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Evaluation of 3 northwest European seaweed species on enteric methane production and lactational performance of Holstein-Friesian dairy cows

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ABSTRACT

Seaweeds have been studied for their ability to reduce enteric methane emissions of ruminants when fed as a feed supplement. In vivo research with dairy cattle is mainly limited to the seaweed species *Ascophyllum nodosum* and *Asparagopsis taxiformis*, whereas in vitro gas production research covers a broader range of brown, red, and green seaweed species from different regions. The objective of the present study was to determine the effect of *Chondrus crispus* (Rhodophyta), *Saccharina latissima* (Phaeophyta), and *Fucus serratus* (Phaeophyta), 3 common northwest European seaweeds, on enteric methane production and lactational performance of dairy cattle. Sixty-four Holstein-Friesian dairy cattle (16 primiparous, 48 multiparous) averaging (mean ± standard deviation) 91 ± 22.6 d in milk and 35.4 ± 8.13 kg/d fat- and protein-corrected milk yield (FPCM) were randomly assigned to 1 of 4 treatments in a randomized complete block design. Cows were fed a partial mixed ration [54.2% grass silage, 20.8% corn silage, and 25.0% concentrate; dry matter (DM) basis] with additional concentrate bait in the milking parlor and the GreenFeed system (C-Lock Inc.). The 4 treatments consisted of a control diet without seaweed supplement (CON), or CON supplemented with 150 g/d (fresh weight of dried seaweed) of either *C. crispus* (CC), *S. latissima* (SL), or a 50/50 mix (DM basis) of *F. serratus* and *S. latissima*. Milk yield (28.7 vs. 27.5 kg/d, respectively), fat- and protein-corrected milk (FPCM) yield (31.4 vs. 30.2 kg/d, respectively), milk lactose content (4.57 vs. 4.52%, respectively), and lactose yield (1,308 vs. 1,246 g/d, respectively) increased for SL compared with CON. Milk protein content was lower for SL compared with the other treatments. Milk fat and protein contents; yields of fat, protein, lactose, and FPCM; feed efficiency; milk nitrogen efficiency; and somatic cell count did not differ between CON and the other treatments. Depending on week of experiment, milk urea content was higher for SL compared with CON and CC. No effects were observed of the treatments compared with CON for DM intake, number of visits to the GreenFeed, or gas emission (production, yield, or intensity) of CO_2, CH_4, and H_2. In conclusion, the seaweeds evaluated did not decrease enteric CH_4 emissions and did not negatively affect feed intake and lactational performance of dairy cattle. Milk yield, FPCM yield, milk lactose content, and lactose yield increased, and milk protein content decreased, with *S. latissima.*
Key words: dairy cow, cattle, methane, seaweed, milk yield, performance

INTRODUCTION

Seaweeds (macroalgae) have been incorporated in the diet of livestock to substitute a part of the diet in times of scarcity, mainly in areas close to the coast with regular wash-off or natural growth of seaweeds (Evans and Critchley, 2014). For use in animal diets, seaweeds have been researched as a novel protein source, a source of carbohydrates to stimulate gut health, and as a source of bioactive compounds for both human and animal health (Makkar et al., 2016). In recent years, the evaluation of different seaweed species in vitro and in vivo as a feed supplement to reduce enteric methane (CH_4) in ruminants has gained interest (e.g., Machado et al., 2014; Kinley and Fredeen, 2015; Stefenoni et al., 2021).

The livestock supply chain emits approximately 7.1 Gt of CO_2 equivalents (CO_2-eq) worldwide per year, of which 2.2 Gt of CO_2-eq consists of enteric CH_4 emitted by beef and dairy cattle (Gerber et al., 2013). Strategies to reduce the enteric CH_4 production of ruminants have been an important research topic in the past decades. These strategies have focused, among others, on influencing the VFA profile during digestion (e.g., starch-rich feed), offering alternative hydrogen (H_2) sinks (e.g., nitrate, sulfate), adding antimicrobial components (e.g., tannins), and adding additives to inhibit specific enzymes (3-nitrooxypropanol, halogenated me-

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tabolites), and such strategies are integral to meeting targets to limit global warming (Arndt et al., 2022).

The use of seaweeds to reduce enteric methane production in ruminants has garnered increasing interest because of the methane mitigation potential of 2 red (Rhodophyta) seaweed species: *Asparagopsis taxiformis* and *Asparagopsis armata*. These 2 species are rich in bromoform and other halogenated metabolites, which are concentrated in special glands (Paul et al., 2006). Bromoform and other halogenated compounds in *Asparagopsis* spp. can strongly reduce enteric methane production by 0 to 98%, depending on dosage, basal diet, and storage conditions, which has been shown in recent ruminant trials (e.g., Roque et al., 2019; Kinley et al., 2020; Stefenoni et al., 2021). The use of *A. taxiformis* and *A. armata* may result in decreased voluntary feed intake in dairy cattle (Roque et al., 2019; Stefenoni et al., 2021), abnormalities or damage to the rumen wall of sheep and dairy cattle (Li et al., 2018; Muizelaar et al., 2021), and transfer of bromoform and other metabolites to the milk and urine of lactating dairy cattle (Muizelaar et al., 2021; Stefenoni et al., 2021), although other studies did not find negative effects on feed intake or transfer of bromoform in meat, organs, or feces (e.g., Kinley et al., 2020). Other seaweed species, like the brown (Phaeophyta) seaweeds *Fucus serratus* and *Ascophyllum nodosum*, can be rich in polyphenols, specifically phlorotannins, a seaweed-specific tannin-like metabolite (Connan et al., 2006; Heffernan et al., 2015). Phlorotannins extracted from *Laminaria digitata* decreased methane production in vitro without negatively affecting the total gas production at inclusion levels <40 g/kg (Vissers et al., 2018). Moreover, the addition of fermented *Saccharina latissima* (Phaeophyta) reduced methane production in vitro (Thorsteinsson et al., 2021). Carrageenan is a highly sulfated carbohydrate often found in red (Rhodophyta) seaweed species (Campo et al., 2009). Depending on type (kappa, iota, or lambda), seaweed species, and batch, the sulfate content of carrageenan can range from 20 to 40% by weight (de Ruiter and Rudolph, 1997). Sulfate can act as an alternative hydrogen sink when added to the diet of ruminants and thereby reduce enteric methane production (van Zijderveld et al., 2010). The red seaweed *Chondrus crispus* (Rhodophyta) was, in the past, harvested and processed for carrageenan collection (Collén et al., 2014) and has been demonstrated in vitro to reduce methane production (Kinley and Fredeen, 2015). Total carrageenan content in *C. crispus* can range from 30 to 45% of dry weight depending on life stage, location, and time of year (Tasende et al., 2012). It remains unclear whether the observed methane reduction by Kinley and Fredeen (2015) was due to the carrageenan, and thus sulfate, content in *C. crispus*. There is little information available on the potential of these or similar seaweed species to reduce enteric methane emissions in dairy cattle.

