Feeding Seaweed to Accelerate Enteric Methane Emissions Reductions

California Livestock Methane Measurement, Mitigation and Thriving Environments Research Program
(CLIM3ATE-RP)

John Gibbons
Mooteric LLC

Grant Agreement 23-0504-000-S

This project and a final report were supported by the California Department of Food and Agriculture (CDFA), Office of Agricultural Resilience and Sustainability (OARS) through the CLIM3ATE research program, grant 23-0504-000-SG. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the CDFA.

Contents

About the CLIM3ATE Research Program

The California Livestock Methane Measurement, Mitigation, and Thriving Environments Research Program (CLIM3ATE-RP) is a research funding initiative administered by the California Department of Food and Agriculture's Office of Agricultural Resilience and Sustainability (OARS).

CLIM3ATE-RP was launched with funds from the Budget Act of 2021 (SB 170, Chapter 240) to support applied research that advances California's climate goals and strengthens the long-term environmental and economic sustainability of the state's livestock sector.

Research Program Focus Areas

CLIM3ATE-RP funded research in three critical areas related to methane emissions and manure management in livestock operations:

1. Verification of Methane Reduction Strategies

Supporting work to improve and validate greenhouse gas estimates for projects funded under CDFA's Alternative Manure Management Program (AMMP) and the Dairy Digester Research and Development Program (DDRDP). This includes evaluating methane reductions resulting from AMMP practices and enhancing quantification tools used in California's climate-smart agriculture programs.

2. Alternative Methane Reduction Strategies

1. Field studies evaluating seaweed-based feed additives for their potential to reduce methane in livestock digestion.
2. Nutritional research investigating the potential of by-product feeding as a methane mitigation strategy.
3. Development of a standardized framework for designing and evaluating enteric methane research protocols, creating more consistency and comparability across studies.

3. Manure Recycling and Innovative Product Development

a. Evaluating the use of lemna for nutrient recovery from digester effluent.

In the 2022 funding cycle, CDFA awarded six research projects totaling $4.7 million in funding.

How Mooteric's Project "Feeding Seaweed to Accelerate Enteric Methane Emissions Reductions" Accomplishes CLIM3ATE-RP Goals

This project supports goal two of the CLIM3ATE program to evaluate alternative methane reduction strategies.

The study, conducted at a test farm, was designed to simulate conditions on typical Central Valley dairies. Cows from local dairies were divided into three groups: a control group and two groups fed different methane reducing feed additives. Cows were fed typical rations and housed in a standard dairy shed for roughly 90 days. Enteric methane measurements were taken during feeding. Differences in enteric methane from the three groups were compared along with the economic benefit of reduced methane calculated and analyzed.

The project had three main objectives:

1. Conduct a rigorous on-farm feeding trial to verify the methane-mitigating qualities of seaweed-based feed.
2. Establish long-term economically viable supply chain for seaweed-based products, including an assessment on the pathways to federal product approval.
3. Conducted financial modeling for California dairies to foster acceptability and understanding among producers to use seaweed-based feed additives.

The following report will evaluate each objective and its findings.

Objective 1: Conduct a rigorous on-farm feeding trial to verify the methane-mitigating qualities of seaweed-based feed.

Key Take-Aways:

- *Asparagopsis taxiformis*, a red seaweed, and an algae-based product from Alga Biosciences, were evaluated for efficacy of enteric methane emissions reduction.
- Alga Biosciences and the Toki (Shilai Feeds/AT) products proved highly effective in reducing enteric methane emissions in dairy cows vs. the control group. Immediate dramatic drop in enteric methane was measured when feed additives were included into feed rations.
- Samples of both milk and blood were submitted to monitor bromoform and iodine levels.
    - Bromoform was not detected at levels less than 5 ug/L
    - Iodine levels were elevated from baseline within test groups consuming both Alga and Toki *(Asparagopsis taxiformis)* feed additives.
- Dry matter intake (DMI) reduction raised concerns about proper dosage and administration of both feeds.
- Exhibit A is a full text research paper for this project and can be found at: Feed Additive Study.

Executive Summary of the Project:

The objective of this study was to evaluate the effects of seaweed-based feed additives containing bromoform on milk yield and composition, dry matter intake, feed efficiency, body weight, enteric emissions, and nutrient digestibility. A total of 60 Holstein cows on their 2nd or 3rd and 4th parity at 204 ± 7 days in milk were blocked by milk yield and randomly assigned to: a) control (no seaweed added to the TMR); b) ShiLai™ *Asparagopsis taxiformis* pellets added to the TMR at a rate of 0.50% of DM; or, c) Alga Biosciences product added to the TMR also at a rate of 0.50% of DM from enrollment to day on supplementation 21. The rate was reduced to 0.25% of DM from day 22 to the end of the study (day 42). Cows were housed in a single group and fed ad libitum. Individual cow TMR intake was recorded through the Biocontrol CRFI feed intake control and measurement system, and enteric methane emissions were measured using GreenFeed units. Individual milk yield was recorded using AfiMilk electronic milk meters, and milk fat and protein were measured using optical in-line analyzers at each of two daily milkings. Treatment and treatment by time effects claimed at $P<0.05$ were assessed by multiple linear regression. Supplementation of AB or AT at 0.05% of DM resulted in an approximate 90% decrease of enteric methane emissions, however, it also resulted in an overtime decrease and overall lower milk production compared to control cows, potentially driven by lower DMI observed in cows supplemented with both products. Supplementation of TMR with AB or AT at 0.25% of DM resulted in a 36-41% decrease of enteric methane emissions, and although a trend to decrease DMI, the magnitude of the effect was smaller compared with the higher dose, as well as the differences in production parameters as detected by time conditional effects. In conclusion, both seaweed-based feed additives effectively decreased enteric methane emissions in a dose-response manner and additional research is needed to evaluate the optimal dose to achieve an important reduction in methane emission without compromising the cow's performance, as well to evaluate the long-term methane inhibiting effects of these seaweed-based feed additives containing bromoform.

Objective 2: Establish long-term economically viable supply chain for seaweed-based products, including an assessment on the pathways to federal product approval.

Key Take-Aways

- Supply chains from both China and Vietnam appear to be most viable long-term
- FDA approval process is complex and lengthy
- Farmer acceptance is not guaranteed even with FDA approval and substantial economic benefit to farmers

1) Supply Chains

There is currently no large-scale farming of *Asparagopsis*. The seaweed is native to regions in the South Pacific, off the coast of Portugal and Spain and North Africa. Most of the research of the product is being done in Australia and New Zealand. As a result, the Australian livestock industry is well ahead of the U.S. in product development, government approval and on farm adaptation. However, with no large-scale seaweed farms and expensive regional labor the economic benefit in feeding *Asparagopsis* to Australian livestock is nominal.

China and Vietnam both have ambitions to establish large-scale *Asparagopsis* farming operations. Mooteric visited both countries to assess. Vietnam has a well-developed fish farming industry with fish farming operations being very similar to seaweed operations. Companies like Costco buy fish (Barramundi) direct from the supplier visited by Mooteric. As a result, the supply chain from the waters off Vietnam to Costco's freezers in the USA is transparent and traceability is clean. The seaweed industry is nascent, and it will be several years before supply is significant enough to serve the California dairy industry. China shares a similar profile as far as cost structure and where they are in the development cycle. The main issue with China is a lack of transparency. As an example, Mooteric met with the Chinese supplier at their offices but were unable to go inside the drying plant.

2) FDA Approval Process

Mooteric engaged NutraSteward (nutrasteward.com) to evaluate the FDA approval process. NutraSteward is a regulatory consulting company whose services include helping food companies navigate the FDA approval process. NutraSteward provided Mooteric a mapping of how to get the products used in the trials through the FDA approval process. While unadulterated *Asparagopsis* is completely natural, the active ingredient in the seaweed is bromoform which is a known carcinogen. In the midst of the trials, the Innovative Feed Act (iFeed) was passed. This act categories the feed additives in the study as a Zootechnical Feed Additives. While this legislation clearly brought in FDA oversight for *Asparagopsis* and thus added hurdles to regulatory approval, it defined the steps to approval and removed significant ambiguity on what is required to use on farm.

Similar feed additives to what was used in the Mooteric trials are now going through the FDA approval process. The cost to go through the approval process is significant and out of the scope of the trials. Mooteric is monitoring the regulatory environment and may partner with a feed additive company on merchandising once there is a FDA approvals.

3) Farmer Acceptance

The trials demonstrated that the farmer benefit of using seaweed-based feed additives can add in excess of $150/head/year in profits. It makes economic sense for farmers to use the additives. There are, however, substantial risks. The dosages of the product are very small with active ingredient being very potent. An ingredient mix up resulting in even a small change in the dosage could have a terrible impact on the milk. The U.S. dairy industry has experienced such catastrophes in the past. There has been recent press about similar feed additives with a 2024 BBC article broadly referring to methane reducing feed additives as “poison”. Even with perfect dosing and monitoring, there is a stigma with the additives that may offset the positive economic benefit to farmers.

Objective 3: Conducted financial modeling for California dairies to foster acceptability and understanding among producers to use seaweed-based feed additives.

Key Take-Away:

- Wide use of the product would not be expected if milk yields are down, no matter what the improvement in feed efficiency. Milk yields were down 7% and 12% in the higher dosage scenarios. Body weights were down less significantly.

A summary of the financial model is below:

	Dosage	DMI	CO2e	CO2e	CO2	Carbon	FE	Product Cost	Product Cost	P&L
Feed Additive	Grams/Day	KG/Day	Mt/Yr	Redu/Day	Redu/Yr	Rev/YR	Revenue	Day	Per Year	Per Cow/YR
Control 21 Days	NA	25.95	3.78	NA	NA	NA	NA	NA	NA	NA
Control 11 Days	NA	26.69	3.78	NA	NA	NA	NA	NA	NA	NA
Asparagopsis T	100.8	20.16	0.43	0.886	3.35	$268	TBD	$0.323	$117.73	$150.27
Asparagopsis T	61.65	24.66	2.54	0.327	1.24	$99	TBD	$0.197	$72.01	$27.19
Alga	103.65	20.73	0.34	0.910	3.44	$275	TBD	$0.285	$104.04	$171.16
Alga	61.1	24.44	2.65	0.305	1.14	$91	TBD	$0.168	$61.33	$29.87

The cows were fed the recommended dosage of feed additives for 21 days after being acclimated at Dairy Experts in Tulare. The reduction in methane was approximately 90% for the 40 cows who consumed the feed additive.

The cost for the Alga (AB) product is $.28/day and the cost for the *Asparagopsis* (AT) product is $0.32/day. Revenue consists of monetizing carbon reduction, as either an inset or offset, and an improvement in feed efficiency. With a carbon credit price of $80/mt (indicative price from global milk buyer) the net P&L to the farmer would be $150/cow/year for cows consuming AT and $171/cow/year for cows consuming the Alga Bioscience product.

During the trial a significant drop in DMI was noticed as well as a drop in milk yield. As a result, the dosage of the feed additive was cut in half after day 21. While DMI did improve, vs the control group of cows, the cows continued to produce less milk vs. cows in the control group. The financial benefits to the farmer were less for cows consuming the lower dosage, roughly $30/cow/year.

The table below shows the drop in DMI as well as milk yields and body weight vs the control group.

Feed/Cow Weight/Milk Output					
High Dosage	Control	Alga	Change	Aspara T	Change
Dry Matter Intake (kg/day)	25.95	20.73	-20%	20.16	-22%
Body Weight (kg)	671.1	652	-3%	641.4	-4%
Milk Yield (kg/day)	31.71	29.44	-7%	27.99	-12%
Low Dosage					
Dry Matter Intake (kg/day)	26.69	24.44	-8%	24.66	-8%
Body Weight (kg)	661.7	641	-3%	628	-5%
Milk Yield (kg/day)	30.1	29.04	-4%	27.85	-7%

Results and Discussion

Both products, fed at the recommended dosage, generated enough methane reduction to make them economically viable for the dairy farmer. Other studies have noted that a significant revenue source for the dairy farmer would be an improvement in feed efficiency. This is accurate but any drop in milk yield, milk revenue, is a non-starter for the dairy farmer. Further studies are required to adapt product palatability. With the same DMI and the same improvement in feed efficiency achieved in this study, the dairy farmer would achieve a substantial additional revenue boost from the use of both feed additives.

The focus of this trial was on building an economic viability model for dairy farmers for using these two seaweed feed additives. Without FDA approval, the product cannot be merchandised. In addition, even with FDA approval there will need to be consumer acceptance of milk from cows fed with seaweed, which has a Bromoform as an active ingredient. Importantly, any traces of bromoform in the milk were not statistically significant and were well below the maximum allowable amounts in drinking water.

