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Effects of feeding dairy cows seaweed mixtures on feed intake, methane emission, milk production, and milk mineral and fatty acid composition

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ABSTRACT

There has been much recent research interest in the potential of macroalgae (seaweed) as a methane-suppressing feed supplement for ruminants, but there are numerous potential seaweed species available that have varying compositions of potentially beneficial or deleterious consequence. Therefore, the objective of the present study was to determine the effects of feeding 3 mixtures of 3 seaweeds at levels within EU regulatory limits for ration iodine concentration on methane production and milk yield and composition of dairy cows. Forty lactating (204 ± 5.6 DIM) Holstein cows were randomly assigned to 1 of 4 treatments (10 per treatment) in blocks based on DIM, parity, BW, and milk yield. Treatments were a control TMR and 3 seaweed mixtures replacing TMR grass silage: *Ascophyllum nodosum*, *Fucus vesiculosus,* and *Asparagopsis taxiformis* (AN, FV, and AT, respectively; 5:45:50 and 1.5 g/kg DM), AN and FV (AN:FV at 90:10 and 6.5 g/kg DM), or FV and AN (FV:AN at 90:10 and 17.5 g/kg DM). Cows were individually fed for ad libitum DMI in freestall housing and treatments continued for 7 wk, with daily measurements of milk yield and DMI and weekly measurements of milk composition. Methane emission was estimated using 2 GreenFeed head chambers in wk 5 to 7 of treatment, milk mineral concentrations were measured in wk 1 to 6, and milk FA composition was measured in wk 6. Feeding the AN:FV and FV:AN mixtures had no effect on methane emission (g/d) or yield (g/kg DMI), whereas cows fed AT (0.075% of DM) in combination with AN and FV tended to have a lower methane emission (9%) and methane yield (12%) than cows fed the control diet and lower (20%) methane intensity (g/kg ECM) than cows fed the AN:FV mixture. Cows fed all 3 seaweed mixtures had 1.7- to 2.2-fold higher milk iodine concentrations, which showed a week-by-treatment interaction, in that cows fed the AN:FV mixture had higher milk iodine concentrations in wk 3 and 5 of the study. Cows fed the AN:FV also had lower milk urea concentration, suggesting effects of AN or FV bioactive components on rumen protein degradation. Cows fed the AT:AN:FV mixture had lower concentrations of C18:3n-6, and higher concentrations of C15:1 and C17:0, but there were no other effects of feeding seaweed on milk yield or composition. In conclusion, feeding a relatively small amount of AT (0.75 g/kg diet DM) with AN and FV reduced methane emission and yield by 9% and 12%, respectively, and there was no effect of feeding mixtures of AN and FV without AT. Feeding a higher level of AN with FV (5.85 g/kg DM) reduced milk urea concentration, suggesting inhibitory effects of phytotannins or other bioactive components on protein degradation.
Key words: macroalgae, methane, iodine, heavy metals

INTRODUCTION

Enteric methane from cattle is a major source of GHG emissions included in global inventories attributable to food production, thus sustainable approaches to reduce methane emissions by dairy cattle and other ruminant livestock are considered key to meeting commitments for GHG reductions and the development of more sustainable livestock production practices. Recent reviews of potential mitigation strategies include feed additives and other dietary strategies that reduce rumen methane production through enzyme inhibition or modification of the rumen environment (Honan et al., 2021; Beauchemin et al., 2022; Kreuzer, 2025). In this regard, feeding macroalgae (seaweed) has been highlighted as a potential

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mitigation strategy (Belanche et al., 2016; Vijn et al., 2020; Honan et al. 2021; Min et al., 2021; Beauchemin et al., 2022). The potential to inhibit methanogenesis was demonstrated in an in vivo study that showed up to 80% reduction at 3% dietary OM inclusion of the red seaweed *Asparagopsis taxiformis* (**AT**) in sheep (Li et al., 2018). Similar effects were reported in lactating dairy cows fed *Asparagopsis armata* (Roque et al., 2019) and in numerous more recent studies in which lower levels of AT were included in the diets of cattle and sheep (Hristov et al., 2022; Wasson et al., 2022; Hristov, 2024). However, the current availability of red seaweeds used in these studies is unlikely to meet the potential worldwide demand for methane reducing feed supplements. Sourcing seaweed locally reduces the carbon footprint of using seaweed as a feed supplement, although growing *Asparagopsis sp.* commercially in Northern Europe will potentially have a high energy requirement. In this regard, recent studies have investigated effects of feeding brown and red seaweeds from the North Atlantic to dairy cattle (Antaya et al., 2019; Muizelaar et al., 2023; Thorsteinsson et al., 2023b), albeit without observing significant effects on methane emissions.

In addition to effects on methane production, seaweed supplementation may have other beneficial effects, such as improved meat or milk quality and increased efficiencies of production, in part due to some seaweeds being a rich source of antioxidants, nutrients, and minerals (Vijn et al., 2020). Seaweeds are typically a rich source of iodine (Antaya et al., 2019; Newton et al., 2023) and previous studies have demonstrated that feeding red and brown seaweeds to lactating dairy cows increases milk concentrations of iodine and other minerals (e.g., Antaya et al., 2019; Newton et al., 2023). This may be useful in iodine deficient populations, such as women of childbearing age in Norway (Johnsen et al., 2025), although excess levels must be avoided (Vijn et al., 2020). In this regard *Asparagopsis spp*. are often extremely high in iodine content (Min et al., 2021; Wasson et al., 2022), as well as some brown seaweeds such as *Saccahrina latissima* (Thorsteinsson et al., 2023b).

Although seaweeds have been shown to be rich dietary sources of beneficial macro- and microminerals, they can also contain potentially toxic elements (**PTE**) and halogenated compounds that can be toxic and as such, are a concern for consumers if these constituents are transferred to meat and milk (Vijn et al., 2020). The inhibitory effect of AT and *A. armata* has been attributed primarily to their high concentrations of the halomethanes and in particular bromoform, which is associated with their high concentrations of iodine and bromine (Glasson et al., 2022; Hristov et al., 2022; Wasson et al., 2022). As a potential carcinogen, transfer of bromoform to meat and milk products is a concern for product safety. Although studies have shown no transfer of dietary bromoform to milk in dairy cows (Roque et al., 2019; Stefenoni et al., 2021), other studies have reported significant increases in milk bromoform and bromide concentrations when *A. armata* was fed to lactating dairy cows (Williams et al., 2024), which is a concern for consumer trust in dairy products. Seaweed, especially brown seaweed, can contain high levels of arsenic, cadmium, lead, and mercury; therefore, the levels of these PTE must be monitored in seaweed intended as feed (EFSA, 2023). In addition, there are limits to the amount of dietary iodine that can be fed to food-producing animals in the European Union (5 mg/kg diet for lactating cattle; EFSA, 2013), as high levels of iodine in milk and other animal products are a particular concern for children. These limits on the amount of iodine that can be fed to food-producing animals may limit the amount of specific seaweeds that can be fed in practice as methane-suppressing feed supplements. The objectives of the present study were therefore to determine the effect of feeding lactating dairy cows combinations of seaweeds, selected on the basis of their inhibitory effects on methane in vitro and iodine content, on feed intake, methane emission, milk production, and milk composition, including minerals and PTE.