The objective of the present study was to determine the effect of *C. crispus*, *S. latissima*, and a 50/50 mix of *S. latissima* and *F. serratus*, 3 common northwest European seaweeds, on enteric methane production and lactational performance of Holstein-Friesian dairy cattle. We hypothesized that the inclusion of these seaweeds through specific metabolites would affect rumen methanogenesis and lead to reduced enteric methane production without negatively affecting feed intake or lactational performance.

MATERIALS AND METHODS

Experimental Design

Before the start of the experiment, we performed a literature search on potential active components, abundance, cultivability, and potential to reduce methane production in vitro of relevant seaweed species. Based on this analysis, 10 seaweed species with an inclusion rate of approximately 4.5% on a DM basis were tested in an in vitro gas production experiment and evaluated for their potential to reduce methane production (results not shown). The top 3 species were identified based on the available information and selected for this experiment. These seaweeds reduced in vitro methane production (mL of CH_4/g of OM) by 4 to 10% after 72 h of incubation, without negatively affecting total gas production.

The experiment was conducted from July to October 2019 at the animal research facilities of Wageningen University and Research (Leeuwarden, the Netherlands) and was in accordance with Dutch law on animal experiments. The experiment lasted 13 wk in total: 1 wk of adaptation to the barn (all cows received the same control diet), 2 wk of covariate measurements (again, all cows receiving the control diet), and 10 wk of experimental treatments. The experiment followed a randomized complete block design with 4 dietary treatments and 64 Holstein-Friesian dairy cows (16 primiparous cows and 48 multiparous cows). The 4 dietary treatments consisted of a control diet without seaweed supplement (**CON**), CON supplemented with *C. crispus* (**CC**), CON supplemented with *S. latissima* (**SL**), and CON supplemented with a 50/50 mix (DM basis) of *F. serratus* and *S. latissima* (**FS**). The 50/50 mix of *F. serratus* and *S. latissima* was chosen in view of the high arsenic content in samples of *F. serratus* obtained before actual harvest; it was expected that a mix of *F. serratus* with *S. latissima* would be below the maximum level of arsenic allowed. After harvesting,

Table 1. Chemical composition (g/kg of DM, unless otherwise stated) of the partial mixed ration (PMR), GreenFeed bait (GF bait), milking carousel bait (MC bait), and individual seaweeds

				Seaweed[3]		
Item	PMR[1,2]	GF bait	MC bait	CC	SL	FS
DM (g/kg of product)	541	877	888	843	908	887
Ash	87	66	86	197	314	266
CP[4]	147	140	153	141	102	87
Crude fat	34	19	49	6	9	18
Starch	63	ND[5]	ND	63	3	5
Sugar	89	118	93	5	3	12
NDF	411	380	368	176	323	158
ADF	225	237	200	42	241	285
ADL	19	19	48	13	<6	71
Arsenic (mg/kg of DM)	ND	ND	ND	8	70	63
Cadmium (mg/kg of DM)	ND	ND	ND	0.4	0.6	0.9

[1]Grass silage composition: DM: 458 g/kg, ash: 114 g/kg of DM, CP: 166 g/kg of DM, crude fat: 41 g/kg of DM, sugar: 111 g/kg of DM, NDF: 442 g/kg of DM, ADF: 245 g/kg of DM, ADL: 20 g/kg of DM. Corn silage composition: DM: 344 g/kg, ash: 44 g/kg of DM, CP: 67 g/kg of DM, crude fat: 32 g/kg of DM, starch: 308 g/kg of DM, sugar: 12 g/kg of DM, NDF: 403 g/kg of DM, ADF: 220 g/kg of DM, ADL: 15 g/kg of DM. Concentrate composition: DM: 880 g/kg, ash 62 g/kg of DM, CP: 172 g/kg of DM, crude fat: 22 g/kg of DM, starch: not determined, sugar: 104 g/kg of DM, NDF: 350 g/kg of DM, ADF: 184 g/kg of DM, ADL: 21 g/kg of DM.
[2]Ratio PMR was 54.2%, 20.8%, and 25.0% for grass silage, corn silage, and concentrate, respectively (DM basis).

[3]CC = *Chondrus crispus*; SL = *Saccharina latissima*; FS = 50/50 mix (DM basis) of *Fucus serratus* and *Saccharina latissima*.

[4]Calculated as N × 6.25.

[5]ND = not determined.

analyses indicated higher arsenic levels than expected based on preharvest analyses (Table 1), and approval to use these seaweed supplements was granted by the relevant authorities. Cows were blocked according to parity (2.7 ± 1.56; mean ± standard deviation, **SD**), fat- and protein-corrected milk yield (**FPCM**; 35.4 ± 8.13 kg/d), and DIM (91 ± 22.6) at the start of the trial. Cows within a block were randomly assigned to 1 of the 4 treatments.

Diets, Feeding, and Housing

All cows received the same partial mixed ration (**PMR**) during the trial, supplemented (except for the CON treatment) with 150 g (fresh weight) of dried seaweed (126–136 g of DM) per cow per day at the start of the treatment period. The inclusion rate of 126 to 136 g of DM/d of seaweed is within the range previous studies have used in diets of dairy cattle (57–360 g/d; Antaya et al., 2015; Hong et al., 2015; Chaves Lopez et al., 2016) and was expected to make a minimal contribution to total arsenic levels in the ration. The specific seaweed was mixed with the PMR for each treatment, and the seaweed inclusion level achieved was 0.56 to 0.60% (DM basis) of total DMI. Additional concentrate feed was provided through the GreenFeed (**GF**; C-Lock Inc.) system and the milking carousel as bait. The basal PMR consisted of 54.2% grass silage, 20.8% corn silage, and 25.0% concentrate (DM basis). The chemical composition of the PMR, GF bait, milking carousel bait, and seaweeds is given in Table 1, and the chemical composition of the complete diet is given in Table 2. All seaweeds were harvested from the wild in a single batch in spring 2019, washed with drinking water, air-dried at 70°C to an average moisture content of 12%, and milled at 1 mm before being packaged by Wild Irish Seaweeds Ltd. Seaweed CC was first sun bleached before washing, drying, and milling as previously described.