There are several areas that require further study:

1. Feed efficiency

   A potential major per cow P&L boost could come from gains in feed efficiency. This was beyond the scope of this study. In the economic model above, the DMI intake of cows taking Alga dropped by 20% while the milk yield dropped by 7% and body weight by 3% vs the control group. The conclusion is that the cows favor feed without the feed additives. If the cows were to consume the same amount of DM they would presumably produce more milk with the same rations. Further study on improving palatability of the rations is recommended.

2. Bromoform and Iodine

   While no traces of bromoform were found in the milk or blood of the non-control groups, the active ingredient in the seaweeds is bromoform, which is a carcinogen. Dairy farmers are very hesitant, rightly so, to introduce a new feed additive that has a carcinogen regardless of the positive financial impact and the lack of traces in the blood and milk. More trials and more scientific studies are required to prove out the elimination of bromoform during the digesting process.

3. FDA Approval and Consumer Acceptance

   Until one or more seaweed-based feed additives has broad FDA support and approval, as well as consumer acceptance, it will be a challenge to make further progress with the dairy community. While Mooteric is a stakeholder and would benefit substantially from FDA product approval, the company does not have the financial resources to go through an FDA approval process.

4. Supply Chain

Asparagopsis is most economically grown and harvested in China, Vietnam and other Asian and South Pacific countries. Mooteric traveled to China to meet with Toki. While China can be extremely competitive on pricing, it is apparent that transparency may be an issue long term. Mooteric also traveled to Vietnam to meet with another potential supplier. The supply chain has a long way to go in terms of transparency, bulk large-scale farming (most likely in Vietnam) and long-term supply contracts with Emerging Market suppliers. It is difficult to get too far along with these items without a ready market in the U.S.

Exhibit A – Feed Additive Study

REPORT / PROJECT

No. DE230802
CONFIDENTIAL

DATE PREPARED:	March, 2024
PROJECT NUMBER:	DE230802
STUDY TITLE:	Feeding Seaweed to Accelerate Enteric Methane Emissions Reductions in Central Valley Dairies
PRINCIPAL INVESTIGATORS:	Alfonso Lago, DVM, Dipl. ABVP-Dairy, PhD and Bruna Calvo, BS, PhD
BIOSTATISTICIAN:	Ainhoa Valldecabres, DVM, PhD
SPONSOR:	Mooteric LLC
OBJECTIVE:	Evaluate the methane-mitigating benefits of seaweed-based feed additives within the regular feed rations of Central Valley dairy cows on milk yield and composition, dry matter intake, feed efficiency, body weight, enteric emissions and nutrient digestibility.
DATE INITIATED:	September 18th, 2023
DATE CONCLUDED:	November 20st, 2023

Dairy Experts
expediting innovation

Signature: Date:

Table of Contents

EXECUTIVE SUMMARY

The objective of this study was to evaluate the effects of seaweed-based feed additives containing bromoform on milk yield and composition, dry matter intake, feed efficiency, body weight, enteric emissions, and nutrient digestibility. A total of 60 Holstein cows on their 2nd or 3rd and 4th parity at 204 ± 7 days in milk were blocked by milk yield and randomly assigned to: a) control (no seaweed added to the TMR); b) ShiLai™ *Asparagopsis taxiformis* pellets added to the TMR at a rate of 0.50% of DM; or, c) Alga Biosciences product added to the TMR also at a rate of 0.50% of DM from enrollment to day on supplementation 21. The rate was reduced to 0.25% of DM from day 22 to the end of the study (day 42). Cows were housed in a single group and fed *ad libitum*. Individual cow TMR intake was recorded through the Biocontrol CRFI feed intake control and measurement system, and enteric methane emissions were measured using GreenFeed units. Individual milk yield was recorded using AfiMilk electronic milk meters, and milk fat and protein were measured using optical in-line analyzers at each of two daily milkings. Treatment and treatment by time effects claimed at $P<0.05$ were assessed by multiple linear regression. Supplementation of AB or AT at 0.50% of DM resulted in a ~90% decrease of enteric methane emissions, however, it also resulted on an overtime decrease and overall lower milk production compared to control cows, potentially driven by lower DMI observed in cows supplemented with both products. Supplementation of TMR with AB or AT at 0.25% of DM resulted in a 36-41% decrease of enteric methane emissions, and although a trend to decrease DMI, the magnitude of the effect was smaller compared with the higher dose, as well as the differences in production parameters as detected by time conditional effects. In conclusion, both seaweed-based feed additives effectively decreased enteric methane emissions in a dose-response manner and additional research is needed to evaluate the optimal dose to achieve an important reduction on methane emission without compromising the cow's performance, as well to evaluate the long-term methane inhibiting effects of these seaweed-based feed additives containing bromoform.

OBJECTIVE

The objective of the present study was to evaluate the effects of seaweed-based feed additives within a common Central Valley dairy cow feed ration on milk yield and composition, dry matter intake, feed efficiency, body weight, enteric emissions, and nutrient digestibility.

METHODS

Study Design

A total of 60 Holstein cows in their 2nd or 3rd lactation were brought to the DairyExperts Research Barn 21 days prior to the experimental phase of the study to adapt to facilities and feeding system. Cows were milked twice a day during the adaptation period; milk yield from four consecutive days of the adaptation period was used to block cows by baseline milk yield prior to random assignment to study treatments when cows were 204±7 DIM. Thus, the study was a randomized complete block design with block being baseline milk yield.

Experimental Treatments

Three treatment groups:

- CO (n = 20 cows): No seaweed added to the TMR (CONTROL)
- AT (n = 20 cows): ShiLai™ *Asparagopsis taxiformis* Feed pellets added to the TMR at a rate of 0.50% of DM from enrollment to day 21 of supplementation, and at a rate of 0.25% of DM from day 22 to the end of the study (day 42). Pellets contained 0.38 ± 0.08% bromoform [n = 3 samples analyzed by Gas Chromatography Mass Spectrometry (GC-MS)]
- AB (n = 20 cows): Alga Biosciences product added to the TMR at a rate of 0.50% of DM from enrollment to day 21 of supplementation, and at a rate of 0.25% of DM from day 22 to the end of the study.

Housing and Milking

Cows in all treatments shared the same housing space. Cows' housing is a roof covered loose system pen with a compost bedded resting area which has adjacent a cow traffic alley with feed mangers and waterers. Next to the cows' housing area there is a double 10 parallel parlor with each stall equipped with milk meters (MPC™, AfiMilk, Israel) and optical in-line milk component analyzer (AfiLab™, AfiMilk, Israel) that allows for milk yield and composition determination at each milking. The milk meters and component analyzer were calibrated every 4 weeks. Cows were milked twice a day during the adaptation and experimental period.

Feeding

Cows in the study had *ad libitum* access to water and to a TMR fed once a day. The TMR formulated to meet or exceed the predicted requirements of energy, protein, minerals, and vitamins (NASEM, 2021) was prepared daily at the research facility that includes feed storage, feed mixer wagon and concrete floors allowing neat feed handling for the preparation of the different rations. A base TMR was prepared once a day in a single batch, afterwards, it was divided into 3 piles in an amount equivalent to the previous day feed intake for each group plus 5%. Dry matter (DM) of the base TMR was measured on a daily basis. The TMR pile assigned to CON cows was reloaded into the mixer wagon for additional mixing and distribution into the mangers assigned to this group. Then, AT and AB were added to the second and third piles, respectively, and reloaded each pile separately for additional mixing and distribution of each load into the mangers assigned to each treatment. In order to avoid cross contamination between batches approximately 75 kg of Bermuda grass was loaded into the mixing wagon and have the augers running for about 4 min before discharging it to sweep away TMR residues. A total of 27 feed mangers (9 mangers for CON, 9 mangers for AT, and 9 mangers for AB) were sequentially assigned to each treatment in series of 3: CON, AT, AB. Feed mangers are equipped with a feed intake control and measurement system (CRFI™, BioControl, Rakkestad, Norway) that allows to control the access of cows to feed mangers with different diets, access of cows to multiple mangers within the same treatment diet, and to measure individual cow feed intake, number of visits, and feeding time. Mangers scales were calibrated every 4 weeks.

Data Collection and Study Outcomes

Data collection was initiated two weeks prior to supplementation start (baseline period) and finished on the last day of supplementation (42 days after supplementation start).

Performance

a) Milk yield and components

Individual cow milk yield and composition (concentration of fat, protein, and lactose) measured at each milking with the milk meters and optical in-line milk component analyzers was downloaded using AfiFarm™ software (AfiMilk, Israel; Kaniyamattam and De Vries, 2014). Energy-corrected milk yield [milk yield value corrected for 3.5% fat and 3.2% true protein as: (0.3246 × kg of milk) + (12.86 × kg of fat) + (7.04 × kg of true protein) and 3.5% fat-corrected milk yield [(0.4324 × kg of milk) + (16.216 × kg of fat)], were calculated for each cow using the information described above, according to the NRC (2021).

Cows' milk urea nitrogen (MUN) and somatic cell count (SCC) were determined at the a.m. and p.m. milking once during the baseline period and twice a week during the treatment administration period. Samples were collected into vials and taken to Tulare DHIA (Tulare, CA) for analysis.

b) Dry matter intake and feed efficiency

Individual cow TMR intake was continuously recorded through the feed intake control and measurement system described above (Ternman et al., 2021; Chapinal et al., 2007). Dry matter (DM) of the base TMR was 64%, individual daily dry matter intake determination as: (kg of TMR consumed × DM of TMR). Daily feed efficiency was defined as kg of energy-corrected milk produced per kg of DM consumed on an individual basis.

c) Body weight

Cows were individually weighed after the morning milking using an electronic scale (PS-3000 scale; Salter Brecknell, Fairmont, MN) on the last day of the adaptation period and on days 14, 28, and 42 of the experimental period.

Methane emissions

Enteric gaseous emissions (CH_4 and CO_2) were measured using 2 GreenFeed system units (C-Lock Inc., Rapid City, SD) which were permanently available for cows to visit, and during visits, enteric gas emissions from individual cows were measured. Alfalfa pellets were available at each cow visit and the weight of pellets dispensed was recorded and included in the daily DMI estimation. Cows were adapted to using the GreenFeed before the beginning of the experiment. GreenFeed units were calibrated following the manufacturer's recommendations. The GreenFeed is equipped with a head position sensor and gas emission data are rejected when the cow's head position criteria are not met. Each cow was allowed a maximum of 6 visits in 24 h, with a 4-h interval between visits, and not more than 10 feed drops of approximately 30 g each per visit.

Apparent total-tract digestibility and fecal dry matter flow

Fresh feed and individual ingredient (alfalfa hay, corn silage, and grain mix) samples were collected before supplementation start (baseline), and at days 14, 28 and 42 during the experimental phase (treatment administration period). Feed refusals and individual cow fecal samples were collected the day after fresh feed collection. A total of 200 g of fresh feed, feed refusals and feces were stored at −20°C until laboratory analysis at Cumberland Valley Analytical Services (Waynesboro, PA) using the NIR Plus package (Righi et al., 2017). Nutrient intakes (OM, CP, NDF, uNDF and starch) were calculated as kg of feed consumed per cow on a dry matter basis, times the nutrient concentration on the TMR fed that day, corrected for TMR refusals.

Digestibilities of DM, OM, CP, NDF, and starch were calculated from the respective nutrient intake and fecal flow. Undegraded NDF (uNDF) was used as an internal marker to calculate DM and nutrient digestibility (Cochran et al., 1986). Apparent total-tract DM digestibility was calculated as: 1 − (diet uNDF, %/feces uNDF, %). Apparent total-tract for each nutrient was calculated as: 1 − [(diet uNDF, % × feces Nutrient, %)/(feces uNDF, % × diet Nutrient, %)]. Fecal dry matter flow was calculated as the ratio of feces uNDF concentration to uNDF intake.