MATERIALS AND METHODS

Animals and Housing

The experiment was conducted using 40 lactating Holstein dairy cows at the University of Reading's Centre for Dairy Research (CEDAR, Arborfield, UK). All procedures were approved and monitored under the UK Home Office Animals (Scientific Procedures) Act 1986 (Project License PA75D3A9E) and by the University of Reading's Home Office Animals Procedures Committee. Cows were randomly assigned to 1 of 4 treatments (n = 10 per treatment) in blocks based on measurements obtained during the week immediately before the start of the study. Average (± SEM) values for blocking factors were 204 ± 5.6 DIM, 3.5 ± 0.2 lactations parity, 737 ± 8.7 kg BW, and 34.0 ± 0.64 kg/d milk yield. The cows were loose-housed in a yard with sand-bedded cubicles and continuous access to fresh water. Cows were fed individually using an electronic Calan Broadbent (American Calan, Northwood, NH) gate system as described by Hammond et al. (2016) and milked twice daily on a 50-point rotary parlor (Dairy Master Ltd., Bromsgrove, UK) with electronic milk yield recording. The study ran continuously for 49 d with cows remaining on the same diet treatment throughout the study.

Table 1. Formulation of experimental diets (g/kg DM)

Item	Treatment[1]			
	Control	AN:FV:AT	AN:FV	FV:AN
Grass silage	195	193.5	188.5	177.5
Maize silage	362	362	362	362
Concentrate blend[2]	346	346	346	346
Soda wheat[3]	76	76	76	76
Megalac[4]	10	10	10	10
NaCl	4	4	4	4
Limestone	2	2	2	2
Premix[5]	5	—	—	—
I free premix[6]	—	5	5	5
AN	—	0.075	5.85	1.75
FV	—	0.675	0.65	15.75
AT	—	0.75	—	—
Total	1,000	1,000	1,000	1,000

[1]Treatment diets: Control, no seaweed; AN:FV:AT = *Ascophyllum nodosum* (AN), *Fucus vesiculosus* (FV) and *Asparagopsis taxiformis* (AT) at 5:45:50 and 1.5 g/kg diet DM; AN:FV at 90:10 and 6.5 g/kg diet DM; FV:AN at 90:10 and 17.5 g/kg diet DM.

[2]Table 2.

[3]NaOH treated wheat grain (KW Feeds, Atherstone, UK).

[4]Volac Wilmar Feed, Orwell, UK.

[5]Containing per kg as fed: 194 g Ca, 80 g Mg, 110 g Na, 10 g P, 7.5 g Zn, 5.5 g Mn, 1.25 g Cu, 500 mg I, 70 mg Co, 45 mg Se, 120 mg biotin, 0.5 mg vitamin B_{12}, 600,000 IU vitamin A, 230,000 IU vitamin D_3, and 3,200 IU vitamin E.

[6]Containing per kg as fed: 187 g Ca, 80 g Mg, 110 g Na, 10 g P, 7.5 g Zn, 5.5 g Mn, 1.25 g Cu, 70 mg Co, 45 mg Se, 120 mg biotin, 0.5 mg vitamin B_{12}, 600,000 IU vitamin A, 230,000 IU vitamin D_3, and 3,200 IU vitamin E.

Table 2. Formulation of the concentrate blend fed

Item	g/kg DM
Rapeseed meal	525
Barley	267
Soya hulls	58
Megalac[1]	33
Limestone	28
Palm kernel expeller	26
Molasses	25
Urea	21
NaCl	17
Total	1,000

[1]Volac Wilmar Feed, Orwell, UK.

Diets

Treatment diets (Tables 1 and 2) included a control TMR based on maize and grass silage (56:44 forage: concentrate, DM basis) or the same TMR with the addition of 1 of 3 seaweed mixtures, which replaced grass silage in the TMR on a DM basis. Treatment diets included varying proportions of 3 North Atlantic seaweed accessions obtained for a project funded by the European Institute of Innovation and Technology Food (EIT Food, 2025) and shown to reduce methane emission in vitro (Künzel et al., 2022; Yergaliyev et al., 2024). Seaweed fed during the trial was from the same batch for each seaweed. The 3 seaweed species were commercially sourced by Matis: *Ascophyllum nodosum* (**AN**) from Iceland, *Fucus vesiculosus* (**FV**) from Scotland, and AT from Portugal (seaExpert, Azores). The AN and FV were harvested in August and October, respectively, and dried by industry standards, which included air drying (FV) and gentle drying (AN), and subsequently stored dry at room temperature until used. The AT was freeze-dried in June and stored frozen until fed beginning in October, to preserve the bromoform content. Iodine concentration of the seaweeds fed in the present study were measured before the trial to calculate the permissible seaweed inclusion. Proportions and amounts of the seaweeds added to the TMR fed were adjusted based on their measured iodine content to limit estimated total TMR concentration of iodine to less than 5 mg/kg total diet (as-fed basis) as legislated for lactating dairy cows (EFSA, 2013). Diets containing seaweed included an iodine free trace mineral premix, but iodine in the other TMR components was not accounted for. Cows were fed once daily for ad libitum intake (10% refusals) with weekly adjustments to the amounts fed based on changes in forage DM concentration.

Measurements

Feed samples were taken 3 times a week (Monday, Wednesday, Friday) to measure DM concentration (100°C for 24 h) and were used to adjust amount of feed offered and to determine DMI; BW (measured using an electronic scale) and BCS were measured weekly. Two consecutive milk samples (a.m. and p.m.) for each cow were obtained weekly and analyzed for composition as described below. Methane emission of individual cows was monitored continuously using 2 GreenFeed automated head chambers (C-Lock Inc., Rapid City, SD), much as described by Hammond et al. (2016). Using the GreenFeed head chambers, measurements of methane emission for short periods of 5 to 7 min were obtained up to 6 times daily at dispersed time points for each cow. The bait used to generate visits to the GreenFeed was a commercial calf pellet used previously (Hammond et al., 2016; chemical composition [g/kg DM] of ash, 85.1; ether extract, 46.5; ADF, 174; NDF, 289; starch, 259; water soluble carbohydrate [**WSC**], 91.3; nitrogen [N], 27.3; and CP, 171). GreenFeed bait consumption was limited by the maximum number of visits allowed (6 visits) and the amount of pellet fed at each visit, to 1,080 g as fed daily. The cows were trained to using the GreenFeed system in the first week of the study and continued to use the GreenFeed units over the entire course of the study. GreenFeed units were calibrated based on quantitative recovery of carbon dioxide immediately before and after the measurement period (Hammond et al., 2016).

Diet Samples

Representative samples of the individual forages, concentrates, and the TMR (~500 g/d of each) were collected thrice weekly as described above and added to a bulked sample stored in sealed bags at −20°C. At the end of each measurement week, the bulked sample was mixed and divided to produce 3 samples (500 g each). Samples were either submitted for analysis fresh, dried at 60°C for 72 h, milled (1-mm screen), and retained in airtight containers at room temperature, or stored frozen at −20°C until needed for analysis. A representative sample of refused feed from each cow was taken and bulked by treatment. Analysis of refused feed bulk sample DM concentration (100°C) was used for calculation of feed DMI, which included both TMR and GreenFeed bait concentrate intake.