The PMR was automatically mixed 3 times per day using a Trioliet feed mixing robot (Triomatic HP 2 300, Trioliet BV) and distributed in Insentec feed bins (**FB**;

Table 2. Chemical composition (g/kg of DM, unless otherwise stated) of the complete diet fed to cows in the different treatment groups

	Treatment[1]			
Item	Control	CC	SL	FS
DM (g/kg in product)	618	619	617	616
Ash	85	85	86	86
CP[2]	145	146	145	145
Crude fat	34	34	34	34
Sugar	94	94	93	93
NDF	406	405	406	405
ADF	225	224	225	225
ADL	23	23	22	23

[1]Control = no seaweed; CC = *Chondrus crispus*; SL = *Saccharina latissima*; FS = 50/50 mix (DM basis) of *Fucus serratus* and *Saccharina latissima*.

[2]Calculated as N × 6.25.

RIC system, Hokofarm Group BV) for automatic individual feed intake registration as described in detail by van Gastelen et al. (2022). To avoid cross-contamination, a rinsing diet, which was not fed to the cows in the experiment, was mixed between mixing of the different treatment diets. Cows were fed ad libitum, allowing 10% refusals. The dairy cows were housed as one group in a freestall barn with 64 cubicles with commercially available rubber mats and covered with wood shavings as bedding material. One FB per 2 cows belonging to the same treatment was available; that is, every cow had access to 8 FB containing her allocated diet. The assignment of the cows to the FB was established at the start of the experiment and remained the same throughout the experiment. The FB were equipped with an automated identification system (monitor ID system based on transponders within the collar of the dairy cows) to enable access. The experimental diets were equally distributed over the FB to avoid potential barn location effects. For each visit of a cow to the FB, the start and end times and the start and end weights of the FB were recorded. The FB were calibrated with a standard weight on a weekly basis. Three GF systems were equally distributed in the barn on the other side of the cubicles from the FBs and were available for all cows. Cows had free access to clean drinking water throughout the experiment and were exposed to light from 0500 to 2300 h. Cows were milked twice daily at 0500 and 1500 h at the milking carousel (AutoRotor PerFormer, GEA Farm Technologies), and 1 kg (fresh weight) of bait per milking was offered.

Sample Collection and Measurements

Samples of all individual feed components and concentrate feeds were taken weekly. These samples were subsequently pooled per 3 wk, subsampled, and stored at −20°C pending analysis. Milk samples were collected from all animals on Wednesday evening and Thursday morning on a weekly basis. A milk sample (10 mL) of each milking event was collected in a tube containing sodium azide (5 μL) for preservation and stored no longer than 1 d at 4°C. A weighted average daily milk composition was calculated from the milk composition and milk yield of both milking events.

Measurements of enteric CH_4, H_2, and CO_2 emissions were recorded using 3 GF systems, as described in detail by van Gastelen et al. (2022) for the same barn as in the present experiment. The GF systems were calibrated at the start and end of the experiment. A known amount of CO_2 gas was released at the start and end of the trial in the GF units near where the animal's nose would be when consuming the bait during a GF visit to check the recovery of expired gases. The average recovery of CO_2 of all units combined was 99.2%; for individual GF units, average recovery was between 98.3% and 99.8%. Cows were encouraged to visit the GF systems with a pelletized bait (Table 1). Average weight of the pellet cup drops was recorded weekly per system and used for the DMI calculations. Maximum intake of the GF bait allowed was based on the actual milk yield of the cows at the start of the experiment, and settings of the GF system were changed accordingly based on the average weight of the cup drop. The settings did not vary during the experiment and allowed for a maximum of 8 visits per day, 8 cup drops per visit, a 30-s interval per cup drop, and minimum of 3 h between visits. Only GF gas emission data based on at least a 2-min uninterrupted visit were used for further calculations (gas production, g/d; gas yield, g/kg of DMI; gas intensity, g/kg of milk or g/kg of FPCM).

Chemical Analysis

Feed ingredient samples were analyzed by Eurofins Agro (Wageningen, the Netherlands) for DM drying at 103°C, ash by combustion at 550°C (International Organization for Standardization, 2002), CP by Kjeldahl (similar to International Organization for Standardization, 2005), crude fat without hydrolysis (similar to International Organization for Standardization, 1999), starch by enzymatic determination with amyloglucosidase (International Organization for Standardization, 2004, only corn silage), reducing sugar similar to van Vuuren et al. (1993), NDF gravimetric determined as the remaining insoluble organic fraction after hydrolysis with neutral detergent reagents and enzymatic incubation (similar to Van Soest et al., 1991), ADF gravimetric determined as the remaining insoluble organic fraction after hydrolysis with acid detergent reagents (International Organization for Standardization, 2008), and ADL gravimetric determined as the remaining insoluble organic fraction after hydrolysis with acid detergent reagents followed by incubation with sulfuric acid (International Organization for Standardization, 2008). Crude protein was calculated as N × 6.25 for all feedstuffs. Seaweed samples were analyzed by Nutricontrol (Veghel, the Netherlands) for DM, ash, CP, crude fat, starch, reducing sugar, NDF, ADF, ADL, arsenic, and cadmium. The red and brown seaweed species used in this study do not contain true starch but contain similar storage polysaccharides (floridean starch and laminarin, respectively) that could be detected as starch (Rioux et al., 2010; Ball et al., 2011).

Milk samples were analyzed for fat, CP, lactose, and urea content by mid-infrared spectroscopy (International Organization for Standardization, 2013) and SCC by flow cytometry (Qlip BV). Fat- and protein-corrected milk yield was calculated according to the equation FPCM (kg/d) = (0.337 + 0.116 × fat % + 0.06 × protein %) × milk yield (kg/d) (CVB, 2016).