DATA ANALYSES

Data was analyzed using the SAS software (Version 9.4; SAS Institute Inc., Cary, NC, USA). Baseline

values were calculated averaging the information from the blocking baseline period for milk yield and components data and feed intake and efficiency outcomes; averaging information from the last two weeks prior to treatments start for enteric methane emissions; averaging information for the last AM and PM milking prior to treatment start for SCC and MUN; and using the information from the day prior to supplementation start for body weight and nutrients intake and digestibility. Baseline values were compared among treatments using the MIXED procedure; where baseline differences were detected (Tables 2 and 3), models were adjusted for the effect of baseline.
Data analysis was performed separately for the periods of 0.50 (High-dose) and 0.25% of DM (Low-dose) of methane mitigation product feeding, allowing for an 11-day period between them. The High- and Low-dose periods comprised 21 and 10 consecutive days, respectively. The washout period was established based on records for enteric methane emissions and not included in the analysis; cows were receiving treatments at 0.25% of DM during this period. Figure 1 shows a timeline for the aforementioned study periods. Washout period data is presented in the accompanying figures for completeness.

Performance

a) Milk yield and components

Daily milk yield [calculated as the sum of both morning and afternoon milk weights (kg)], energy-corrected milk [calculated as the sum of both morning and afternoon milk weights (kg)], fat-corrected milk [calculated as the sum of both morning and afternoon milk weights (kg)], milk fat and protein concentrations (calculated as the average of both morning and afternoon milking readings) and yields (calculated multiplying daily milk fat and protein concentrations by the daily milk yield) were calculated using the SQL procedure. When both, AM and PM milking information was not available for either of the variables of study, information for that day and that variable was not used in the analysis. Raw data plots were generated for the identification of outliers using the SGPLOT procedure; no outliers were identified.
Multiple linear regression was used to analyze productive data with the MIXED procedure. All models included the fixed effects of treatment, time (day), and treatment by time. Time was included in the models as a categorical variable. For each outcome, the variance-covariance structure leading to the lowest Akaike's and Bayesian information criterion was used to model the correlation of multiple measures within cow, with cow as the subject of the repeated statement. Unstructured, compound symmetry, autoregressive 1, heterogeneous autoregressive 1, Toeplitz, and Toeplitz heterogeneous were the variance-covariance structures evaluated. The LSMEANS statement with Bonferroni adjustment was used to quantify the association between treatment and the outcome of interest. Impact of influential observations (studentized residuals >|4|) was assessed removing them from the models prior to results interpretation; if influential observations did not alter interpretation, results from models including all observations are presented. Results are presented as LSM with the corresponding SEM. Overall model fit was assessed with final models' residuals plots generated with the residual option in the model statement.
To comply with model assumptions of normality and homoscedasticity of residuals, milk SCC was analyzed as Log10 SCC. To allow for Log transformation of all observations, 1 was added to all SCC determinations. MUN and SCC were analyzed as described above, except for the Low-dose period as only one observation per animal was available (4 observations per animal were available for the High-dose period). Given the observed differences at baseline (Table 2), baseline MUN was included in the statistical models.

b) Feed intake and efficiency

Daily DM intake and feed efficiency were evaluated as described for production data.

c) Body weight

Body weight data was evaluated as described for production data without the repeated measures statement.

Apparent total-tract digestibility and fecal dry matter flow

Apparent total-tract digestibility and fecal DM flow data was analyzed as body weight data.

Methane emissions

Daily methane production represents g of CH_4 g/day. Enteric CH_4 (and CO_2) emission yield (g/kg of DMI) and intensity (g/kg of ECM) weekly averages were calculated based on the available CH_4 measurements and DMI and ECM daily records using the SQL procedure. Methane emissions data was analyzed as milk yield and components data (repeated measurements model) and using all the observations by dose period without the repeated measurements (dose period model) to allow for a higher number of observations per cow and to decrease the uncertainty associated to smaller number of records. Treatment by time contrasts were generated within day on supplementation using the PLM procedure.

RESULTS

Data from a total of 60 cows (20 cows/ treatment group) was used for the analyses. Baseline description of study outcomes is presented in Tables 2 and 3. Treatment differences at baseline were detected for MUN and ADF digestibility.

Performance

a) Milk yield and components

Overall treatment effects and additional effects included in the models are presented in Table 4. Treatment by time effects are depicted in Figures 2 to 10.

High-dose period

Treatment by time effects were observed for milk yield (*P* < 0.001; Figure 2), ECM yield (*P* = 0.04; Figure 3) and milk protein yield (*P* = 0.001; Figure 6) during the High-dose supplementation period. Numerical differences were observed from study day 5. Statistical differences were consistently observed for AT compared to control cows from study day 16; compared to control, milk, ECM and protein yields were lower for AT cows at study days 6 [4.3 kg (*P* = 0.08), 4.6 kg (*P* = 0.10), protein yield was not different], 14 [4.3 kg (*P* = 0.08), ECM and protein yields were not different], 16 [5.0 kg (*P* = 0.03), 5.1 kg (*P* = 0.04) and 0.20 kg (*P* = 0.03)], 17 [5.0 kg (*P* = 0.04), 5.2 kg (*P* = 0.06) and 0.16 kg (*P* = 0.10)], 18 [5.5 kg (*P* = 0.02), 5.0 kg (*P* = 0.04) and 0.19 kg (*P* = 0.02)], 19 [6.7 kg (*P* = 0.005), 6.3 kg (*P* = 0.02) and 0.22 kg (*P* = 0.03)], 20 [5.2 kg (*P* = 0.02), 5.0 (*P* = 0.04) and 0.17 kg (*P* = 0.07)], and 21 [6.7 kg (*P* = 0.004), 6.3 kg (*P* = 0.02) and 0.20 kg (*P* = 0.02)]. While compared to control cows, milk, ECM and protein yields were lower for AB cows at study days 16 [4.2 kg (*P* = 0.10), 4.5 kg (*P* = 0.10) and 0.16 kg (*P* = 0.09)], 18 [5.1 kg (*P* = 0.03), 4.8 kg (*P* = 0.05) and 0.20 kg (*P* = 0.01)], 20 [4.3 kg (*P* = 0.07), ECM and protein yields were not different], and 21 [5.6 kg (*P* = 0.02), 5.4 kg (*P* = 0.05) and 0.17 kg (*P* = 0.06)]. Milk yield was similar for AB and AT cows at all study days.

No effects involving treatment were observed for FCM yield, fat yield, fat concentration, protein concentration, milk SCC and MUN during the High-dose supplementation period.

Low-dose period

A treatment by time effect was observed for milk yield during the Low-dose supplementation period; however, no statistical differences within study day were observed for any of the treatment comparisons. Additionally, a treatment by time effect was detected for milk protein concentration (*P* = 0.02); AB cows had lower milk protein concentration compared to control cows at study day 37 (0.27 units of percentage; *P* = 0.03), and compared to control (0.27 units of percentage; *P* = 0.03) and AT cows (0.33 units of percentage; *P* = 0.005) at study day 38. A trend for a treatment by time effect was observed for MUN (*P* = 0.10), however, treatment comparisons within study day were not statistically significant.

No effects involving treatment were observed for ECM yield, FCM yield, fat yield, protein yield, fat concentration, and milk SCC during the Low-dose supplementation period.

b) Feed intake and efficiency

Overall treatment effects and additional effects included in the models are presented in Table 5. Treatment by time effects are depicted in Figures 11 and 12.

High-dose period

Treatment and treatment by time effects were observed for DMI (*P* < 0.001 for both) and feed efficiency (*P* < 0.001 and 0.002, respectively) during the High-dose supplementation period.

Overall, compared to control cows, DMI was 5.2 and 5.8 kg/d lower for AB and AT cows, respectively (*P* <

0.001 for both). Statistical differences on DMI were consistently observed from study day 4 (3.58 to 9.71 kg/d; $P \leq 0.05$; Figure 11) except for study days 11 and 12 where DMI was similar for control and AB cows ($P > 0.10$); DMI was also lower for AB cows compared to control cows at study day 1 [4.4 kg ($P = 0.01$)]. Dry matter intake was similar for AB and AT cows during the High-dose period.

Overall, compared to control cows, feed efficiency was 0.22 and 0.23 units higher for AB and AT cows, respectively ($P < 0.001$ for both). For AB cows compared to control cows, differences on feed efficiency were observed at study days 1 (0.27 units; $P < 0.001$), 4 (0.21 units; $P = 0.08$), 7 (0.29 units; $P = 0.002$), 9 (0.22 units; $P = 0.09$), 13 (0.27 units; $P = 0.002$), 14 (0.27 units; $P = 0.01$), 15 (0.22 units; $P = 0.04$), 17 (0.35 units; $P < 0.001$), 18 (0.38 units; $P < 0.001$), 19 (0.31 units; $P = 0.01$), 20 (0.29 units; $P < 0.001$), and 21 (0.34 units; $P < 0.001$). For AT cows compared to control cows, differences on feed efficiency were observed at study days 4 (0.23 units; $P = 0.04$), 5 (0.31 units; $P = 0.03$), 6 (0.30 units; $P = 0.03$), 7 (0.22 units; $P = 0.04$), 12 (0.23 units; $P = 0.08$), 13 (0.17 units; $P = 0.09$), 15 (0.43 units; $P < 0.001$), 16 (0.44 units; $P = 0.008$), 17 (0.26 units; $P = 0.02$), 18 (0.34 units; $P < 0.001$), 19 (0.33 units; $P = 0.007$), 20 (0.41 units; $P < 0.001$), and 21 (0.21 units; $P = 0.08$). Feed efficiency was similar for AB and AT cows during the High-dose period (Figure 11).

Low-dose period

Influential observations (n = 7) from 1 cow (control) were detected for DMI (DMI of 0 to 1.4 kg/d). Excluding influential observations, a trend for an overall treatment effect on DMI was observed ($P = 0.06$); overall DMI tended to be 2.3 kg/d lower for AB compared to control cows ($P = 0.10$). However, keeping these observations in the model no effects involving treatment were observed for DMI (control: 25.95 ± 0.93 kg/d; AB: 24.44 ± 0.93 kg/d; AT: 24.66 ± 0.93 kg/d; $P = 0.47$).

A treatment by time effect was observed for feed efficiency during the Low-dose period ($P = 0.05$); feed efficiency tended to be lower for AT compared to AB at study day 37 (0.13 units; $P = 0.07$; Figure 12).

c) Body weight

Overall treatment effects and additional effects included in the models are presented in Table 5.

High-dose period

Body weight was similar among treatments during the High-dose period (control: 1476.5 ± 27.7 kg; AB: 1434.3 ± 27.7 kg; AT: 1411.1 ± 27.7 kg; $P = 0.25$).

Low-dose period

Body weight was similar among treatments during the Low-dose period (control: 1455.8 ± 28.7 kg; AB: 1410.2 ± 28.7 kg; AT: 1381.6 ± 28.7 kg; $P = 0.19$).

Methane emissions

Treatment and additional effects included in the statistical models are presented in Table 6. Treatment by time effects are depicted in Figures 13 to 15.

a) Methane production

High-dose period

Overall treatment and treatment by time effects were observed for methane emissions during the High-dose supplementation period ($P < 0.001$ for both). Compared to control (345.11 ± 6.30 g/d), methane emissions were reduced by 91% (31.42 ± 6.02 g/d) and 89% (38.29 ± 6.19 g/d) for AB and AT cows, respectively in the repeated measures model ($P < 0.001$); methane emissions were similar for AB and AT cows ($P = 1.00$). The conditional effect of time was driven by treatment differences at study day 1; afterwards, treatment differences resemble those presented as overall treatment effects ($P < 0.001$ for all; Figure 13).

When all observations from the High-dose period were combined, compared to control (347.71 ± 3.80 g/d), methane emissions were reduced by 90% (31.33 ± 3.59 g/d) and 89% (39.72 ± 3.79 g/d) lower for AB and AT cows, respectively ($P < 0.001$); while similar for AB and AT cows ($P = 0.32$).

Low-dose period

Overall treatment and treatment by time effects were observed for methane emissions during the Low-dose supplementation period ($P < 0.001$ for both). Compared to control (388.25 ± 13.82 g/d), methane emissions were reduced by 36% (246.75 ± 15.26 g/d) and 38% (242.52 ± 14.39 g/d) for AB and AT cows, respectively in the repeated measures model (*P* < 0.001); methane emissions were similar for AB and AT cows (*P* = 1.00). Overtime, differences resembled those stated for the overall treatment effects (*P* ≤ 0.06 for all), except for study day 39 where methane emissions were similar for AB and control cows (*P* = 0.23), while still lower for AT compared to control cows (*P* = 0.006; Figure 13).