Milk Samples

Weekly consecutive a.m. and p.m. milk samples were treated with bronopol preservative (Labtek Services, Yeovil, UK) and stored at 4°C until analyzed for concentrations of fat, CP, casein, lactose, and urea using a CombiFoss (Foss, Hillerød, Denmark) infrared analyzer (National Milk Laboratories, Wolverhampton, UK). Energy-corrected milk and 4% FCM yields were then calculated based on milk yield and composition, as described by Hammond et al. (2016). Weekly milk samples for wk 1 to 6 of treatments were also composited according to a.m. and p.m. yield for mineral and fatty acid (**FA**) analysis. At each initial weekly milking, a 100-mL milk sample was stored at 4°C until sampling for the subsequent milking was completed. The milk samples were then heated in a water bath at 38°C, the contents were gently mixed and a yield-based composited sample was produced for each cow and then stored frozen (−20°C) until analyzed for mineral, trace elemental, and FA concentration.

Milk FA analysis was conducted, much as described by Livingstone et al. (2015). Total lipids (**TL**) of the milk samples were extracted according to the method of Bligh and Dyer (1959), and the TL concentration was determined gravimetrically. The extraction of the FA was done following the American Oil Chemists' Society official method Ce 1b-89 (AOCS, 2005) with minor adjustments for use of a flame ionization detector. Approximately 2 mL of TL extract was evaporated at 55°C under nitrogen until total evaporation of the chloroform. Between 60 and 90 mg of oil from the Blight and Dyer (1959) extraction was mixed with 1.5 mL of 0.5 *N* NaOH in methanol, mixed, and then heated at 100°C for 7 min, then cooled, and 2 mL of boron trichloride (12% in methanol) was added. The mixture was heated at 200°C for 30 min. Once cooled, 1 mL of methyl tricosanoate in isooctane standard was added, as well as 5 mL of saturated NaCl solution and vortexed for 30 s. The isooctane layer was transferred to a clean tube with natrium sulfate to remove any aqueous particles. Then 1 mL of isooctane was added to the former tube and vortexed again for 30 s. The isooctane layer was transferred again. The sample was then transferred to a glass vial for injection into the GC. The FA methyl esters were separated on a GC (Varian 3900, Varian Inc., Walnut Creek, CA) equipped with a fused silica capillary column (Supelco Omegawax 250, 30 m × 0.25 mm i.d. × 0.25 μm df) and using a flame ionization detector. A split injection ratio of 200:1 was used with the injector temperature at 225°C. After 4 min at 100°C the column was raised at 3°C/min to 240°C and held at that temperature for 15 min. The column flow was helium at 0.8 mL/min. The FA concentrations were expressed as grams per 100 g of TL.

Feed Analysis

Dry matter concentration of feeds was determined at sampling by oven drying at 100°C for 24 h. Frozen bulk samples of the TMR constituents for each diet were thawed and analyzed for DM and N (macro Kjeldahl method; AOAC International method 954.01; AOAC International, 2001) and CP estimated as N × 6.25. Dried samples were analyzed for ash (combustion at 500°C for 16 h), NDF and ADF (expressed inclusive of residual ash; Mertens et al., 2002; Robertson and Van Soest, 1981), starch (Fuller, 1967; MacRae and Armstrong, 1968), and WSC, and frozen forage samples were analyzed for volatile component concentrations and pH as described for previous studies at our location (Kliem et al., 2017). Concentrations (g/kg DM) of CP, NDF, ADF, ash, starch, and WSC in each TMR were then calculated based on constituent inclusion rates.

Total polyphenolic content (**TPC**) in seaweeds was determined by dissolving freeze-dried sample in ultrapure H_2O at a concentration of 10 mg DM/mL and shaken in 50-mL cap tubes (Sarstedt) on a plate shaker for ~1 h at room temperature at 400 rpm. Samples were then centrifuged at 5,100 × *g* and 4°C for 20 min and the water phase was used for subsequent analysis. The method of Singleton and Rossi (1965) adapted to a microplate format was used to measure TPC. Results were calculated as gram of phloroglucinol equivalents per 100-g sample and gallic acid equivalent per 100-g sample. Bromoform in the seaweed was analyzed according to method 8260B (EPA, 1996) by ALS Global.

Mineral and Trace Elemental Analyses

The trace elements Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sn, Hg, and Pb and macrominerals Na, Mg, P, K, and Ca were quantified in silage, seaweeds, TMR, and milk samples after a digestion using an Ultra-wave Acid Digestion System (Milestone Inc.) followed by analysis using an Agilent 7900 quadrupole inductively coupled plasma mass spectrometer (**ICP-MS**; Agilent Technologies). Briefly, 0.150 to 0.200 g of the sample was weighed in quartz digestion vessels (MLS GmbH, Leutkirch im Allgäu, Germany), 1 mL of concentrated nitric acid and 1 mL of hydrogen peroxide (30%) were added, and digests were heated to 70°C for 30 min. After the digestion, the samples were diluted to 50 mL with Milli-Q water, and further diluted 50-fold in 2% (vol/vol) HNO_3 for minerals analysis. Samples were prepared in duplicate. Certified reference materials (**CRM**) were analyzed with each instrumental analysis for quality control with a z-score below 2: skim milk powder (BCR-063R; Joint Research Center) and either brown seaweed hijiki (CRM 7305-a; National Metrology Institute of Japan), or fish proteins (CRM DORM-4; National Research Council of Canada). Iodine analysis was performed using an Agilent 8800 triple quad ICP-MS. Milk (0.1 mL) was diluted in 9.9 mL of 2% (vol/vol) of tetramethylammonium hydroxide, and the samples were then shaken for 10 s and filtered before analysis using a sterile 0.45-μm polyvinylidene fluoride syringe filter. A skim milk powder CRM for mineral analyses (ERM-BD150; Joint Research Centre) was included as a quality control for the I analysis.

Statistical Analysis

Measurements of methane emission and associated milk, FCM, and ECM milk production were averaged for the last 3 wk of treatments before statistical analysis using the Mixed procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) and a model testing fixed effects of diet (3 df), the random effect of cow within treatment, and measurements the week before the start of the study (wk 0) as a covariates. For methane emission (g/d) and yield (g/kg DMI) measurements of DMI in wk 0 was used as a covariate, whereas for methane intensity (g/kg ECM), ECM in wk 0 was used as the covariate. In addition, treatment least squares means were compared using the probability of difference procedure of the LSMEANS statement. Weekly measurements of feed intake and milk production and composition over the first 7 wk of the study (wk 1–6 for mineral composition) were analyzed using the same mixed models, but with their measurement in wk 0 as a covariate, the addition of week as a repeated measure, and week-by-treatment interaction, and using the covariance structure giving the best fit based on corrected Akaike's information criteria (unstructured, compound symmetry, heterogeneous compound symmetry, autoregressive, or heterogeneous autoregressive). Days in milk, parity, milk yield, DMI, and BW in wk 0 were used as covariates for all variables if their probability was less than 0.30 in an initial analysis. Probability levels of 0.05 or less were considered significant and greater than 0.05 and less than 0.10 were considered a tendency for treatment diet affects or differences between treatment means.

RESULTS

Diet Composition

The seaweeds fed in the present study were relatively high in ash, and this was particularly true for AT (Table 3). Although AT was also relatively high in CP concentration (170 g/kg DM), the AN and FV were relatively low in CP (75 and 30 g/kg DM, respectively). As regards iodine concentration, the FV fed had the lowest iodine concentration (0.156 g/kg DM), whereas AT was very high in iodine concentration at 4.1 g/kg DM. Although only traces of bromoform were measured in the AN and FV fed, bromoform concentration in the AT was 3.6 mg/kg DM. In contrast, total phenolic concentration was lowest in AT compared with AN and FV. Although several PTE were present in seaweed at measurable levels (Table 4), the inclusion rate of the seaweeds in the treatment diets was relatively low to meet restrictions on diet iodine concentrations, therefore replacement of grass silage with seaweed mixtures had little effect on measured TMR concentration of organic components (Table 5) or minerals (Table 6). Although iodine concentrations were measured in seaweeds to formulate dietary inclusion rates, iodine concentrations for the treatment diets fed are not available.