Data and Statistical Analysis

One cow in treatment FS had to be replaced due to an accident in the first week of feeding the treatment diet. We decided to delete all data of both cows from the data set due to the accident of the first cow and no covariate data of the replacement cow. Data of the first 2 wk of the treatment period were excluded from data and statistical analysis to allow for adaptation to the treatments. All parameters related to feed intake, milk production, milk composition, and GF visits were averaged per cow per week. Week 10 (relative to first week of feeding the respective treatments) of the experiment consisted of 3 full days of measurement only. At d 4 of wk 10, the treatments were stopped because of insufficient amounts of seaweed due to unforeseen loss of material during storage. Therefore, wk 10 was excluded for parameters related to feed intake, milk production, milk composition, and GF visits. One CH_4 sensor in one of the GF systems was determined to have malfunctioned during the trial, resulting in approximately one-third of CH_4 measurements being deleted. Therefore, all parameters related to gaseous exchange were averaged per cow per 2 wk to ensure a minimum of 20 uninterrupted visits per cow in a 2-wk period, the minimal amount of visits needed according to Manafiazar et al. (2017). The average number of uninterrupted visits per cow per 2-wk period to the 2 properly functioning GF systems was 80 ± 11.6. The data from the 3 full days in wk 10 were included in the analyses of gaseous exchange by calculating a 2-wk average with data from wk 9.

Data were subjected to a repeated-measurements REML analysis in Genstat (19th edition, VSN International). Treatment, experimental week, the interaction treatment × week, and the baseline measurement from the covariate period were considered fixed effects. Blocking factors were considered random effects, and a first-order autoregression term was estimated for timelag-dependent correlation of residual effects within cow. Differences between treatment means were compared using the least squares means and the Fisher's least significant difference method for multiple comparisons when an interaction was detected at $P \leq 0.05$. All results are reported as least squares means, with significance of effects declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

DMI and GreenFeed Visits

No treatment × week interaction or treatment effects were observed for the DMI of the GF bait or for the number of visits to the GF per cow per day. There was a significant treatment × week interaction for DMI of the PMR ($P < 0.001$; Table 3) and total DMI ($P < 0.001$), which is mainly driven by the DMI of the PMR. At wk 5, the DMI of the PMR for SL was higher than that for CON (Figure 1A). The DMI of the PMR for CC was higher than that for both CON and FS, and SL was higher than FS at wk 6. Similarly, the total DMI of SL was higher than CON at wk 5, and total DMI of CC and SL were both higher than that of CON and FS at wk 6 (Figure 1B). There were no differences between treatments for DMI of the PMR or total DMI. A week effect was observed for total DMI, DMI of the PMR, and DMI of the GF bait.

Milk Production, Milk Composition, Feed Efficiency, and N Efficiency

Treatment × week interactions were observed for feed efficiency ($P = 0.004$), milk urea content ($P < 0.001$), protein yield ($P = 0.024$), lactose yield ($P = 0.032$), and milk N efficiency ($P = 0.002$). A trend for a treatment × week interaction was observed for milk yield ($P = 0.071$; Table 4). Feed efficiency (kg of

Table 3. GreenFeed (GF) visits and DMI of lactating dairy cattle fed diets containing different seaweeds

	Treatment[1]					P-value[2]		
Item	Control	CC	SL	FS	SEM	T	W	T × W
GF visits (no./cow per day)	6.4	6.0	6.2	6.2	0.19	0.310	0.150	0.479
DMI GF bait (kg/d)	2.25	2.21	2.17	2.19	0.119	0.779	<0.001	0.668
DMI partial mixed ration (kg/d)	18.5	18.7	19.1	18.8	0.21	0.138	<0.001	<0.001
DMI total[3] (kg/d)	22.2	22.5	22.8	22.4	0.22	0.217	<0.001	<0.001

[1]Control = no seaweed; CC = *Chondrus crispus*; SL = *Saccharina latissima*; FS = 50/50 mix (DM basis) of *Fucus serratus* and *Saccharina latissima*.

[2]Effect of T = treatment; W = week; T × W = treatment and week interaction.

[3]All cows received an additional 1.6 kg of DM/d of bait in the milking parlor.