When all observations from the High-dose period were combined, compared to control (394.04 ± 7.74 g/d), methane emissions were reduced by 38% (243.20 ± 9.84 g/d) and 41% (233.95 ± 8.78 g/d) for AB and AT cows, respectively (*P* < 0.001); methane emissions were similar for AB and AT cows (*P* = 1.00).

b) Methane yield

High-dose period

Overall treatment and treatment by time effects were observed for methane yield during the High-dose supplementation period (*P* < 0.001 for both). Compared to control, methane yield was 11.79 ± 0.25 g/kg DMI and 11.47 ± 0.26 g/kg DMI lower for AB and AT cows, respectively in the repeated measures model (*P* < 0.001); methane yield was similar for AB and AT cows (*P* = 0.62). The conditional effect of time was driven by treatment differences at study day 1; compared to control, methane yield was 7.17 ± 1.24 g/kg DMI (*P* < 0.001) lower for AB cows, tended to be 2.94 ± 1.25 g/kg DMI (*P* = 0.06) lower for AT cows, and was 4.22 ± 1.22 g/kg DMI lower for AB compared to AT cows (*P* = 0.002), afterwards treatment differences resemble those presented as overall treatment effects (*P* < 0.001 for all; Figure 14).

When all observations from the High-dose period were combined, methane yield was 11.88 ± 0.22 g/kg DMI and 11.52 ± 0.23 g/kg DMI lower for AB and AT cows compared to control, respectively (*P* < 0.001); methane yield was similar for AB and AT cows (*P* = 0.32).

Low-dose period

Overall treatment and treatment by time effects were observed for methane yield during the Low-dose supplementation period (*P* < 0.001 for both). Compared to control, methane yield was 4.32 ± 0.70 g/kg DMI and 4.56 ± 0.67 g/kg DMI lower for AB and AT cows, respectively in the repeated measures model (*P* < 0.001); methane yield was similar for AB and AT cows (*P* = 1.00). Overtime for AB cows compared to control cows, differences on methane yield were observed at study days 33 (2.42 g/kg DMI; *P* = 0.07), 35 (6.90 g/kg DMI; *P* < 0.001), 36 (4.18 g/kg DMI; *P* = 0.006), 37 (4.43 g/kg DMI; *P* = 0.005), 41 (7.59 g/kg DMI; *P* < 0.001), and 42 (9.63 g/kg DMI; *P* < 0.001; Figure 15); for AT cows compared to control cows, differences on methane yield were observed at study days 33 (4.10 g/kg DMI; *P* < 0.001), 34 (3.64 g/kg DMI; *P* = 0.03), 35 (9.06 g/kg DMI; *P* < 0.001), 36 (4.03 g/kg DMI; *P* = 0.005), 37 (4.84 g/kg DMI; *P* < 0.001), 38 (5.07 g/kg DMI; *P* = 0.004), 39 (3.35 g/kg DMI; *P* = 0.02), 41 (4.81 g/kg DMI; *P* < 0.001), and 42 (4.89 g/kg DMI; *P* < 0.001; Figure 14). Additionally, methane yield was lower for AB compared to AT at study days 41 (2.78 g/kg DMI; *P* = 0.07) and 42 (4.73 g/kg DMI; *P* = 0.002).

When all observations from the Low-dose period were combined, methane yield was 4.70 ± 0.49 g/kg DMI and 4.95 ± 0.46 g/kg DMI lower for AB and AT cows compared to control, respectively (*P* < 0.001); methane yield was similar for AB and AT cows (*P* = 1.00).

c) Methane intensity

High-dose period

Overall treatment and treatment by time effects were observed for methane intensity during the High-dose supplementation period (*P* < 0.001 for both). Compared to control, methane intensity was 9.24 ± 0.30 g/kg ECM and 8.99 ± 0.30 g/kg ECM lower for AB and AT cows, respectively in the repeated measures model (*P* < 0.001); methane intensity was similar for AB and AT cows (*P* = 1.00). The conditional effect of time was driven by treatment differences at study day 1; compared to control, methane intensity was 5.89 ± 1.02 g/kg ECM (*P* < 0.001) and 2.87 ± 1.03 g/kg ECM (*P* = 0.02) lower for AB and AT cows, and 3.01 ± 1.00 g/kg ECM lower for AB compared to AT cows (*P* = 0.008), afterwards treatment differences resemble those presented as overall treatment effects (Figure 15).

When all observations from the High-dose period were combined, methane intensity was 9.36 ± 0.17 g/kg ECM and 9.07 ± 0.18 g/kg ECM lower for AB and AT cows compared to control, respectively (*P* < 0.001); methane intensity was similar for AB and AT cows (*P* = 0.28).

Low-dose period

Overall treatment and treatment by time effects were observed for methane intensity during the Low-dose supplementation period (*P* < 0.001 for both). Compared to control, methane intensity was 4.15 ± 0.71

g/kg ECM and 3.48 ± 0.69 g/kg ECM lower for AB and AT cows, respectively in the repeated measures model (P < 0.001); methane intensity was similar for AB and AT cows (P = 1.00). The conditional effect of time was driven by treatment effect differences on methane intensity relative to those presented for the overall treatment effects at study days 34 (statistically similar for AB and control cows; P = 0.17), 38 (statistically similar for AB and control cows; P = 0.10), 39 (statistically similar for all treatment groups), 40 (statistically similar for AT and control cows; P = 0.68); 41 (lower for AB compared to AT; P = 0.02) and 42 (lower for AB compared to AT; P = 0.003; Figure 15).
When all observations from the Low-dose period were combined, methane intensity was 4.65 ± 0.42 g/kg ECM and 4.11 ± 0.40 g/kg ECM lower for AB and AT cows compared to control, respectively (P < 0.001); methane intensity was similar for AB and AT cows (P = 0.70).

Nutrients intake, apparent total-tract digestibility and fecal dry matter flow

Treatment and additional effects included in the statistical models are presented in Tables 7 and 8. Treatment by time effects are depicted in Figures 16 and 17.

High-dose period

Intakes of organic matter, crude fat, crude protein, NDF, ADF, starch and ash were 5.29 ± 1.59, 0.32 ± 0.09, 0.94 ± 0.30, 1.31 ± 0.45, 0.92 ± 0.27, 1.67 ± 0.48 and 0.36 ± 0.11 g/d lower for AB compared to control cows (P < 0.01 for all), respectively. Similarly, intakes of organic matter, crude fat, crude protein, NDF, ADF, starch and ash were 5.06 ± 1.59, 0.31 ± 0.09, 0.94 ± 0.30, 1.51 ± 0.45, 0.89 ± 0.27, 1.61 ± 0.48 and 0.36 ± 0.11 g/d lower for AT compared to control cows (P < 0.01 for all), respectively.
Apparent total tract digestibility of dry matter, organic matter, crude protein, NDF and ADF was 8.36 ± 0.85, 14.30 ± 1.80, 9.65 ± 1.34, 16.51 ± 1.17 and 14.30 ± 1.80 units of percentage higher for AB compared to control cows (P < 0.001 for all), respectively. Similarly, apparent total tract digestibility of dry matter, organic matter, crude protein, NDF and ADF was 7.51 ± 0.85, 16.64 ± 1.93, 7.82 ± 1.34, 18.09 ± 1.17 and 16.64 ± 1.93 units of percentage higher for AB compared to AT cows (P < 0.001 for all), respectively. Apparent total tract starch digestibility tended to be 1.28 ± 0.58 units of percentage higher for AB compared to control cows (P = 0.10), but it was similar for AB and AT cows (P = 0.30). Apparent total tract digestibility of the evaluated nutrients was similar for control and AT cows (P > 0.10 for all).
Treatment effects were observed on fecal dry matter flow (P = 0.002); fecal dry matter flow was 11.32 ± 2.65 kg/d slower for AB compared to control cows (P = 0.001) while similar among the other treatment groups.

Low-dose period

An influential observation from one cow (DMI 1.3 kg; control) was detected. Excluding this observation, intakes of organic matter, crude fat, NDF, ADF, starch and ash were 3.11 ± 1.12, 0.17 ± 0.06, 1.14 ± 0.31, 0.81 ± 0.19, 0.89 ± 0.35 and 0.23 ± 0.08 g/d lower for AB compared to control cows (P < 0.05 for all), respectively. Similarly, intakes of organic matter, crude fat, NDF, ADF, starch and ash were 3.14 ± 1.12, 0.17 ± 0.06, 1.20 ± 0.31, 0.84 ± 0.19, 0.90 ± 0.35 and 0.25 ± 0.08 g/d lower for AT compared to control cows (P < 0.05 for all), respectively. Intakes of crude protein were similar among treatments (P = 0.13). In contrast, maintaining this observation, treatment differences were only observed for NDF (P = 0.08) and ADF (P = 0.02), and these followed the same pattern described above.
Apparent total tract digestibility of dry matter, organic matter, crude protein and NDF was 3.50 ± 0.75, 3.58 ± 0.79, 5.25 ± 1.05 and 4.12 ± 1.19 units of percentage higher for AB compared to control cows (P < 0.01 for all), respectively. Similarly, apparent total tract digestibility of dry matter, organic matter and crude protein was 2.49 ± 0.75, 2.73 ± 0.79 and 3.36 ± 1.05 units of percentage higher for AB compared to AT cows (P < 0.01 for all), respectively. Additionally, apparent total tract ADF digestibility was 4.86 ± 1.27 units of percentage higher for AB compared to AT cows (P = 0.001), and apparent total tract starch digestibility tended to be 1.31 ± 0.58 units of percentage higher for AT compared to control cows (P = 0.08).
An influential observation from one cow (fecal dry matter flow 282.6 kg/d; control) was detected. Excluding this observation, treatment effects on fecal dry matter flow were observed (P < 0.001); fecal dry matter flow was respectively 3.67 ± 0.95 and 3.22 ± 0.95 kg/d slower for AB and AT cows compared to control cows. In contrast, maintaining this observation, fecal dry matter flow was similar across treatments (P = 0.55).

CONCLUSIONS

Supplementation of AB or AT at 0.50% of DM resulted in a ~90% decrease of enteric methane emissions, however, it also resulted on an overtime decrease and overall lower milk production compared to control cows, potentially driven by lower DMI observed in cows supplemented with both products. Supplementation of TMR with AB or AT at 0.25% of DM resulted in a 36-41% decrease of enteric methane emissions, and although a trend to decrease DMI, the magnitude of the effect was smaller compared with the higher dose, as well as the differences in production parameters as detected by time conditional effects. In conclusion, both seaweed-based feed additives effectively decreased enteric methane emissions in a dose-response manner and additional research is needed to evaluate the optimal dose to achieve an important reduction on methane emission without compromising the cow's performance, as well to evaluate the long-term methane inhibiting effects of these seaweed-based feed additives containing bromoform.

REFERENCES

Chapinal, N., Veira, D. M., Weary, D. M., & Von Keyserlingk, M. A. G. (2007). Validation of a system for monitoring individual feeding and drinking behavior and intake in group-housed cattle. Journal of Dairy Science, 90(12), 5732-5736.

Cochran, R. C., Adams, D. C., Wallace, J. D., & Galyean, M. L. (1986). Predicting digestibility of different diets with internal markers: evaluation of four potential markers. Journal of Animal Science, 63(5), 1476-1483.

Kaniyamattam, K., & De Vries, A. (2014). Agreement between milk fat, protein, and lactose observations collected from the Dairy Herd Improvement Association (DHIA) and a real-time milk analyzer. Journal of Dairy Science, 97(5), 2896-2908.

NASEM (National Academies of Sciences, Engineering, and Medicine). Nutrient Requirements of Dairy Cattle. 8th rev. ed. The National Academies Press, Washington, DC. 2021.

Righi, F., Simoni, M., Visentin, G., Manuelian, C. L., Currò, S., Quarantelli, A., & De Marchi, M. (2017). The use of near infrared spectroscopy to predict faecal indigestible and digestible fibre fractions in lactating dairy cattle. Livestock Science, 206, 105-108.

Ternman, EM, Terré, M, Bouchon, M, Meunier, B, Munksgaard, L & Veissier, I 2021, Validation of eating duration assessment from automatic feeder systems. in E Strandberg, L Pinotti, S Messori, D Kenny, M Lee, JF Hocquette, VAP Cadavez, S Millet, R Evans, T Veldkamp, M Pastell & G Pollott (eds), Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science. Wageningen Academic Publishers, EAAP Book of Abstracts, vol. 27, pp. 613-613, 72nd Annual Meeting of the European Federation of Animal Science, Davos, Switzerland.

TABLES AND FIGURES

Table 1: Ingredients and nutrient composition of formulated study cows' diet.