Feed Intake, Milk Yield and Composition, and BW and Condition

Over the course of the 7-wk feeding trial TMR DMI ($P < 0.10$) tended to be affected by treatment, with cows fed the highest level of FV (FV:AN) having 1.8 kg/d lower intake of TMR (Table 7) compared with cows fed the AT containing mixture, which was reflected by a similar difference in total feed DMI, as GreenFeed bait intake was not affected by treatment. There were treatment-by-week interactions ($P < 0.001$) for TMR and total DMI, as well as milk yield ($P < 0.06$), reflecting weekly variation across the 4 diets over the course of the study (Figure 1). There were no effects of treatment on milk, FCM, or ECM yield (Table 7; $P \geq 0.478$). Milk urea concentration was lower ($P < 0.09$) when the AN:FV diet was fed,

Table 3. Measured chemical composition (g/kg DM unless indicated) of grass silage (GSilage), maize silage (MSilage), concentrate blend (Blend), NaOH-treated wheat (Wheat), *Ascophyllum nodosum* (AN), *Fucus vesiculosus* (FV), and *Asparagopsis taxiformis* (AT)

Item	GSilage	MSilage	Blend	Wheat	AN	FV	AT
pH	3.62	3.78					
DM	316.4	340.0	868.5	663.0	918.0	911.0	935.0
Ether extract	35.4	32.7	69.3	22.8	40.0	40.3	16.1
Ash	76.5	36.4	120.3	63.3	207.0	165.8	515.5
CP	107.1	83.1	295.1	111.6	75.2	29.6	170.1
ADF	268.2	229.2	158.6	24.3			
NDF	461.7	407.1	232.2	51.3			
WSC[1]	84.7	ND[2]	73.5	27.9	23.2	27.0	21.4
Starch		291.5	130.5	535.4			
Iodine, mg/kg					632	156	4,100
Bromoform, μg/kg					3.0	0.7	3,600
Total phenolics, μg/kg					7.5	10.2	0.3
Lactate	31.0	15.6					
Volatiles, mg/kg DM							
Ethanol	10,188	1,485					
Propan-1-ol	ND	195					
Acetic acid	6,549	5,084					
Propionic acid	139	338					
iso-Butyric acid		74					
Propane-1,2-diol	173	611					
n-Butyric acid	53	212					
iso-Valeric acid	72	57					
n-Valeric acid	ND	ND					
Hexanoic acid	ND	ND					
Heptanoic acid	32	ND					

[1]Water soluble carbohydrate.

[2]Not detectable.

but milk fat, protein, and lactose concentration and yield were not affected by diet (Table 7). Cow BW and BCS were not affected by treatment (Table 7).

Milk concentrations of trace elements, including PTE such as arsenic and cadmium, was largely unaffected by dietary seaweed inclusion, except for an increase in

Table 4. Measured mineral concentrations of experimental forages and TMR fed (mg/kg DM)

	Silage		Seaweed[1]		
Item	Grass	Maize	FV	AN	AT
Calcium	4,031	1,821	12,991	14,289	12,639
Phosphorus	1,235	1,407	705	1,349	1,347
Sodium	1,172	46.8	20,402	26,942	78,348
Potassium	22,572	9,202	23,758	21,575	15,834
Magnesium	987	1,011	5,670	6,633	9,685
Zinc	60.2	47.6	15.2	9.3	16.1
Iron	177.3	157.1	132.7	463.1	5,255
Manganese	145.9	23.6	123.8	33.9	86.7
Copper	6.8	7.7	0.9	1.7	3.6
Molybdenum	3.1	3.1	0.22	0.89	1.23
Selenium	0.1	0.0	0.05	0.31	0.7
Aluminum	174.2	89.5	79	263	3,112
Cobalt	0.3	0.2	0.8	1.4	3.2
Chromium	3.9	13.5	0.6	0.6	42.8
Nickel	2.7	7.1	2.4	1.5	16.0
Arsenic	0.1	0.1	28.8	34.9	16.8
Tin	0.0	0.0	0.013	0.030	0.113
Lead	0.4	0.2	0.377	0.039	0.406
Cadmium	0.1	ND[2]	0.46	1.74	0.37
Mercury	ND	ND	0.014	0.027	0.010

[1]*Ascophyllum nodosum* (AN), *Fucus vesiculosus* (FV), and *Asparagopsis taxiformis* (AT).

[2]Not detectable.

Table 5. Measured composition of experimental diets[1]

Item	Control	AN:FV:AT	AN:FV	FV:AN
pH	5.00	5.00	4.98	5.08
DM	381.2	393	392.8	402.2
Ether extract	49.9	54	49.9	48
Ash	81.3	80.9	84.5	83.5
CP	156.3	158.3	154.3	157.4
ADF	198.9	193.3	199.6	196.4
NDF	330.3	324.7	327.5	325.9
WSC[2]	37.7	41.4	28.9	43.5
Starch	211.3	218.7	195.2	214.5
Lactate	16.1	16.0	16.2	16.5
Volatiles, mg/kg DM				
Ethanol	6,603	6,628	7,088	7,382
Propan-1-ol	58	64	50	52
Acetic acid	4,325	4,341	4,470	4,221
Propionic acid	159	180	177	145
iso-Butyric acid	ND[3]	ND	ND	ND
Propane-1,2-diol	267	265	296	290
n-Butyric acid	75	86	87	78
iso-Valeric acid	41	37	44	37
n-Valeric acid	ND	ND	ND	ND
Hexanoic acid	ND	ND	ND	ND
Heptanoic acid	ND	ND	ND	ND

[1]Treatment diets: Control, no seaweed; AN:FV:AT = *Ascophyllum nodosum* (AN), *Fucus vesiculosus* (FV), and *Asparagopsis taxiformis* (AT) at 5:45:50 and 1.5 g/kg diet DM; AN:FV = AN:FV at 90:10 and 6.5 g/kg diet DM; FV:AN at 90:10 and 17.5 g/kg diet DM.

[2]Water soluble carbohydrate.

[3]Not detectable.