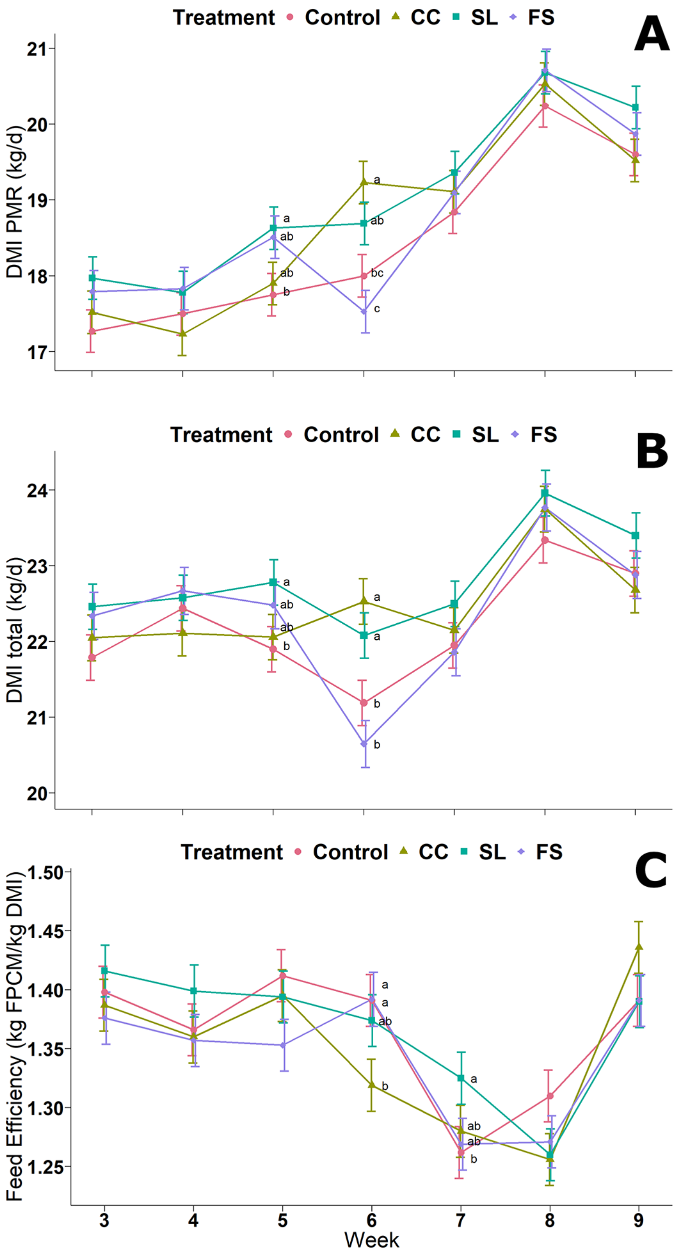

Figure 1. Average DMI of the partial mixed ration (PMR) (A), total DMI (B), and feed efficiency [fat- and protein-corrected milk (FPCM)/DMI; C] of lactating dairy cattle fed diets containing different seaweeds: Control = no seaweed; CC = *Chondrus crispus*; SL = *Saccharina latissima*; FS = 50/50 mix (DM basis) of *Fucus serratus* and *Saccharina latissima*. Values with different letters (a–c) indicate a significant ($P < 0.05$) difference between the diets in the specific week indicated. Week is expressed relative to first week of feeding the respective treatment diets. Data points are the LSM ± SEM.

FPCM/kg of DMI) was higher for CON and FS than for CC at wk 6, and higher for SL than for CON at wk 7 (Figure 1C). Milk urea content was higher for SL and CON than for CC and FS at wk 3; higher for FS than for CC and CON and higher for SL than for CC at wk 6; higher for SL than all other treatments and higher for CC than for CON at wk 7; higher for FS than for CC at wk 8; and higher for FS than for CON at wk 9 (Figure 2E). Protein yield (g/d) was higher for SL than for CC at wk 4 (Figure 2B). Lactose yield (g/d) was higher for SL than for CC at all weeks (Figure 2C). Additionally, lactose yield was higher for SL than CON at wk 4, higher for CON than CC at wk 5, higher for SL than FS at wk 6, higher for FS than CC at wk 8, and higher for SL than CON at wk 7 and 9. Milk N efficiency (g/g) was higher for CON than SL at wk 5, and higher for CON and FS than SL and CC at wk 6 (Figure 2F).

Milk yield ($P = 0.020$), FPCM yield ($P = 0.046$), milk protein content ($P = 0.002$), milk lactose content ($P = 0.009$), milk urea content ($P < 0.001$), and milk lactose yield ($P = 0.006$) differed between treatments (Table 4). Milk yield (kg/d; Figure 2A) was higher for SL than for both CON and CC, but did not differ from FS. Yield of FPCM (kg/d; Figure 2D) was higher for SL than for all other treatments. Milk protein content (%) was lower for SL than for all other treatments. Milk lactose content (%) was higher for SL than for all other treatments. Lactose yield (g/d) was higher for SL than for CON and CC, but did not differ from FS. Trends for a treatment effect were observed for both SCC ($P = 0.064$) and fat yield ($P = 0.099$). A week effect was observed for all milk- and efficiency-related parameters except SCC.

Gaseous Exchange

There were no treatment × week interactions or treatment effects for CO_2 and H_2 emissions, except for a tendency for a treatment × week interaction of CO_2 yield ($P = 0.073$; Table 5). A treatment × week interaction was observed for CH_4 yield ($P = 0.037$; Table 5). In the 2-wk period of wk 3 to 4, the CH_4 yield (g of CH_4/kg of DMI) of CC was higher than that of CON (Figure 3B). Trends for a treatment × week interaction were observed for CH_4 production ($P = 0.078$) and CH_4 milk intensity ($P = 0.074$). No treatments effects were observed for CH_4 emissions. A week effect was observed for all gaseous emissions ($P < 0.05$) except for CH_4 intensity (g/kg of FPCM), which showed a trend ($P = 0.053$).

DISCUSSION

Feed Intake

In the present study, total DMI and DMI of the PMR differed between treatments in wk 5 and 6, but these effects were not consistent before or after these weeks

(Figure 1A). At wk 6, a change in batch of corn silage occurred, which might explain the difference between treatments. At wk 8, the total DMI of all treatments increased (Figure 1B), which corresponds with the change in batch of grass silage. Similarly, milk yield in wk 8 increased (Figure 2A) and, together with the increase in DMI, resulted in reduced feed efficiency in wk 8. Similar results with no effect on DMI were observed in studies with dairy cattle that used the brown seaweed *Ascophyllum nodosum* (Antaya et al., 2015; Silva et al., 2022) or a mix of the brown seaweeds *A. nodosum* and *L. digitata* (Newton et al., 2021). When *A. nodosum* was fed to grazing Jersey cows, estimated herbage DMI increased but total DMI did not differ from the control (Antaya et al., 2019). In the study of Maheswari et al. (2021), Murrah buffaloes were fed a mixture of the red seaweed species *Kappaphycus alvarezii* and *Gracilaria salicornia* and no effect on DMI was observed. Both *K. alvarezii* and *G. salicornia* are known to contain substantial amounts of sulfated carbohydrates similar to *C. crispus* in the present study, mainly carrageenan and agar, respectively (Campo et al., 2009; Torres et al., 2019).