Item	% of DM[1]
Ingredient	
Corn silage	33.45
Ground corn	10.39
Rolled corn	10.33
Soybean meal	7.33
Wheat bran	7.27
Dried distillers grains	7.01
Alfalfa hay	6.03
Almond hulls	4.25
Molasses	2.68
Canola meal	1.75
Rumen protected fat	1.22
Sodium sesquicarbonate	0.841
Limestone	0.84
Urea	0.469
Magnesium oxide	0.204
Zinc methionine	0.193
Salt	0.188
Trace mineral mix	0.033
Vitamin A, D and E premix	0.019
Nutrient composition	
Crude Protein	17.13
Ether extract	4.58
ADF	16.11
Ash-free NDF	27.40
NFC	44.36
Starch	28.24
NE_L 3X (Mcal/kg)	1.63

[1]Cows were fed a TMR at 64% of DM (average of daily determinations during the study period).

Table 2: Baseline comparison (LSM ± SEM) for performance, intake and enteric methane emissions measures by treatment group for cows enrolled in the study.

	Treatment[1]			
Variable	CONTROL	ALGA BIOSCIENCE	ASPAROGOPSIS TAXIFORMIS	*P*-value[2]
Production variables				
Milk yield, kg/d	32.97 ± 1.53	32.95 ± 1.53	32.81 ± 1.53	1.00
Energy-corrected milk yield, kg/d	33.98 ± 1.52	33.22 ± 1.52	33.31 ± 1.52	0.93
Fat-corrected milk yield, kg/d	34.06 ± 1.47	33.41 ± 1.47	33.26 ± 1.47	0.92
Fat yield, kg/d	1.22 ± 0.05	1.19 ± 0.05	1.18 ± 0.05	0.80
Protein yield, kg/d	1.03 ± 0.06	0.99 ± 0.06	1.02 ± 0.06	0.90
Fat concentration, %	3.76 ± 0.07	3.67 ± 0.07	3.59 ± 0.07	0.26
Protein concentration, %	3.10 ± 0.06	3.02 ± 0.06	3.10 ± 0.06	0.58
MUN, mg/dl	10.19 ± 0.24	11.35 ± 0.24	10.96 ± 0.24	0.003
Log_{10}SCC, cells/mL	1.79 ± 0.08	1.65 ± 0.08	1.72 ± 0.08	0.51
Dry matter intake, kg/d	25.54 ± 0.69	24.73 ± 0.69	25.58 ± 0.69	0.63
Feed efficiency	1.36 ± 0.06	1.38 ± 0.06	1.39 ± 0.06	0.96
Body weight, kg	671.3 ± 11.7	664.0 ± 11.7	655.6 ± 11.7	0.64
CH_4 (g/d)	344.14 ± 16.85	329.76 ± 16.85	344.42 ± 15.99	0.78
CH_4 yield, (g/kg DMI)	14.22 ± 0.59	13.74 ± 0.59	14.39 ± 0.56	0.71
CH_4 intensity, (g/kg ECM)	10.65 ± 0.56	10.65 ± 0.54	10.88 ± 0.52	0.49

[1]CONTROL: Cows receiving TMR without the methane reduction product; ALGA BIOSCIENCE: Cows receiving TMR with the Alga Bioscience; ASPAROGOPSIS TAXIFORMIS: Cows receiving TMR with the *Asparagopsis taxiformis* (n = 20 cows/group).

[2]Treatment comparisons were Bonferroni adjusted.

Table 3: Baseline comparison (LSM ± SEM) for nutrients intake and digestibility measures by treatment group for cows enrolled in the study.

Variable	Treatment[1]			P-value[2]
	CONTROL	ALGA BIOSCIENCE	ASPAROGOPSIS TAXIFORMIS	
Nutrients intake				
Organic matter, kg/d	23.72 ± 0.65	22.97 ± 0.65	23.76 ± 0.65	0.63
Crude protein, kg/d	4.37 ± 0.12	4.23 ± 0.12	4.37 ± 0.12	0.63
Crude fat, kg/d	1.32 ± 0.04	1.28 ± 0.04	1.32 ± 0.04	0.63
Neutral detergent fiber (NDF), kg/d	6.95 ± 0.19	6.73 ± 0.19	6.96 ± 0.19	0.63
Acid detergent fiber (ADF), kg/d	4.21 ± 0.11	4.08 ± 0.11	4.22 ± 0.11	0.63
Starch, kg/d	6.92 ± 0.19	6.70 ± 0.19	6.93 ± 0.19	0.63
Ash, kg/d	1.81 ± 0.05	1.76 ± 0.05	1.82 ± 0.05	0.63
Nutrients digestibility				
Dry matter, %	70.54 ± 0.53	70.58 ± 0.54	71.60 ± 0.53	0.28
Organic matter, %	71.71 ± 0.53	71.84 ± 0.54	72.55 ± 0.53	0.58
Crude protein, %	69.80 ± 0.93	70.16 ± 0.95	71.27 ± 0.93	0.51
Neutral detergent fiber (NDF), %	42.52 ± 0.66	42.23 ± 0.68	43.56 ± 0.66	0.34
Acid detergent fiber (ADF), %	31.59 ± 0.93	29.67 ± 0.95	33.40 ± 0.93	0.03
Starch, %	97.91 ± 0.30	97.28 ± 0.31	97.90 ± 0.30	0.27
Fecal DM flow, kg/d	13.63 ± 0.50	13.90 ± 0.52	14.17 ± 0.50	0.75

[1]CONTROL: Cows receiving TMR without the methane reduction product; ALGA BIOSCIENCE: Cows receiving TMR with the Alga Bioscience; ASPAROGOPSIS TAXIFORMIS: Cows receiving TMR with the *Asparagopsis taxiformis* (n = 20 cows/group).

[2]Treatment comparisons were Bonferroni adjusted.

Table 4: Milk yield and components LSM and SEM during treatment administration by study group for cows enrolled in the study.

	Treatment[1]			Fixed Effects *P*-value		
Outcome[2]	CONTROL	ALGA BIOSCIENCE	ASPAROGOPSIS TAXIFORMIS	Treatment	Study day	Treatment x Study day
High-dose period						
Milk yield, kg/d	31.71 ± 1.27	29.44 ± 1.27	27.99 ± 1.27	0.12	<0.001	<0.001
ECM yield, kg/d	33.10 ± 1.33	30.56 ± 1.33	29.34 ± 1.33	0.14	<0.001	0.04
FCM yield, kg/d	33.38 ± 1.31	30.97 ± 1.31	29.61 ± 1.31	0.13	<0.001	0.13
Fat yield, kg/d	1.21 ± 0.05	1.13 ± 0.05	1.08 ± 0.05	0.15	0.002	0.56
Protein yield, kg/d	0.98 ± 0.05	0.88 ± 0.05	0.86 ± 0.05	0.19	<0.001	0.001
Fat concentration, %/d	3.84 ± 0.06	3.87 ± 0.06	3.88 ± 0.06	0.87	<0.001	0.26
Protein concentration, %/d	3.06 ± 0.06	2.99 ± 0.06	3.05 ± 0.06	0.65	<0.001	0.80
MUN[4], mg/dl	9.50 ± 0.16	9.32 ± 0.16	9.06 ± 0.15	0.15	<0.001	0.80
Log_{10}SCC, cells/mL	1.87 ± 0.08	1.74 ± 0.08	1.90 ± 0.08	0.34	0.02	0.35
Low-dose period						
Milk yield, kg/d	30.10 ± 1.42	29.04 ± 1.41	27.85 ± 1.41	0.54	<0.001	0.01
ECM yield, kg/d	31.64 ± 1.45	30.90 ± 1.45	29.47 ± 1.45	0.56	<0.001	0.28
FCM yield, kg/d	31.88 ± 1.43	31.41 ± 1.42	29.75 ± 1.42	0.54	<0.001	0.50
Fat yield, kg/d	1.16 ± 0.05	1.16 ± 0.05	1.09 ± 0.05	0.56	0.001	0.68
Protein yield, kg/d	0.94 ± 0.04	0.88 ± 0.04	0.86 ± 0.04	0.41	<0.001	0.18
Fat concentration, %/d	3.86 ± 0.08	4.04 ± 0.08	3.92 ± 0.08	0.28	0.04	0.13
Protein concentration, %/d	3.11 ± 0.04	3.04 ± 0.04	3.10 ± 0.04	0.41	<0.001	0.02
MUN[3], mg/dl	10.35 ± 0.29	10.82 ± 0.28	10.74 ± 0.27	0.48	0.53	0.10
Log_{10}SCC, cells/mL	1.91 ± 0.07	1.84 ± 0.07	1.95 ± 0.08	0.53	0.005	0.84

[1]CONTROL: Cows receiving TMR without the methane mitigation product; ALGA BIOSCIENCE: Cows receiving TMR with Alga Biosciences Seaweed at a rate of 0.50% of DM for 21 days (High-dose period) and at a rate of 0.25% of DM for 10 days (Low-dose period); ASPAROGOPSIS TAXIFORMIS: Cows receiving TMR with *Asparagopsis taxiformis* at a rate of 0.50% of DM for 21 days (High-dose period) and at a rate of 0.25% of DM for 10 days (Low-dose period; n = 20 cows/group).

[2]ECM: energy-corrected milk; FCM: fat-corrected milk; MUN: milk urea nitrogen.

[3]Model also included the effect of baseline.

Table 5: Dry matter intake, feed efficiency and body weight LSM and SEM during treatment administration by study group for cows enrolled in the study.

Outcome	Treatment[1]			Fixed Effects *P*-value[2]		
	CONTROL	ALGA BIOSCIENCE	ASPAROGOPSIS TAXIFORMIS	Treatment	Study day	Treatment x Study day
High-dose period						
Dry matter intake, kg/d	25.95 ± 0.81[a]	20.73 ± 0.81[b]	20.16 ± 0.81[b]	<0.001	<0.001	<0.001
Feed efficiency	1.29 ± 0.03[a]	1.51 ± 0.04[b]	1.52 ± 0.04[b]	<0.001	<0.001	0.002
Body weight, kg	671.1 ± 12.6	652.0 ± 12.6	641.4 ± 12.6	0.25	-	-
Low-dose period						
Dry matter intake, kg/d[3]	26.69 ± 0.73	24.44 ± 0.72	24.66 ± 0.72	0.06	<0.001	0.43
Feed efficiency	1.22 ± 0.04	1.27 ± 0.04	1.20 ± 0.04	0.33	<0.001	0.05
Body weight, kg	661.7 ± 13.1	641.0 ± 13.1	628.0 ± 13.1	0.19	-	-

[1]Different letter superscripts within a row indicate differences at $P < 0.05$; CONTROL: Cows receiving TMR without the methane mitigation product; ALGA BIOSCIENCE: Cows receiving TMR with Alga Biosciences Seaweed at a rate of 0.50% of DM for 21 days (High-dose period) and at a rate of 0.25% of DM for 10 days (Low-dose period); ASPAROGOPSIS TAXIFORMIS: Cows receiving TMR with *Asparagopsis taxiformis* at a rate of 0.50% of DM for 21 days (High-dose period) and at a rate of 0.25% of DM for 10 days (Low-dose period; n = 20 cows/group).

[2]LSM contrasts were Bonferroni adjusted.

[3]Model excluding 7 influential observations from a control cow (0 to 1.4 kg/d of DMI).

Table 6: Enteric methane emission outcomes LSM and SEM during treatment administration by study group for cows enrolled in the study.