Table 6. Measured mineral concentrations of TMR fed (mg/kg DM)

	TMR[1]			
Item	Control	AN:FV:AT	AN:FV	FV:AN
Calcium	8,455	8,892	7,981	7,364
Phosphorus	2,234	2,820	2,224	2,123
Sodium	6,049	7,878	6,598	6,724
Potassium	12,349	14,576	10,790	12,115
Magnesium	1,778	2,187	1,925	1,814
Zinc	104.5	114.7	71.2	110.4
Iron	272.0	311.3	173.0	261.8
Manganese	106.4	111.6	67.9	98.1
Copper	16.2	16.9	10.3	15.3
Molybdenum	2.0	6.9	1.2	2.0
Selenium	0.5	0.4	0.2	0.3
Aluminum	213.9	220.7	104.5	181.4
Cobalt	0.6	0.7	0.5	0.4
Chromium	3.4	3.1	1.9	2.7
Nickel	2.7	2.3	1.6	2.4
Arsenic	0.1	0.3	0.2	0.6
Tin	0.1	0.1	0.1	0.1
Lead	0.4	0.3	0.2	0.2
Cadmium	0.1	0.1	0.1	0.1
Mercury	ND	ND	ND	ND

[1]Treatment diets: Control, no seaweed; AN:FV:AT = *Ascophyllum nodosum* (AN), *Fucus vesiculosus* (FV), and *Asparagopsis taxiformis* (AT) at 5:45:50 and 1.5 g/kg diet DM; AN:FV at 90:10 and 6.5 g/kg diet DM; FV:AN at 90:10 and 17.5 g/kg diet DM.

iodine concentration ($P < 0.001$), which was greatest for cows fed the AN:FV diet, and an increase in tin concentration for cows fed the AN:FV compared with the control diet (Table 8). There was a significant week-by-treatment interaction for milk iodine concentration ($P < 0.001$), which was numerically higher for cows fed the AN:FV diet in wk 3 and 5 (Figure 2). Milk FA composition in wk 6 (Table 9) was also largely unaffected by treatment diets, although C15:1 and C17:0 were higher and C18:3 n-6 were lower when AT was included in the diet.

Table 7. Feed DMI (kg/d), milk yield, milk composition, BW, and BCS of lactating dairy cows fed different amounts and mixtures of seaweeds for 7 wk (treatment means for 7 wk of treatment are presented)

	Treatment[1]					$P <$		
Item	Control	AN:FV:AT	AN:FV	FV:AN	SEM	Diet	Week	Interaction
Feed DMI								
TMR	23.0[ab]	24.5[a]	23.7[ab]	22.7[b]	0.54	0.096	0.001	0.001
GreenFeed	0.84	0.64	0.85	0.80	0.101	0.400	0.001	0.927
Total	23.8[ab]	25.1[a]	24.5[ab]	23.5[b]	0.53	0.138	0.001	0.001
Milk, kg/d	33.6	33.3	35.6	34.8	1.18	0.478	0.001	0.051
FCM,[2] kg/d	38.2	37.4	38.4	39.6	1.40	0.750	0.001	0.181
ECM, kg/d	37.6	36.5	37.6	38.8	1.45	0.738	0.001	0.226
Fat, %	4.33	4.13	4.24	4.36	0.113	0.501	0.001	0.611
Protein, %	3.29	3.27	3.27	3.23	0.041	0.847	0.001	0.624
Lactose, %	4.40	4.32	4.41	4.40	0.035	0.262	0.001	0.808
Casein, %	2.64	2.60	2.63	2.61	0.030	0.760	0.001	0.523
Fat, kg/d	1.426	1.364	1.510	1.505	0.0541	0.229	0.001	0.138
Protein, kg/d	1.096	1.081	1.143	1.128	0.0369	0.606	0.001	0.359
Lactose, kg/d	1.495	1.435	1.583	1.533	0.0526	0.256	0.001	0.237
Casein, kg/d	0.883	0.866	0.921	0.908	0.0302	0.563	0.001	0.287
Urea	192[a]	193[a]	165[b]	181[ab]	8.2	0.081	0.001	0.001
BW, kg	739[ab]	734[b]	743[a]	736[ab]	2.8	0.151	0.001	0.485
BCS	2.60	2.79	2.65	2.65	0.080	0.339	0.001	0.739

[a,b]Treatment means with different superscripts are significantly different ($P < 0.05$) based on paired *t*-tests.

[1]Treatment diets: Control, no seaweed; AN:FV:AT = *Ascophyllum nodosum* (AN), *Fucus vesiculosus* (FV), and *Asparagopsis taxiformis* (AT) at 5:45:50 and 1.5 g/kg diet DM; AN:FV at 90:10 and 6.5 g/kg diet DM; FV:AN at 90:10 and 17.5 g/kg diet DM.

[2]Fat-corrected (4%) milk.

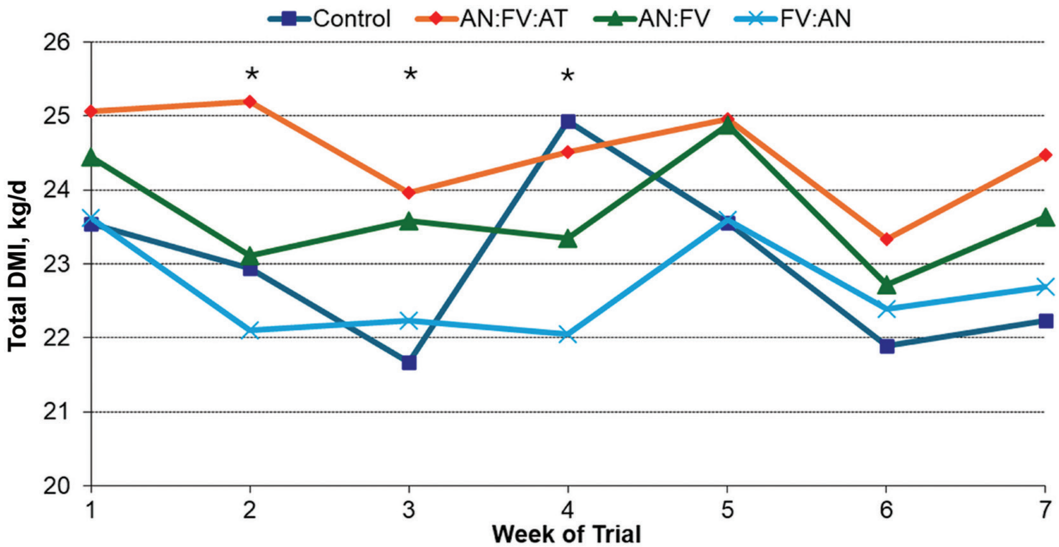

Figure 1. Total DMI of lactating dairy cows over the course of the experiment. Control, no seaweed; AN:FV:AT = *Ascophyllum nodosum* (AN), *Fucus vesiculosus* (FV), and *Asparagopsis taxiformis* (AT) at 5:45:50 and 1.5 g/kg diet DM; AN:FV at 90:10 and 6.5 g/kg diet DM; FV:AN at 90:10 and 17.5 g/kg diet DM. *Effects of treatment for specific weeks at $P < 0.05$.

Methane Emission

Total feed DMI, milk yield, and ECM yield during measurements of methane emission (wk 5–7; Table 10) was not affected by treatments ($P > 0.347$). Although there was no overall effect of treatments ($P > 0.23$) on methane emission (g/d), methane yield (g/kg DMI), or methane intensity (g/kg ECM), paired *t*-tests at $P < 0.08$ showed trends for reduced methane emission (g/d) for the diet containing AT compared with the control diet, and

Table 8. Mineral concentration (DM basis) of milk from cows fed diets containing different amounts and mixtures of seaweeds (means for wk 1 to 6 of feeding)