Milk Production and Efficiency

Treatment SL resulted in a higher milk yield, FPCM yield, milk lactose content, and lactose yield compared with CON, and lower milk protein content compared with CON. The difference in milk lactose and protein content between SL and CON is similar to the relationship described by Nichols et al. (2019). Milk lactose content may increase when a low metabolizable protein supply results in low milk protein content, due to a positive relationship between milk casein and milk salt content (Nichols et al., 2019). However, the lower milk protein content for SL might also be a dilution effect, because SL had a higher milk yield and similar protein yield compared with CON. In the study of Newton et al. (2021), milk protein and casein levels were lower for cows fed the high amount (26–158 g/cow per day) of the seaweed mix with *A. nodosum* and *L. digitata* compared with the control, whereas milk yield and milk lactose content did not differ. When animals were fed only *A. nodosum* or a mix of *K. alvarezii* and *G. salicornia*, milk yield, milk protein, milk casein, and milk lactose content did not differ from the control (Chaves Lopez et al., 2016; Antaya et al., 2019; Maheswari et al., 2021). Lactose is the main driver of milk yield because of its role in the osmotic equilibrium between blood and the alveolar lumen in the mammary gland, and it is simultaneously excreted with water into the alveolar lumen via a secretory vesicle after synthesis in the mammary epithelial cells (Costa et al., 2019). The higher milk yield in the SL treatment is in contrast to studies supplementing *A. nodosum* to the diet of dairy cattle, where no effect on milk yield was found (Antaya et al., 2015, 2019). The brown seaweed *L. digitata* is a close relative to *S. latissima*; both species belong to the Laminariaceae family (Guiry and Guiry, 2020). In the study of Newton et al. (2021), no effects on milk yield were observed when dairy cattle were fed a mix of *A. nodosum* and *L. digitata*. This mix consisted of 91% *A. nodosum* and 9% *L. digitata*, resulting in a

Table 4. Milk yield, fat- and protein-corrected milk (FPCM) yield, milk composition, and feed and milk N efficiency of lactating dairy cattle fed diets containing different seaweeds

Item	Treatment[1]				SEM	*P*-value[2]		
	Control	CC	SL	FS		T	W	T × W
Milk yield (kg/d)	27.5[a]	27.2[a]	28.7[b]	27.9[ab]	0.37	0.020	<0.001	0.071
FPCM yield (kg/d)	30.2[a]	30.1[a]	31.4[b]	30.3[a]	0.38	0.046	<0.001	0.101
FE[3] (kg of FPCM/kg of DMI)	1.36	1.35	1.37	1.34	0.012	0.576	<0.001	0.004
Milk composition								
Protein (%)	3.59[a]	3.64[a]	3.50[b]	3.59[a]	0.026	0.002	<0.001	0.155
Fat (%)	4.75	4.84	4.76	4.69	0.066	0.214	<0.001	0.192
Lactose (%)	4.52[a]	4.51[a]	4.57[b]	4.51[a]	0.020	0.009	<0.001	0.431
Urea (mg/dL)	14.5[ab]	13.6[a]	15.9[c]	15.3[bc]	0.38	<0.001	<0.001	<0.001
Milk SCC (×10³ cells/mL)	148	112	294	91	69.2	0.064	0.371	0.590
Milk component yield								
Protein (g/d)	979	986	1,002	992	11.2	0.371	<0.001	0.024
Fat (g/d)	1,297	1,303	1,346	1,290	22.0	0.099	<0.001	0.175
Lactose (g/d)	1,246[a]	1,214[a]	1,308[b]	1,259[ab]	19.7	0.006	<0.001	0.032
Milk N efficiency (g/g)	0.298	0.295	0.294	0.297	0.0022	0.768	<0.001	0.002

[a–c]Values with a different superscripts indicate a significant ($P < 0.05$) differences between treatments.

[1]Control = no seaweed; CC = *Chondrus crispus*; SL = *Saccharina latissima*; FS = 50/50 mix (DM basis) of *Fucus serratus* and *Saccharina latissima*.

[2]Effect of T = treatment; W = week; T × W = treatment and week interaction.

[3]FE = feed efficiency.