	Treatment[1]			Fixed Effects *P*-value		
Outcome	CONTROL	ALGA BIOSCIENCE	ASPAROGOPSIS TAXIFORMIS	Treatment	Study day	Treatment x Study day
High-dose period						
CH_4 (g/d)	345.11 ± 6.30[a]	31.42 ± 6.02[b]	38.29 ± 6.19[b]	<0.001	<0.001	<0.001
CH_4 (g/d)	347.71 ± 3.80[a]	31.33 ± 3.59[b]	39.72 ± 3.79[b]	<0.001	–	–
CH_4 yield, (g/kg DMI)	13.44 ± 0.18[a]	1.65 ± 0.17[b]	1.97 ± 0.18[b]	<0.001	<0.001	<0.001
CH_4 yield, (g/kg DMI)	13.52 ± 0.16[a]	1.64 ± 0.15[b]	2.00 ± 0.16[b]	<0.001	–	–
CH_4 intensity, (g/kg ECM)	10.33 ± 0.22[a]	1.09 ± 0.21[b]	1.34 ± 0.21[b]	<0.001	<0.001	<0.001
CH_4 intensity, (g/kg ECM)	10.44 ± 0.13[a]	1.08 ± 0.12[b]	1.37 ± 0.12[b]	<0.001	–	–
Low-dose period						
CH_4 (g/d)	388.25 ± 13.82[a]	246.75 ± 15.26[b]	242.52 ± 14.39[b]	<0.001	<0.001	<0.001
CH_4 (g/d)	394.04 ± 7.74[a]	243.20 ± 9.84[b]	233.95 ± 8.78[b]	<0.001	–	–
CH_4 yield, (g/kg DMI)	14.41 ± 0.45[a]	10.09 ± 0.53[b]	9.85 ± 0.49[b]	<0.001	<0.001	<0.001
CH_4 yield, (g/kg DMI)	14.53 ± 0.30[a]	9.83 ± 0.38[b]	9.58 ± 0.34[b]	<0.001	–	–
CH_4 intensity, (g/kg ECM)	12.03 ± 0.47[a]	7.87 ± 0.53[b]	8.54 ± 0.50[b]	<0.001	0.002	<0.001
CH_4 intensity, (g/kg ECM)	12.15 ± 0.26$_{a}$	7.50 ± 0.33[b]	8.04 ± 0.30[b]	<0.001	–	–

[1]Different letter superscripts within a row indicate LSM differences at *P* < 0.05 after Bonferroni adjustment; CONTROL: Cows receiving TMR without the methane mitigation product; ALGA BIOSCIENCE: Cows receiving TMR with Alga Biosciences Seaweed at a rate of 0.50% of DM for 21 days (High-dose period) and at a rate of 0.25% of DM for 10 days (Low-dose period); ASPAROGOPSIS TAXIFORMIS: Cows receiving TMR with *Asparagopsis taxiformis* at a rate of 0.50% of DM for 21 days (High-dose period) and at a rate of 0.25% of DM for 10 days (Low-dose period; n = 20 cows/group).

Table 7: Nutrients intake LSM and SEM during treatment administration by study group for cows enrolled in the study.

	Treatment[1]			
Outcome	CONTROL	ALGA BIOSCIENCE	ASPAROGOPSIS TAXIFORMIS	*P*-value
High-dose period[2]				
Organic matter, kg/d	23.45 ± 1.13[a]	18.16 ± 1.13[b]	18.39 ± 1.13[b]	0.002
Crude protein, kg/d	4.35 ± 0.21[a]	3.41 ± 0.21[b]	3.41 ± 0.21[b]	0.003
Crude fat, kg/d	1.34 ± 0.06[a]	1.02 ± 0.06[b]	1.03 ± 0.06[b]	<0.001
Neutral detergent fiber (NDF), kg/d	6.63 ± 0.32[a]	5.32 ± 0.32[b]	5.12 ± 0.32[b]	0.003
Acid detergent fiber (ADF), kg/d	4.02 ± 0.19[a]	3.10 ± 0.19[b]	3.13 ± 0.19[b]	0.001
Starch, kg/d	7.19 ± 0.34[a]	5.52 ± 0.34[b]	5.58 ± 0.34[b]	0.001
Ash, kg/d	1.68 ± 0.08[a]	1.33 ± 0.08[b]	1.32 ± 0.08[b]	0.003
Low-dose period[3]				
Organic matter, kg/d	25.03 ± 0.80[a]	21.91 ± 0.80[b]	21.89 ± 0.80[b]	0.009
Crude protein, kg/d	4.56 ± 0.15	4.19 ± 0.15	4.20 ± 0.15	0.13
Crude fat, kg/d	1.35 ± 0.04[a]	1.18 ± 0.04[b]	1.18 ± 0.04[b]	0.008
Neutral detergent fiber (NDF), kg/d	7.25 ± 0.23[a]	6.11 ± 0.22[b]	6.05 ± 0.22[b]	<0.001
Acid detergent fiber (ADF), kg/d	4.43 ± 0.14[a]	3.62 ± 0.13[b]	3.59 ± 0.13[b]	<0.001
Starch, kg/d	7.71 ± 0.25[a]	6.82 ± 0.24[b]	6.80 ± 0.24[b]	0.02
Ash, kg/d	1.83 ± 0.06[a]	1.60 ± 0.06[b]	1.57 ± 0.06[b]	0.005

[1]Different letter superscripts within a row indicate LSM differences at P < 0.05 after Bonferroni adjustment; CONTROL: Cows receiving TMR without the methane mitigation product; ALGA BIOSCIENCE (AB): Cows receiving TMR with Alga Biosciences Seaweed at a rate of 0.50% of DM for 21 days (High-dose period) and at a rate of 0.25% of DM for 10 days (Low-dose period); ASPAROGOPSIS TAXIFORMIS (AT): Cows receiving TMR with ShiLai™Asparagopsis taxiformis at a rate of 0.50% of DM for 21 days (High-dose period) and at a rate of 0.25% of DM for 10 days (Low-dose period; n = 20 cows/group).

[2]Number of observations included in the models by treatment group: control (n =7), AB (n = 8), and AT (n = 7).

[3]Models excluding one influential observation; control cow with a 1.3 kg dry matter intake.

Table 8: Nutrients digestibility and fecal dry mater flow LSM and SEM during treatment administration by study group for cows enrolled in the study.

Outcome	Treatment[1] CONTROL	ALGA BIOSCIENCE	ASPAROGOPSIS TAXIFORMIS	*P*-value
High-dose period[2]				
Nutrients digestibility				
Dry matter, %	69.36 ± 0.62[a]	77.71 ± 0.58[b]	70.20 ± 0.62[a]	<0.001
Organic matter, %	70.23 ± 0.61a	78.61 ± 0.57[b]	71.09 ± 0.61[a]	<0.001
Crude protein, %	68.80 ± 0.98[a]	78.45 ± 0.92[b]	70.63 ± 0.98[a]	<0.001
Neutral detergent fiber (NDF), %	38.49 ± 0.85[a]	55.00 ± 0.80[b]	36.91 ± 0.85[a]	<0.001
Acid detergent fiber (ADF), %[3]	30.85 ± 1.26[a]	45.15 ± 1.26[b]	28.51 ± 1.32[a]	<0.001
Starch, %	97.39 ± 0.41	98.66 ± 0.38	97.70 ± 0.41	0.08
Fecal DM flow, kg/d	12.34 ± 1.93[a]	23.66 ± 1.81[b]	17.70 ± 1.93[ab]	0.002
Low-dose period				
Nutrients digestibility				
Dry matter, %	70.14 ± 0.54[a]	73.64 ± 0.53[b]	72.63 ± 0.53[b]	<0.001
Organic matter, %	71.13 ± 0.56[a]	74.72 ± 0.55[b]	73.87 ± 0.55[b]	<0.001
Crude protein, %	67.56 ± 0.75[a]	72.82 ± 0.73[b]	70.93 ± 0.73[b]	<0.001
Neutral detergent fiber (NDF), %	41.75 ± 0.86[a]	45.88 ± 0.83[b]	43.80 ± 0.83[ab]	0.005
Acid detergent fiber (ADF), %[3]	33.45 ± 0.85[ab]	36.04 ± 0.88[a]	31.19 ± 0.85[b]	0.002
Starch, %	96.38 ± 0.42	97.22 ± 0.40	97.69 ± 0.40	0.08
Fecal DM flow, kg/d[4]	12.88 ± 0.69[a]	16.55 ± 0.66[b]	16.10 ± 0.66[b]	<0.001

[1]Different letter superscripts within a row indicate LSM differences at P < 0.05 after Bonferroni adjustment; CONTROL: Cows receiving TMR without the methane mitigation product; ALGA BIOSCIENCE (AB): Cows receiving TMR with Alga Biosciences Seaweed at a rate of 0.50% of DM for 21 days (High-dose period) and at a rate of 0.25% of DM for 10 days (Low-dose period); ASPAROGOPSIS TAXIFORMIS (AT): Cows receiving TMR with ShiLai™Asparagopsis taxiformis at a rate of 0.50% of DM for 21 days (High-dose period) and at a rate of 0.25% of DM for 10 days (Low-dose period; n = 20 cows/group).

[2]Number of observations included in the models by treatment group: control (n =7), AB (n = 8), and AT (n = 7).

[3]Model also included the effect of baseline.

[4]Model excluding one influential observation; control cow with a 282.6 kg/d fecal dry matter flow.

Figure 1. Supplementation periods timeline.

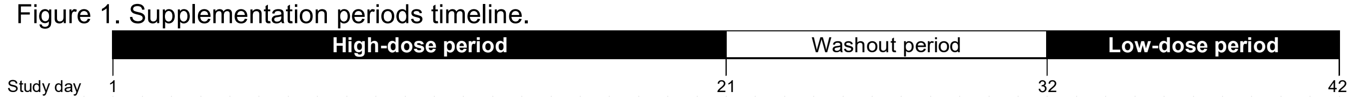


Figure 2. Milk yield LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by model described in Table 4. The middle part of the figure represents the washout period.

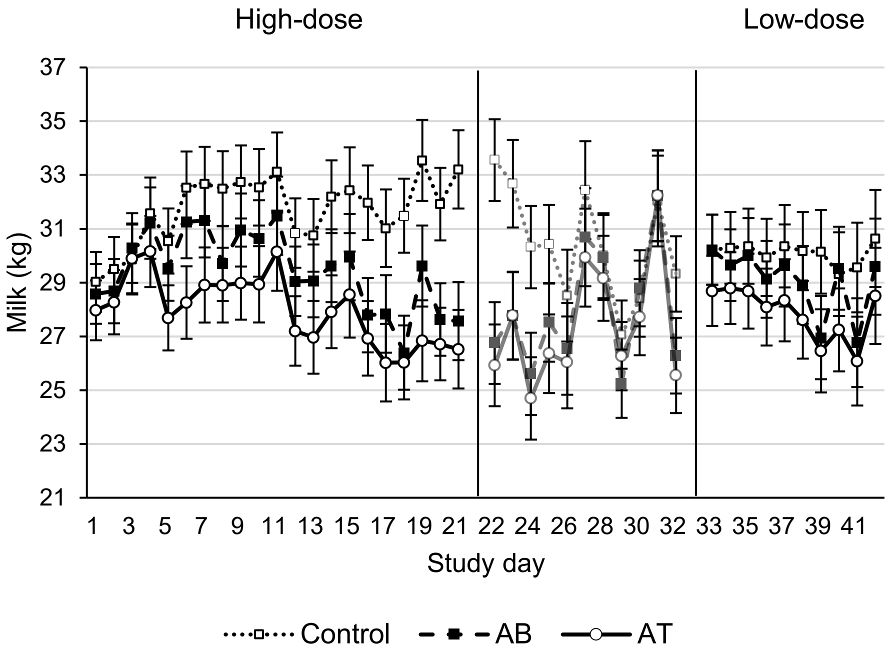


Figure 3. Energy-corrected milk yield LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by model described in Table 4. The middle part of the figure represents the washout period.

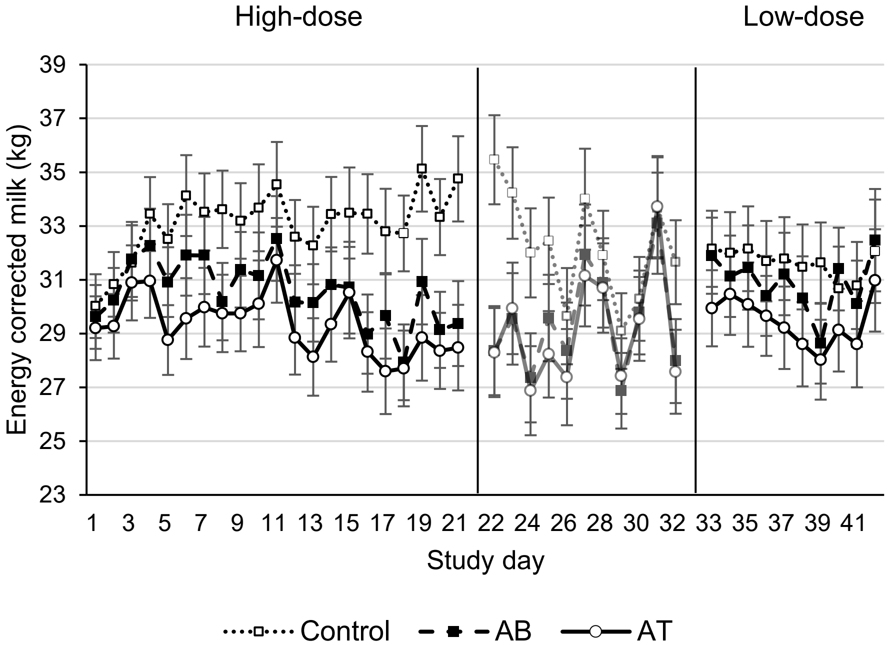


Figure 4. Fat-corrected milk yield LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by model described in Table 4. The middle part of the figure represents the washout period.