Item	Treatment[1]				SEM	P <		
	Control	AN:FV:AT	AN:FV	FV:AN		Diet	Week	Interaction
Concentration, mg/kg								
Calcium	7,625	7,933	7,515	7,487	197	0.372	0.001	0.386
Phosphorus	5,585	5,934	5,545	5,416	161	0.151	0.001	0.586
Sodium	3,085	3,113	3,343	3,402	197	0.582	0.001	0.274
Potassium	10,242	10,703	10,426	10,457	371	0.853	0.001	0.459
Magnesium	778	805	785	772	25.4	0.807	0.001	0.403
Zinc	31.08	30.50	28.66	27.45	1.597	0.367	0.001	0.261
Iron	5.37	2.20	3.60	3.57	1.707	0.631	0.019	0.898
Iodine	0.241[a]	0.423[b]	0.522[c]	0.407[b]	0.0354	0.001	0.001	0.001
Manganese	0.179	0.184	0.158	0.175	0.0086	0.196	0.004	0.212
Copper	0.347	0.323	0.312	0.334	0.0205	0.660	0.155	0.181
Molybdenum	0.636	0.628	0.620	0.643	0.0299	0.951	0.001	0.679
Selenium	0.120	0.111	0.112	0.110	0.0045	0.389	0.001	0.159
Aluminum	0.862	0.721	0.619	0.749	0.1823	0.825	0.014	0.995
Concentration, μg/kg								
Cobalt	2.98	3.01	2.73	2.74	0.157	0.440	0.007	0.535
Chromium	25.39	21.07	43.88	56.56	17.898	0.472	0.005	0.380
Nickel	31.7	34.3	28.7	31.4	0.0036	0.753	0.001	0.922
Arsenic	1.87	2.33	3.85	2.89	0.875	0.424	0.109	0.918
Tin	<LOD[2]	<LOD	<LOD	<LOD				
Lead	3.01	2.92	3.67	2.48	0.646	0.631	0.002	0.936
Cadmium	<LOD	<LOD	<LOD	<LOD				
Mercury	<LOD	<LOD	<LOD	<LOD				

[a–c]Treatment means with different superscripts are significantly different ($P < 0.05$) based on paired *t*-tests.

[1]Treatment diets: Control, no seaweed; AN:FV:AT = *Ascophyllum nodosum* (AN), *Fucus vesiculosus* (FV), and *Asparagopsis taxiformis* (AT) at 5:45:50 and 1.5 g/kg diet DM; AN:FV at 90:10 and 6.5 g/kg diet DM; FV:AN at 90:10 and 17.5 g/kg diet DM.

[2]Limit of detection.

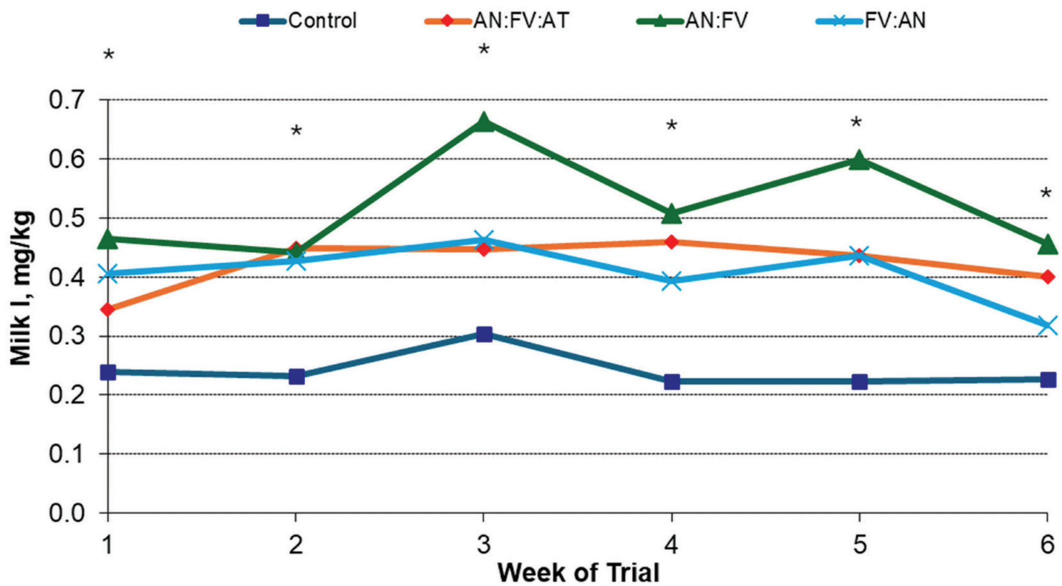

Figure 2. Average iodine concentration of milk of cows fed control or seaweed supplemented diets over the course of the first 6 wk of the experiment. Control, no seaweed; AN:FV:AT = *Ascophyllum nodosum* (AN), *Fucus vesiculosus* (FV), and *Asparagopsis taxiformis* (AT) at 5:45:50 and 1.5 g/kg diet DM; AN:FV at 90:10 and 6.5 g/kg diet DM; FV:AN at 90:10 and 17.5 g/kg diet DM. *Effects of treatment for specific weeks at $P < 0.05$.

a reduction in methane yield compared with the control diet when the diets containing AT and the higher level of FV (FV:AN at 17.5 g/kg diet DM) were fed. Methane intensity also tended to be lower ($P < 0.08$) for the diet containing AT compared with the diet containing the higher concentration of AN (AN:FV at 6.5 g/kg diet DM).

Table 9. Fatty acid concentration of milk (g/100 g of total fatty acids) from cows fed diets containing different amounts and mixtures of seaweeds (wk 6 samples only)

	Treatment[1]					
Item	Control	AN:FV:AT	AN:FV	FV:AN	SEM	$P <$
C6:0	1.67	1.83	1.78	2.06	0.137	0.145
C8:0	1.15	1.23	1.16	1.30	0.062	0.290
C10:0	2.84	2.96	2.68	2.98	0.136	0.373
C12:0	3.67	3.73	3.39	3.46	0.203	0.543
C14:0	11.7	11.5	11.1	11.2	0.25	0.433
C14:1	1.20	1.17	1.02	1.11	0.067	0.248
C15:0	0.38	0.38	0.37	0.38	0.017	0.989
C15:1	0.89[a]	1.00[b]	0.83[a]	0.81[a]	0.035	0.002
C16:0	35.2	34.8	35.0	33.5	0.60	0.161
C16:1n-7	1.90	1.74	1.70	1.70	0.085	0.319
C16:2n-4	0.41	0.41	0.40	0.42	0.021	0.932
C17:0	0.47[a]	0.51[b]	0.47[a]	0.47[a]	0.011	0.032
C17:1	0.15	0.15	0.15	0.60	0.249	0.460
C18:0	8.34	8.64	8.48	8.89	0.541	0.702
C18:1n-9	20.2	20.0	19.7	19.8	0.79	0.981
C18:1n-7	2.16	2.24	2.14	2.26	0.133	0.893
C18:2n-6	2.12	2.09	2.30	2.27	0.087	0.205
C18:3n-6	0.39[a]	0.36[b]	0.41[a]	0.37[ab]	0.012	0.026
C18:3n-4	0.36	0.34	0.40	0.37	0.027	0.414
C20:2	0.11	0.11	0.10	0.12	0.162	0.162
C20:3n-6	0.14	0.12	0.12	0.11	0.009	0.150
SFA	64.9	65.3	64.8	64.6	0.783	0.916
MUFA	26.5	26.3	25.6	26.4	0.735	0.805
PUFA	3.4	3.4	3.7	3.8	0.134	0.136

[a,b]Treatment means with different superscripts are significantly different ($P < 0.05$) based on paired *t*-tests.

[1]Treatment diets: Control, no seaweed; AN:FV:AT = *Ascophyllum nodosum* (AN), *Fucus vesiculosus* (FV), and *Asparagopsis taxiformis* (AT) at 5:45:50 and 1.5 g/kg diet DM; AN:FV at 90:10 and 6.5 g/kg diet DM; FV:AN at 90:10 and 17.5 g/kg diet DM.