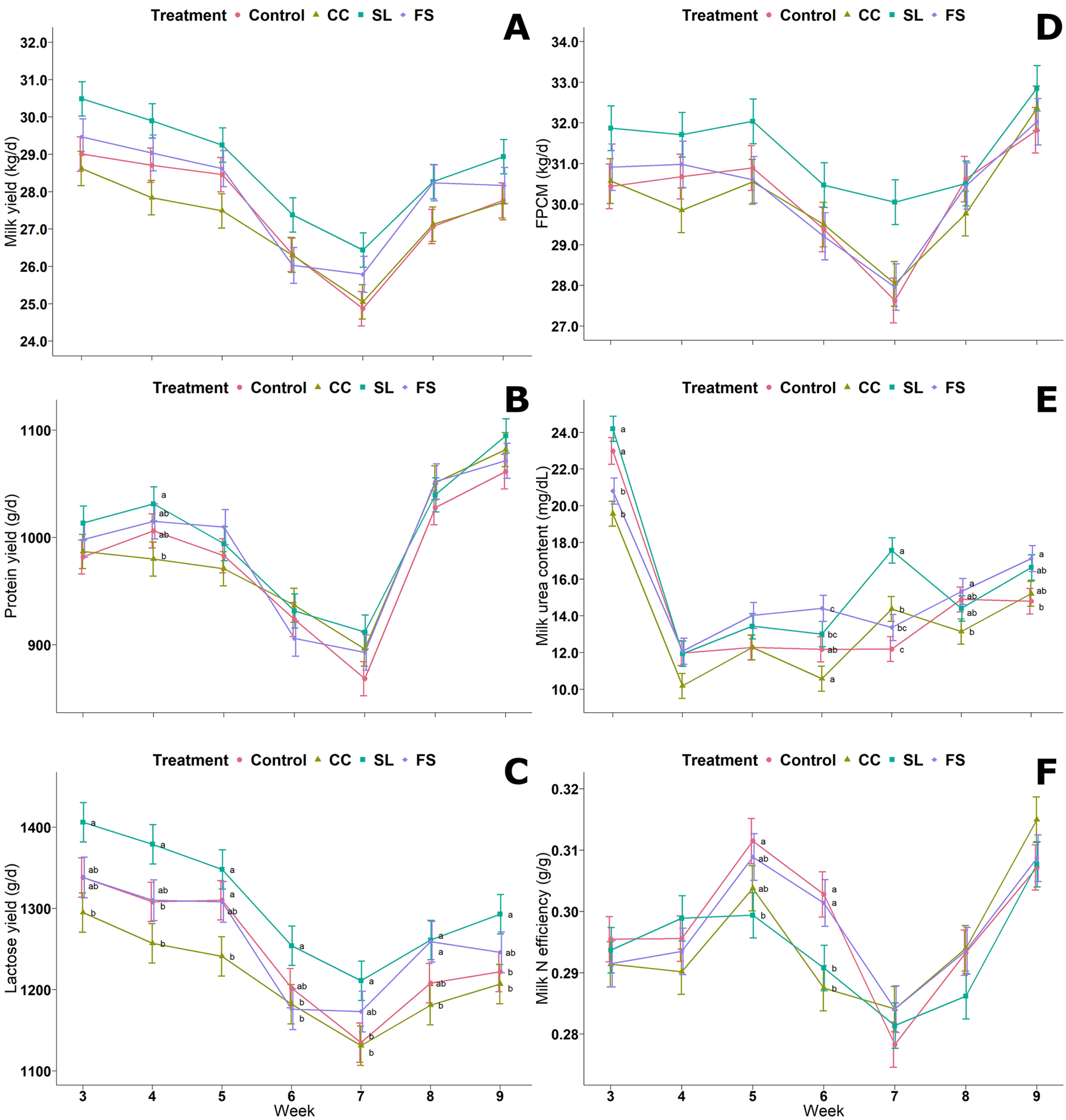

Figure 2. Average milk yield (A), protein yield (B), lactose yield (C), fat- and protein-corrected milk yield (FPCM; D), milk urea content (E), and milk N efficiency (F) of lactating dairy cattle fed diets containing different seaweeds: Control = no seaweed; CC = *Chondrus crispus*; SL = *Saccharina latissima*; FS = 50/50 mix (DM basis) of *Fucus serratus* and *Saccharina latissima*). Values with different letters (a–c) indicate a significant ($P < 0.05$) difference between the diets in the specific week indicated. Week is expressed relative to first week of feeding respective treatment diets. Data points are the LSM ± SEM.

Table 5. Carbon dioxide, methane, and hydrogen emissions of lactating dairy cattle fed diets containing different seaweeds

Item	Treatment[1]				SEM	*P*-value[2]		
	Control	CC	SL	FS		T	W	T × W
CO_2 emissions								
Production (g/d)	13,729	13,611	13,750	13,677	233.4	0.942	<0.001	0.342
Yield (g/kg of DMI)	614	608	608	612	8.8	0.969	<0.001	0.073
Intensity (g/kg of milk)	504	510	483	509	10.8	0.165	<0.001	0.681
Intensity (g/kg of FPCM[3])	451	449	444	459	10.1	0.742	<0.001	0.782
CH_4 emissions								
Production (g/d)	446	459	466	449	7.5	0.179	<0.001	0.078
Yield (g/kg of DMI)	20.1	20.5	20.3	20.2	0.31	0.610	<0.001	0.037
Intensity (g/kg of milk)	16.6	17.2	16.4	16.6	0.31	0.205	<0.001	0.074
Intensity (g/kg of FPCM)	14.8	15.2	14.9	15.1	0.29	0.663	0.053	0.635
H_2 emissions								
Production (g/d)	2.55	2.66	2.68	2.43	0.100	0.246	0.002	0.436
Yield (g/kg of DMI)	0.117	0.120	0.117	0.110	0.0054	0.491	<0.001	0.193
Intensity (g/kg of milk)	0.096	0.100	0.095	0.090	0.0043	0.296	<0.001	0.303
Intensity (g/kg of of FPCM)	0.086	0.090	0.087	0.081	0.0040	0.388	0.003	0.518

[1]Control = no seaweed; CC = *Chondrus crispus*; SL = *Saccharina latissima*; FS = 50/50 mix (DM basis) of *Fucus serratus* and *Saccharina latissima*.

[2]Effect of T = treatment; W = week; T × W = treatment and week interaction.

[3]Fat- and protein-corrected milk.

potentially more pronounced effect of *A. nodosum*. In the current study, treatment FS, a 50/50 mix of the brown seaweeds *F. serratus* and *S. latissima*, did not have a similar effect on milk yield or lactose content as the SL treatment. Both *F. serratus* and *A. nodosum* are closely related and belong to the Fucaceae family (Guiry and Guiry, 2020). This similarity might explain the lack of effect on milk yield or lactose content when they are mixed with a seaweed from the Laminariaceae family. Both *Fucus* spp. and *Ascophyllum* spp. mainly inhabit the intertidal zone, whereas *Laminaria* spp. and *Saccharina* spp. occupy the subtidal zone in coastal regions (Mann, 1972; Lubchenco, 1980). The intertidal and subtidal zones are exposed to different biophysical factors, including wave action, temperature, light availability, UV exposure, and grazing (e.g., by sea urchins). Differences in these biophysical factors affect the composition of the seaweed (Connan et al., 2007; Lee et al., 2017; Manns et al., 2017), which may result in different effects of the same or similar species across studies.

Milk urea content differed between treatments at wk 3 and wk 6 to 9, with a usually inconsistent pattern. In most of these weeks, however, the milk urea content of SL was higher than that of CON and CC. In general, milk urea content may serve as an indicator to monitor milk N efficiency and N excretion (Spek et al., 2013). At similar dietary CP contents of the diets fed, this increased milk urea content indicates less-efficient use of protein with SL. However, this did not coincide with a consistently lower milk N efficiency in these weeks; only in wk 5 and 6 was milk N efficiency lower with SL than with CON. In Antaya et al. (2019), a diet × period interaction was detected for MUN, resulting in a higher MUN for the *A. nodosum* group in period 1 compared with the control, but not in period 2 or 3. Other studies did not find effects of seaweed supplementation (*A. nodosum* or mix of *A. nodosum* and *L. digitata*) on milk urea, MUN, and protein efficiency (Antaya et al., 2015; Chaves Lopez et al., 2016; Newton et al., 2021).