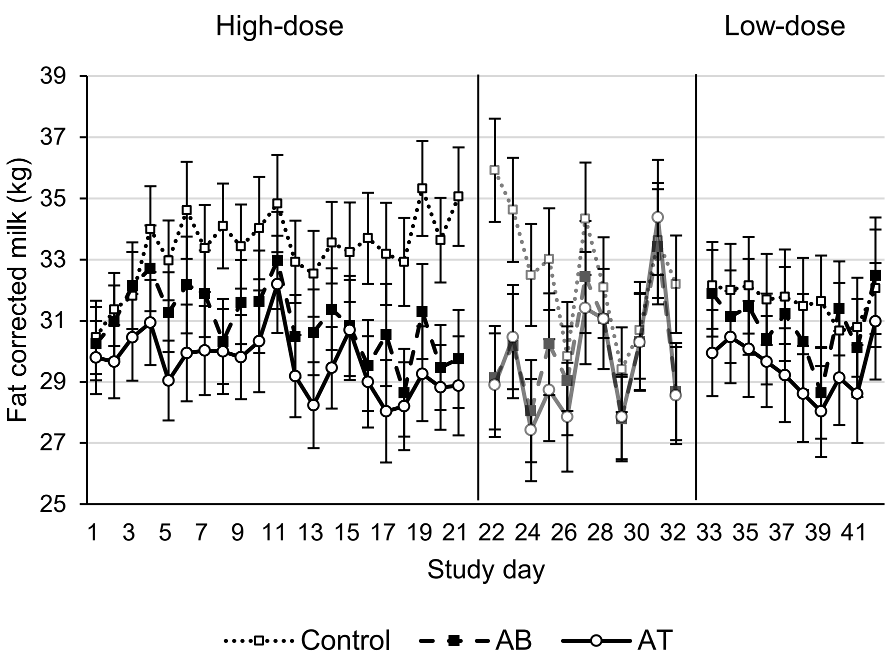


Figure 5. Fat yield LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparagopsis Taxiformis

(AT) by day on study as estimated by model described in Table 4. The middle part of the figure represents the washout period.

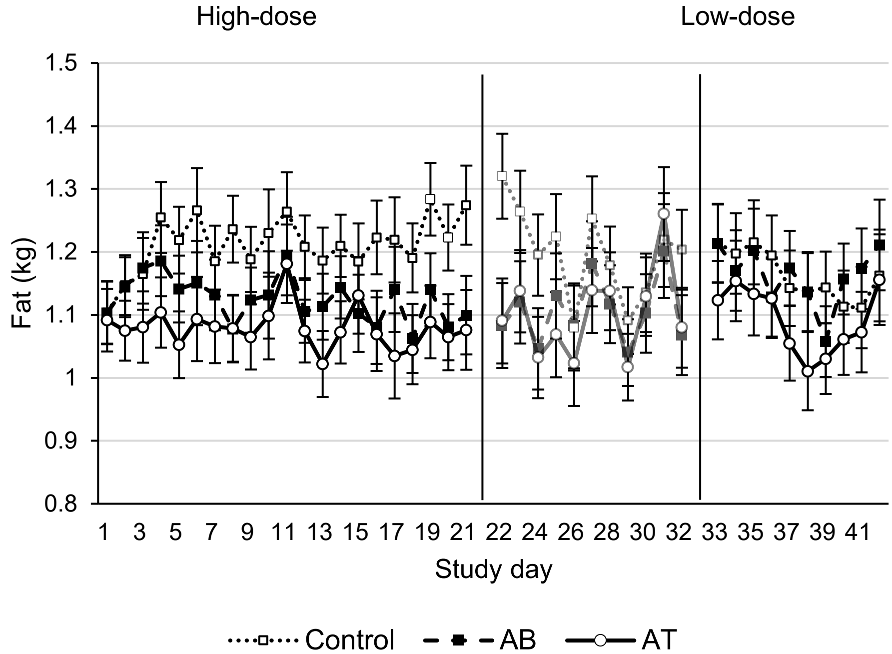


Figure 6. Protein yield LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by model described in Table 4. The middle part of the figure represents the washout period.

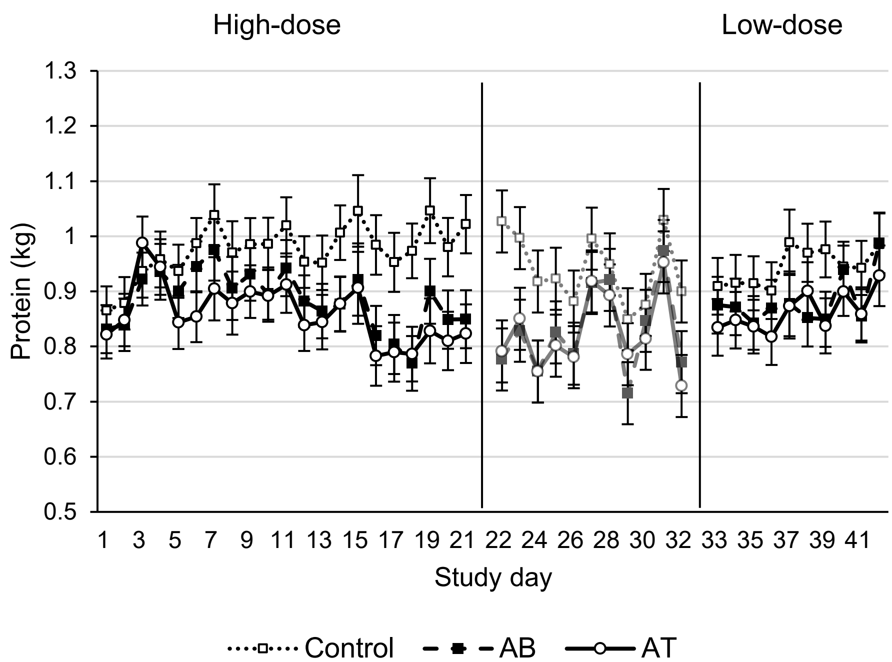


Figure 7. Fat concentration LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by model described in Table 4. The middle part of the figure represents the washout period.

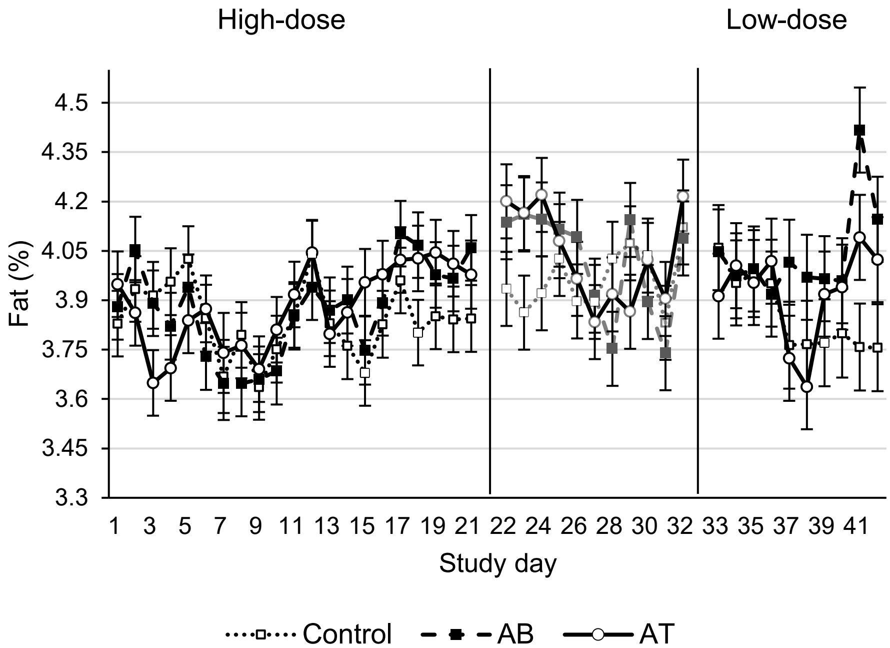


Figure 8. Protein concentration LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by model described in Table 4. The middle part of the figure represents the washout period.

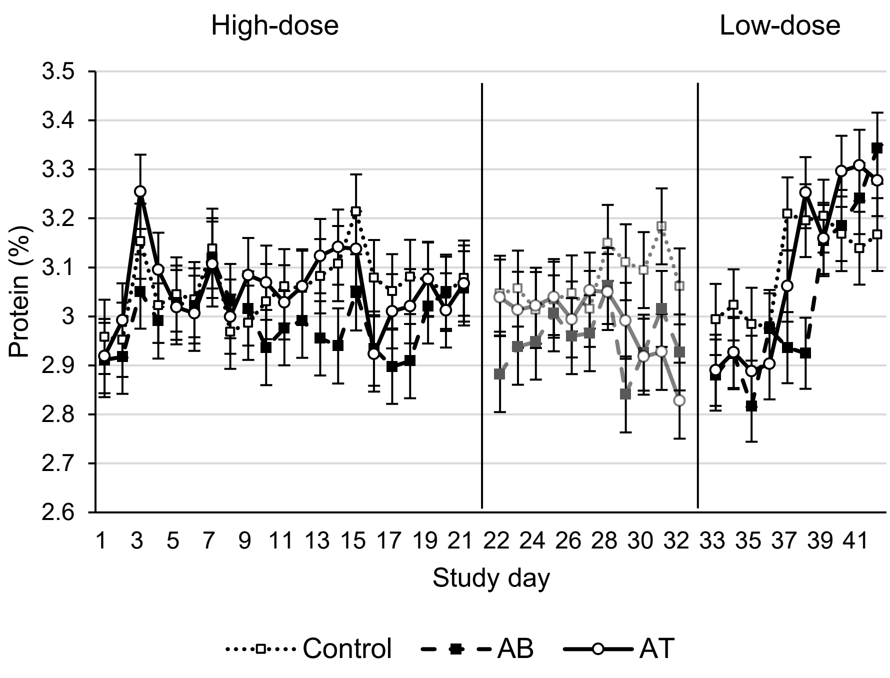


Figure 9. Milk somatic cell count LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis

Taxiformis (AT) by day on study as estimated by model described in Table 4. The middle part of the figure represents the washout period.

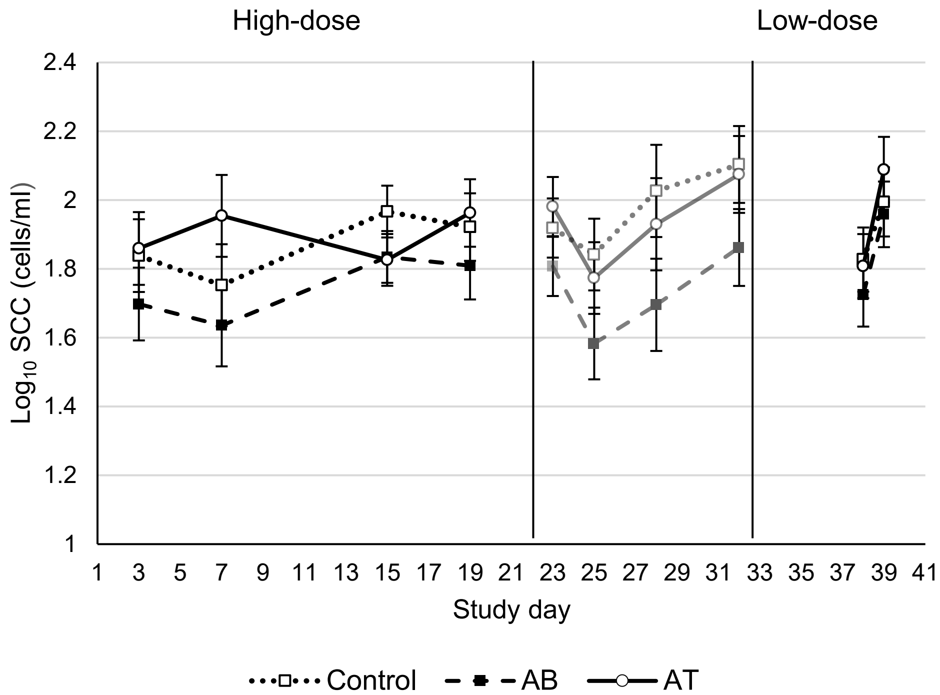


Figure 10. Milk urea nitrogen concentration LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by model described in Table 4. The middle part of the figure represents the washout period.