Table 10. Feed DMI (kg/d), milk yield, and methane emission of lactating dairy cows fed diets containing different amounts and mixtures of seaweeds (averaged for wk 5–7 of treatments)

	Treatment[1]					
Item	Control	AN:FV:AT	AN:FV	FV:AN	SEM	*P* <
TMR DMI	22.6	22.9	24.3	23.7	0.684	0.292
GreenFeed DMI	0.634	0.677	0.544	0.686	0.093	0.698
Total DMI	23.2	23.6	24.8	24.4	0.69	0.347
Milk yield, kg/d	31.5	33.0	31.4	33.1	1.40	0.749
ECM, kg/d	35.9	37.4	34.4	35.3	1.76	0.671
CH_4, g/d	459[a]	417[b]	452[ab]	435[ab]	17.0	0.256
CH_4, g/kg DMI	20.91[a]	18.42[b]	18.72[ab]	18.57[b]	1.065	0.230
CH_4, g/kg ECM	13.36[ab]	11.44[b]	14.21[a]	13.20[ab]	1.09	0.321

[a,b]Treatment means with different superscripts tend to be different at $P < 0.08$) based on paired t-tests.

[1]Treatment diets: Control, no seaweed; AN:FV:AT = *Ascophyllum nodosum* (AN), *Fucus vesiculosus* (FV), and *Asparagopsis taxiformis* (AT) at 5:45:50 and 1.5 g/kg diet DM; AN:FV at 90:10 and 6.5 g/kg diet DM; FV:AN at 90:10 and 17.5 g/kg diet DM.

DISCUSSION

Intake of TMR and total DMI over the 7-wk course of the study (Table 7) was lowest when the higher levels of FV (1.575%; 1.75% total seaweed) were fed and highest when the AT was included in the TMR at 0.075% (0.15% total seaweed). Previous in vivo studies have observed negative effects of feeding *A. armata* (Roque et al., 2019) or AT (Stefenoni et al., 2021) on DMI of dairy cows. In contrast, there was no effect of feeding AT at up to 0.2% of diet DM in beef cattle (Kinley et al., 2020) or up to 3.0% of diet DM in sheep (Li et al., 2018). These differences may be related to the basal diet fed, the level of basal DMI, and the AT composition, including bromoform concentration, and inclusion rate. In dairy cows, the effects of AT on DMI increased with increasing diet inclusion rate (Roque et al., 2019; Stefenoni et al., 2021). A meta-analysis of published in vivo seaweed feeding studies with beef and dairy cattle (Lean et al., 2021) reported a trend ($P = 0.075$) for a negative effect of feeding AT across one beef and 2 lactating dairy cattle studies, but no effect ($P = 0.229$) of feeding AN in 3 studies with lactating dairy cows and no overall effect on DMI of feeding all seaweed per se (including 3 studies with seaweeds other than AT or AN; $P = 0.48$). In contrast a more recent meta-analysis of 23 published seaweed feeding trials in lactating dairy cows (Orzuna-Orzuna et al., 2024) reported that feeding red seaweeds reduced DMI, but feeding brown seaweeds (predominantly AN) had no effect.

As noted previously, over the course of the 7 wk of the present study DMI was lowest when the higher amount of FV was fed (Table 7). Previous in vitro studies of rumen degradability have shown that of 22 Northern Hemisphere macroalgae evaluated, FV had the lowest rumen degradability (Thorsteinsson et al., 2023a). Although it is unlikely that FV degradability directly affected DMI at the levels of diet inclusion in the present study (0.65% or 1.575%), the low levels of degradability may be correlated with other characteristics that affect palatability. In this regard, it is known that the North Ronaldsay island sheep, which since 1832 have survived almost solely on North Atlantic seaweeds for their nutrition, select against FV and *Fucus serratus*, suggesting their palatability is low (Hansen et al., 2003). The difference in DMI between the diet containing AT and the diet containing the higher level of FV was not evident during the last 3 wk of the study (Table 10), suggesting adaptation to the presence of the FV in the diet over the course of the study. In contrast to the present results, Thorsteinsson et al. (2024) fed FV and *Fucus serratus* to lactating dairy cows at 4% of diet DM for 3-wk periods and observed no effects on DMI.

In the present study, there was no effect of dietary inclusion of seaweed in relatively small amounts (1.75% or less) on BW, BCS, milk yield or milk fat, protein, or lactose concentration (Table 7), suggesting no effect on diet digestion or feed utilization efficiency. The meta-analysis of Orzuna-Orzuna et al. (2024) found that feeding red seaweeds reduced milk and ECM yield, but feeding brown seaweeds (AN or *Saccharina latissima*) increased milk yield, but not energy-corrected yield. In 2 recent studies investigating effects of feeding Nordic brown seaweeds, including AN (Thorsteinsson et al., 2023b) or FV (Thorsteinsson et al., 2024) at 4% of diet DM had no effect on DMI, milk yield, or ECM yield of lactating dairy cows. Similarly, Antaya et al. (2019) and Newton et al. (2023) reported no effects of feeding AN on milk yield of dairy cows. In contrast, feeding AT at 1% of diet DM, but not 0.5% of DM, decreased both DMI and milk yield of lactating dairy cows (Stefenoni et al., 2021). These results suggest that negative effects of feeding red seaweeds (Orzuna-Orzuna et al., 2024), AT (Stefenoni et al., 2021), and *A. armata* (Roque et al., 2019) on milk yield are primarily due to effects on DMI and that the effects are dose dependent. In the meta-analysis of Orzuna-Orzuna et al. (2024), there was a decrease in milk protein concentration and an increase in milk fat concentration when seaweed was fed, but these effects would be dependent on the type and amount of seaweed fed. In other studies, feeding different brown seaweeds at 2% to 4% of diet DM had no effect on milk fat, protein, or lactose concentration (Thorsteinsson et al., 2023b, 2024) as observed in the present study, but feeding AT at 1% of diet DM decreased milk protein concentration (Stefenoni et al., 2021). This decrease in milk protein concentration was not observed when the lower level (0.5%) of AT was fed (Stefenoni et al., 2021) and thus, was likely due in part to the decrease in DMI when the higher level of AT was fed. In the study of Roque et al. (2019), incremental decreases in milk protein concentration with increasing diet concentration of *A. armata* were associated with incremental decreases in feed DMI.

In the present study milk urea concentration was lower when the higher level of AN was fed (Table 7). Although the measured CP concentration of the TMR containing the higher level of AN was numerically lower than for the other treatments, the effect may also be due to the presence of phlorotannins in AN fed. Phlorotannins have been shown to bind proteins and inhibit their degradation in the rumen, and previous studies have shown that feeding seaweeds generally (Orzuna-Orzuna et al., 2024), or specifically AN (Thorsteinsson et al., 2023b) or FV (Thorsteinsson et al., 2024), decreases milk urea concentrations. In addition, either AN or FV inclusion in a TMR at 5% of DM reduced CP degradation in vitro (Künzel et al., 2022). In the study of Thorsteinsson et al. (2023b), there was no effect of feeding *Saccharina latissima* at 4% of diet DM or *Sargassum muticum* at 2% of diet DM on milk urea concentration, and the effect of feeding AN on milk urea concentration was associated with a decrease in rumen concentration of ammonia and plasma urea. In this regard, it is known that there are numerous bioactive compounds in seaweeds that could potentially have effects on rumen fermentation and microbial communities, including suppression of methane emissions (Vijn et al., 2020; Wasson et al., 2022).