Gas Emissions

We hypothesized that through specific metabolites (including phlorotannins and carrageenan), the inclusion of the seaweeds evaluated here would affect rumen methanogenesis and lead to reduced enteric methane production. In contrast to our hypothesis, the seaweeds supplemented at 150 g/d [fresh weight basis; 0.56–0.60% (DM basis) of total DMI] did not decrease enteric CH_4 production, yield, or intensity, nor did they affect CO_2 and H_2 emissions. Only in wk 3 to 4 was CH_4 yield (g/kg of DMI) of CC higher than that of CON. Although several in vitro studies have shown the potential of non-*Asparagopsis* seaweed species in decreasing CH_4 emissions (Kinley and Fredeen, 2015; Maia et al., 2016), in vivo data with dairy cattle are scarce. In Antaya et al. (2019), grazing Jersey cows were fed *A. nodosum*, which did not affect CH_4 yield or intensity but resulted in lower CH_4 production during the first period, whereas no differences occurred in the 2 periods thereafter. This suggests an adaptation of the ruminal microbial community to the specific metabolites present in the seaweed fed. In the present study, a decrease in CH_4 emissions upon feeding seaweeds compared with CON did not occur in any 2-wk period from the start of seaweed introduction, indicating either very rapid

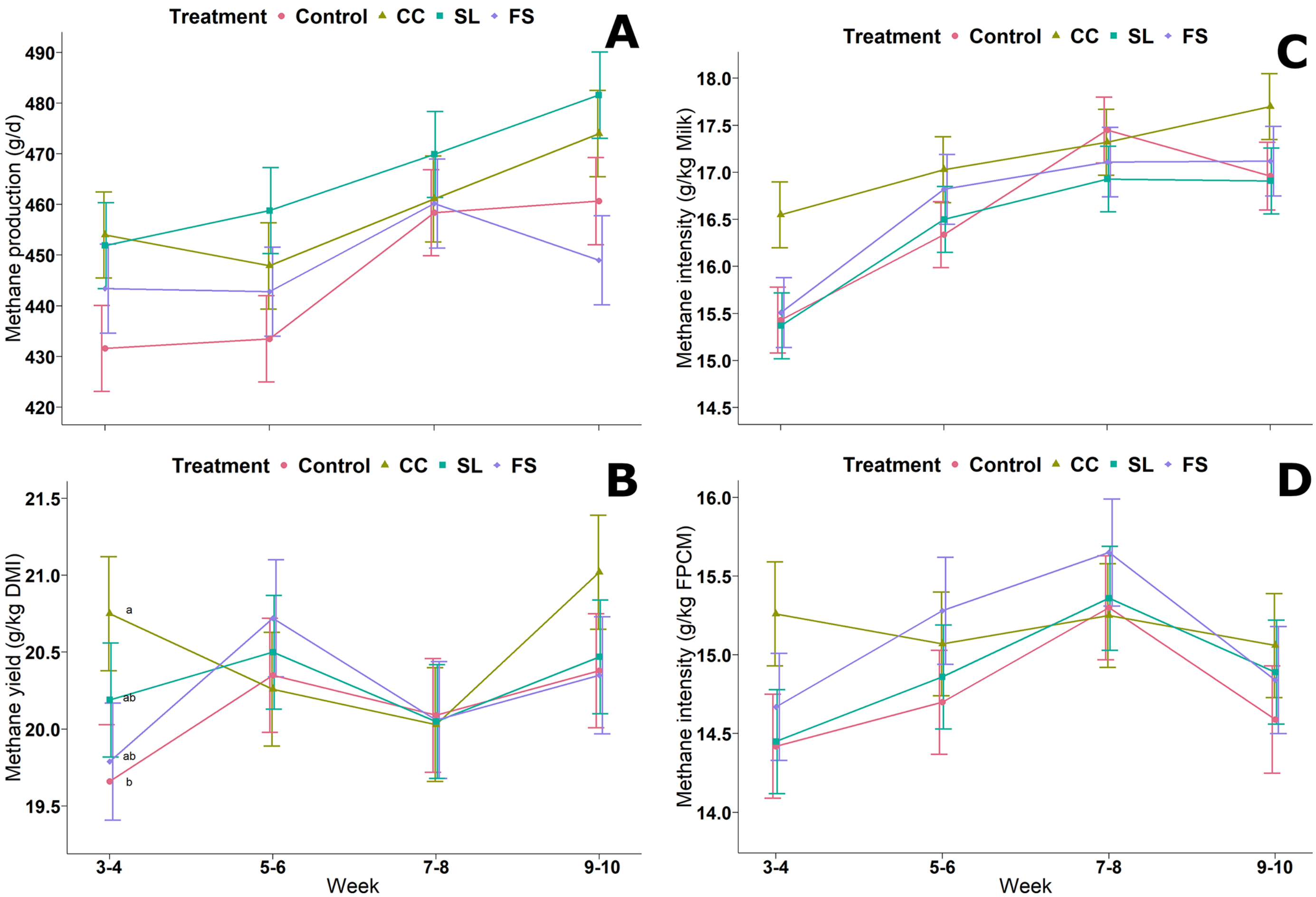

Figure 3. Average methane production (A), methane yield (B), methane intensity (C), and methane intensity [g/kg fat- and protein-corrected milk (FPCM); D] per 2-wk period of lactating dairy cattle fed diets containing different seaweeds: Control = no seaweed; CC = *Chondrus crispus*; SL = *Saccharina latissima*; FS = 50/50 mix (DM basis) of *Fucus serratus* and *Saccharina latissima*. The final 2-wk period comprised 10 full measurement days only. Values with different letters (a, b) indicate a significant ($P < 0.05$) difference between the diets in the specific week indicated. Week is expressed relative to first week of feeding respective treatment diets. Data points are the LSM ± SEM.

adaptation of rumen microbes or that these seaweeds were not effective in mitigating methane emission at the dose fed. When the data were analyzed including the first 2 wk of the treatment, results regarding CH_4 and H_2 emissions were negligibly different compared with the results shown in Table 5. This suggests no adaptation of the cows to the seaweed treatments in these 2 wk. The inclusion level of the seaweeds in the present study might not have been high enough for the specific metabolites to have a substantial effect on methanogenesis in the rumen of the cow.

CONCLUSIONS

None of the seaweeds evaluated had a negative effect on feed intake or lactational performance, indicating that the seaweed species used can be fed at an inclusion rate of 150 g/d (fresh weight of the dried seaweeds) to lactating dairy cows without negatively affecting their performance. Milk yield, FPCM yield, milk lactose content, and lactose yield increased when the diet was supplemented with SL. None of the seaweeds affected enteric gaseous emissions. The inclusion rate might not have been sufficient for the specific metabolites in seaweeds to have an effect on enteric methane production. Further research is needed to evaluate whether greater seaweed inclusion rates may decrease CH_4 emissions in dairy cattle, and to evaluate the reproducibility of the effect of SL on milk yield, FPCM yield, lactose content, and lactose yield, as well as underlying mechanisms.

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