providing financial support for this study. We also extend our appreciation to the Animal Feed Laboratory and Dairy Farm, Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, for facilitating access to laboratory facilities for sample analysis and providing experimental dairy cows. Their support was instrumental in the successful completion of this research.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

AUTHOR CONTRIBUTIONS

Nattapong Saeng-in: contributing to the commencing of the experiment, collecting and analyzing the data, and writing the manuscript.
Trisadee Khamlor: contributing to the critical reviews of the manuscript.
Raktham Mektrirat: contributing to the critical reviews of the manuscript.
Saowaluck Yammuen-art: contributing to the designing, commencing the experiment, collecting and analyzing the data, and writing the manuscript

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How to cite this article;
Nattapong Saeng-in, Trisadee Khamlor, Raktham Mektrirat and Saowaluck Yammuen-art. Effects of Commercial Seaweed Supplementation on *in vitro* Methane Production and Milk Performance in Dairy. Veterinary Integrative Sciences. 2026; 24(3): e2026064-1-17.