Feeding 3 mixtures of seaweeds at varying levels of inclusion had little effect on milk concentrations of minerals and trace elements (Table 8), apart from an increase in iodine concentration that has been observed in numerous previous studies when different types of seaweed have been fed to dairy cattle (e.g., Antaya et al., 2015, 2019; Stefenoni et al., 2021; Newton et al., 2023; Orzuna-Orzuna et al., 2024). The PTE, such as arsenic or cadmium that can be high in seaweed (EFSA, 2023; Sim et al., 2024), were not measurably transferred to the milk in this study. In the present study, milk iodine concentration was highest when the higher level of AN was fed, reflecting a higher concentration of iodine compared with FV (Table 3). Even at the higher level of milk iodine (Table 8), 2.1 kg of milk could be consumed without exceeding the recommended upper limit for iodine intake of adults (Institute of Medicine, Food and Nutrition Board, 2001). Although the iodine concentration of AT was higher than AN or FV, the inclusion rate of AT was relatively low. That said, the increases in milk iodine concentration observed in the present study are lower than observed in numerous previous studies and the overall effect from the meta-analysis of published studies by Orzuna-Orzuna et al. (2024), reflecting the limit on additional dietary iodine used in the present study. Antaya et al. (2015) observed a linear increase in milk iodine concentration with increasing diet AN concentrations in a Latin square experiment with 3-wk periods and 3 levels of AN inclusion. Levels of seaweed inclusion in the present study were based on measured iodine concentrations in the seaweeds fed and EFSA (2013) guidelines on the amount of iodine that can be added to diets of dairy cows producing milk for human consumption. This restriction was most severe for AT, which was much higher in iodine concentration than AN and FV. We included FV in the present study based on in vitro screening for suppressive effects on methane production in batch culture (Künzel et al., 2022; Yergaliyev et al., 2024) and the fact that it is typically lower in iodine concentration than many other seaweeds. The inherently high iodine concentration of *Asparagopsis* sp. seaweeds is linked to their high bromine and thus, bromoform concentrations, but may limit their practical use as a methane-suppressing feed supplement unless technologies for large scale culture or postharvest processing technologies (e.g., Krook et al., 2024) can be developed that reduce iodine concentrations in the product fed (Hristov, 2024) without affecting concentration of bromoform or other methane-suppressing components. As observed by Newton et al. (2021, 2023), there was a significant time-by-treatment interaction for milk iodine concentration, with the highest level of milk iodine observed for the higher AN diet in wk 3 and 5 of the study (Figure 2). As proposed by Newton et al. (2021), the effects of increased dietary iodine intake on milk iodine concentration may have a transitory element associated with adaptation of iodine transporters involved in iodine transfer from the lumen of the gut to milk. In addition to the changes in iodine concentration, we also observed a higher concentration of tin in milk of cows fed higher levels of AN (Table 8), although the concentrations present were very low.

There were only very few minor effects of feeding different mixtures of seaweeds on milk FA concentrations (Table 9). Concentrations of C15:1 and C17:0 were higher and concentration of C18:3 n-6 was lower when the diet containing AT was fed. There are only limited studies reporting effects of feeding AT or other seaweeds on milk FA profiles, but Thorsteinsson et al. (2023b) reported a decrease in milk concentrations of C16:0 and an increase in total PUFA concentration when AN was fed at 4% of DM. In contrast, feeding incremental amounts of AN up to 170 g/d had no effects on milk FA concentrations of Jersey cow (Antaya et al., 2015). Differences in milk concentrations of FAs may be due to differences in diet FA concentrations, their rumen biohydrogenation, or their postabsorptive metabolism. In the present study dietary inclusion rates of the seaweed mixtures was low relative to previous studies, which in part explains the minimal dietary effects on milk FA concentrations observed.

Although there was no overall effect of treatment, methane production, yield, and intensity were all lower for cows fed the AT containing seaweed blend at 0.15% of DM compared with cows fed the control diet with no seaweed inclusion (Table 10). The reduction observed (9% for methane production and 12% for methane yield and intensity) is small compared with previous studies where *A. armata* (Roque et al., 2019) or AT (Stefenoni et al., 2021; Hristov et al., 2022; Hristov, 2024) was fed at higher dietary inclusion rates. As discussed previously, this highlights the limitation that AT (or *A. armata*) iodine concentration may impose on the methane suppression potential of feeding AT or AA in the European Union and other countries with regulations in place to limit potential negative health effects of high dietary iodine concentrations for consumers. In the European Union, it has been proposed that the current maximum limit of 5 mg/kg diet as fed be reduced to 2 mg/kg diet (EFSA, 2013). In the present study feeding the diet with the higher AN inclusion rate (0.65% total seaweed inclusion; 0.585% and 139 g/d for AN) had no effect on methane production or yield. In the study of Antaya et al. (2019) feeding AN at 113 g/d to grazing lactating Jersey cows had no overall effect on methane production or yield, but a significant treatment by period interaction was observed in that methane production was reduced by 10% in the first period of the study but not in 2 subsequent measurement periods. In the present study, cows consumed on average 139 g of AN daily, suggesting that AN fed within limits imposed by its iodine concentration may have limited potential as a strategy for reducing methane emissions of dairy cattle. Thorsteinsson et al. (2023b) also observed no effect of feeding AN to dairy cows at 4% of diet DM (940 g/d) on methane production or yield of lactating dairy cows. In the present study feeding the higher level of FV (1.75% total seaweed inclusion; 1.575% and 358 g/d for FV), there were no effects on methane production, yield, or intensity relative to the control diet. Similarly, Thorsteinsson et al. (2024) observed no effects of feeding lactating dairy cows FV at 4% of diet DM on methane emissions. Thus, although the AN and the FV fed in the present study had methane-suppressing effects in vitro (Künzel et al., 2022), they had no effect on methane production when fed to lactating dairy cows in vivo. This may reflect differences in the relative amounts of dietary inclusion, as well as differences in rumen microbial communities and dynamics of rumen fermentation in vivo compared with in vitro.

CONCLUSIONS

In the present study, when inclusion rates of 3 seaweed mixtures were determined by restrictions on diet iodine concentrations, there was no significant effect of feeding AN- and FV-based seaweed mixtures based on methane production or yield. Feeding AT in combination with AN and FV resulted in a decrease in methane production and yield (9% and 12%, respectively), but the effect was in part limited by the small amount fed. Feeding the higher level of AN decreased milk urea concentration, suggesting effects on ruminal fermentation and protein degradation, whereas all 3 seaweed-containing diets increased milk iodine concentrations. Apart from a small effect of a mixture of AT, AN, and FV, feeding seaweeds from the North Atlantic ocean (AN and FV) at levels restricted by their iodine concentrations had no effects on methane emission, no effect on milk yield, and relatively minor effects on milk composition apart from an increase in iodine concentration.

NOTES

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Nonstandard abbreviations used: AN = *Ascophyllum nodosum*; AT = *Asparagopsis taxiformis*; CRM = certified reference materials; FA = fatty acid; FV = *Fucus vesiculosus*; GSilage = grass silage; ICP-MS = inductively coupled plasma MS; MSilage = maize silage; ND = not detected; PTE = potentially toxic elements; TL = total lipids; TPC = total polyphenolic content; WSC = water soluble carbohydrate.

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