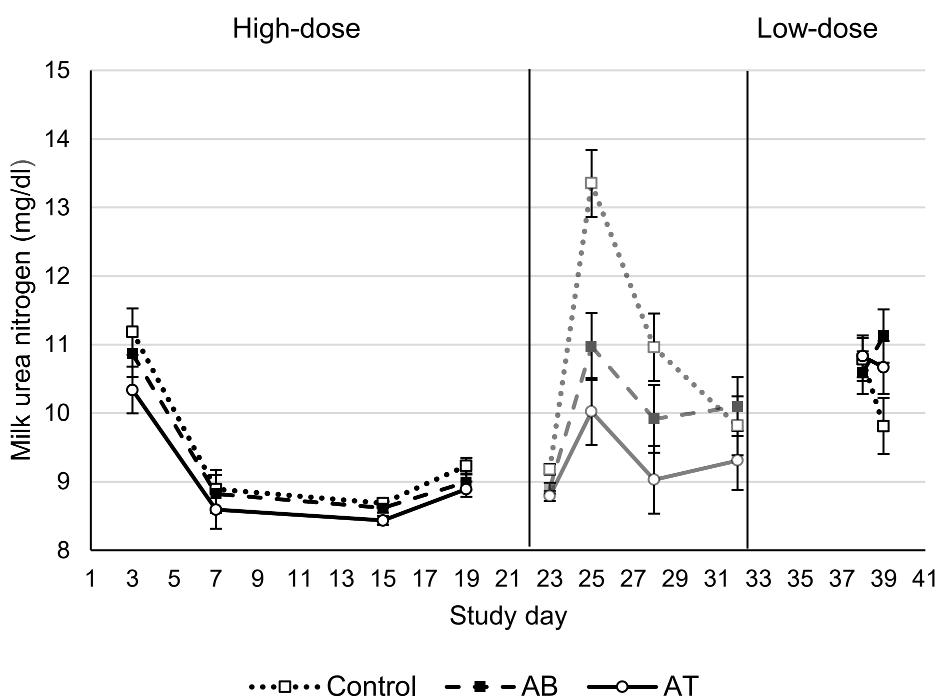


Figure 11. Dry matter intake LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by model described in Table 5. The middle part of the figure represents

the washout period.

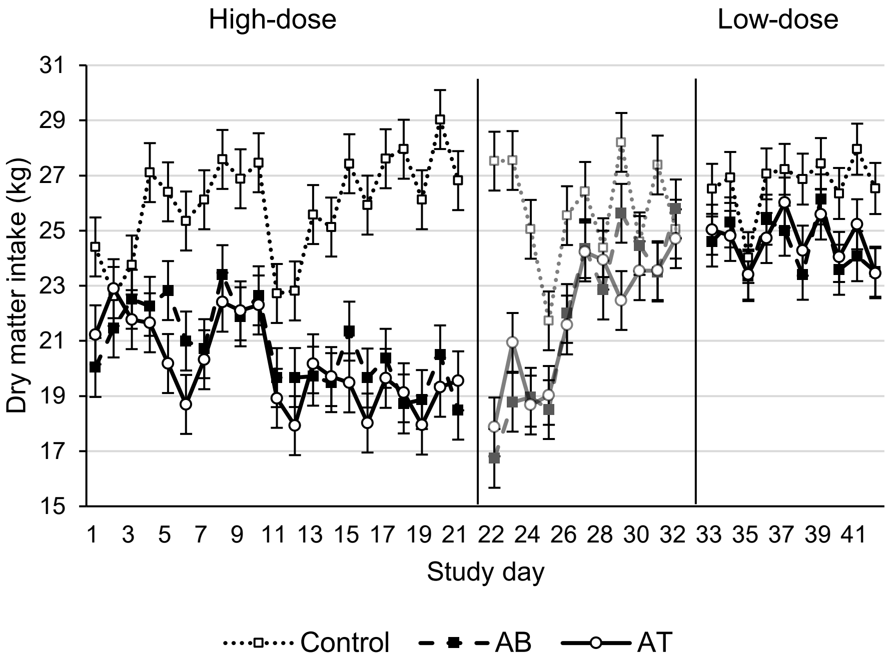


Figure 12. Feed efficiency LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by model described in Table 5. The middle part of the figure represents the washout period.

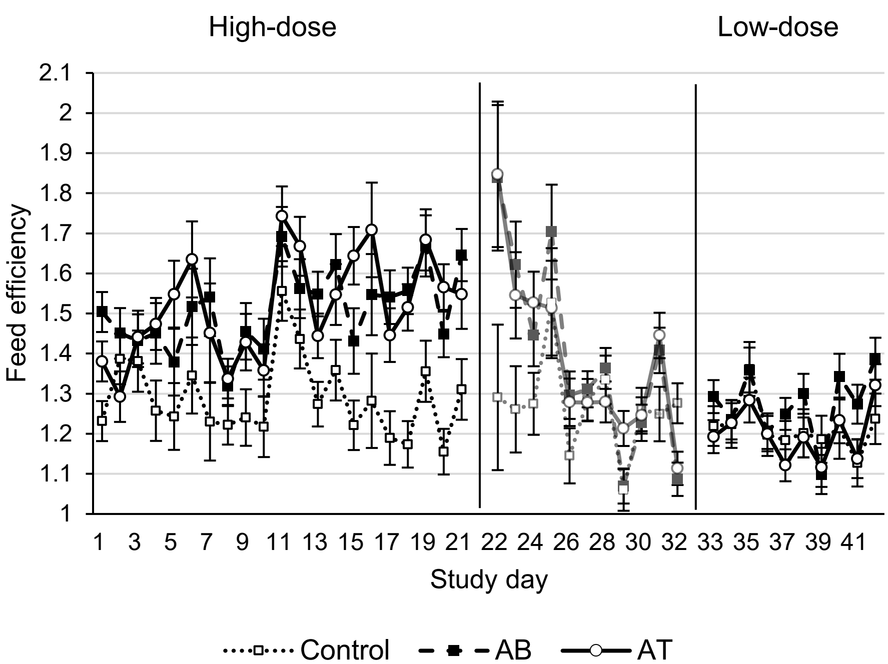


Figure 13. Methane production LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by model described in Table 6. The middle part of the figure represents the washout period.

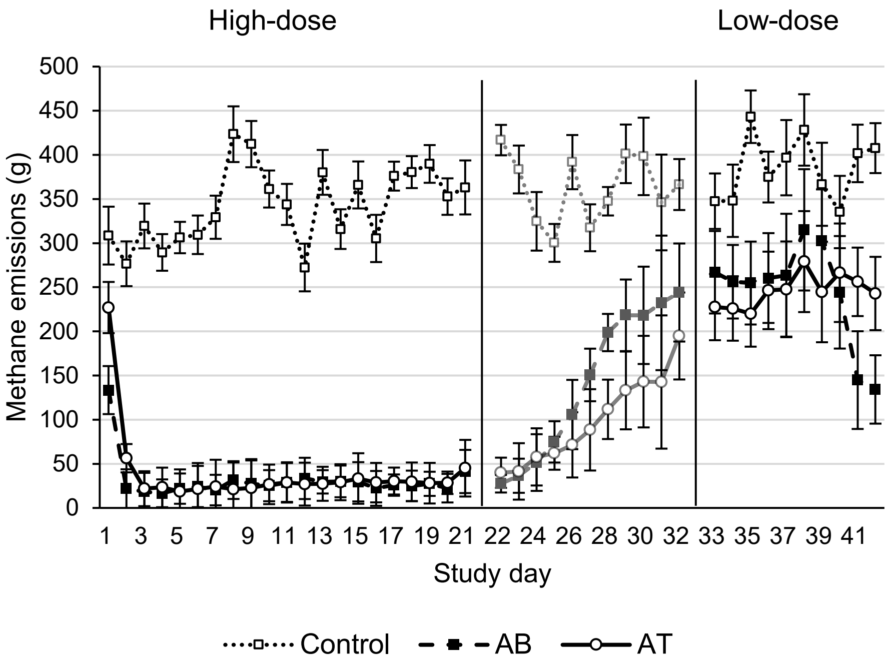


Figure 14. Methane yield LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by model described in Table 6. The middle part of the figure represents the washout period.

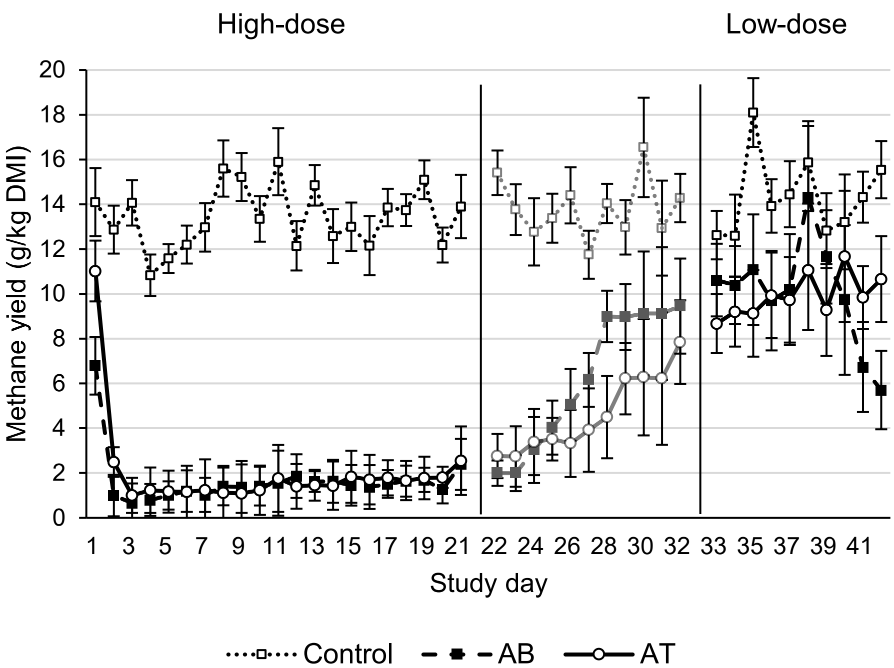
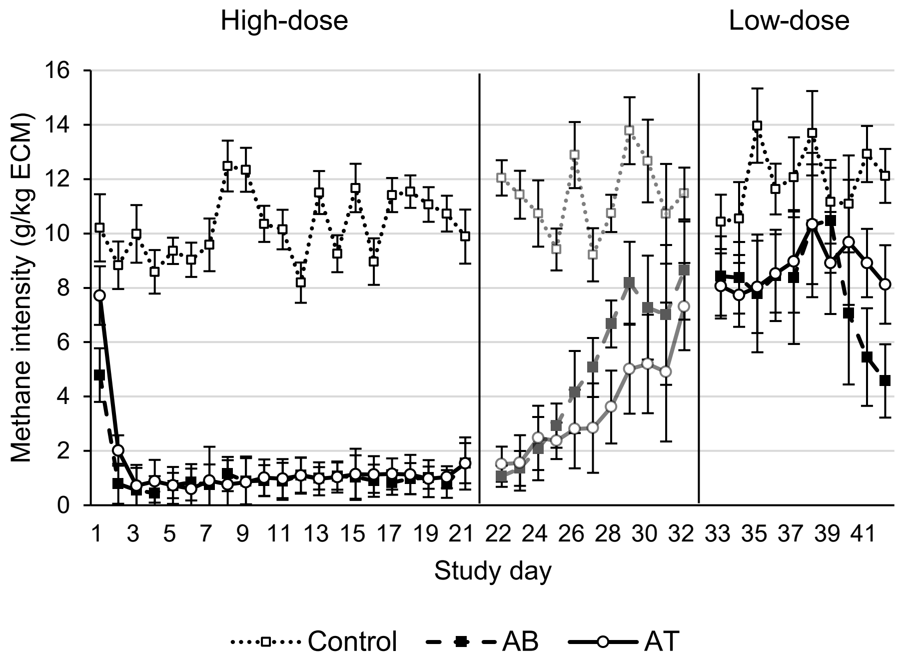


Figure 15. Methane intensity LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by models described in Table 6. The middle part of the figure represents the washout period.



High-dose
Low-dose
Methane intensity (g/kg ECM)
16
14
12
10
8
6
4
2
0
1 3 5 7 9 11 13 15 17 19 21 22 24 26 28 30 32 33 35 37 39 41
Study day
Control
AB
AT

Figure 16. Nutrients intake and fecal dry matter flow LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by models described in Table 7. The middle part of each figure represents the washout period.

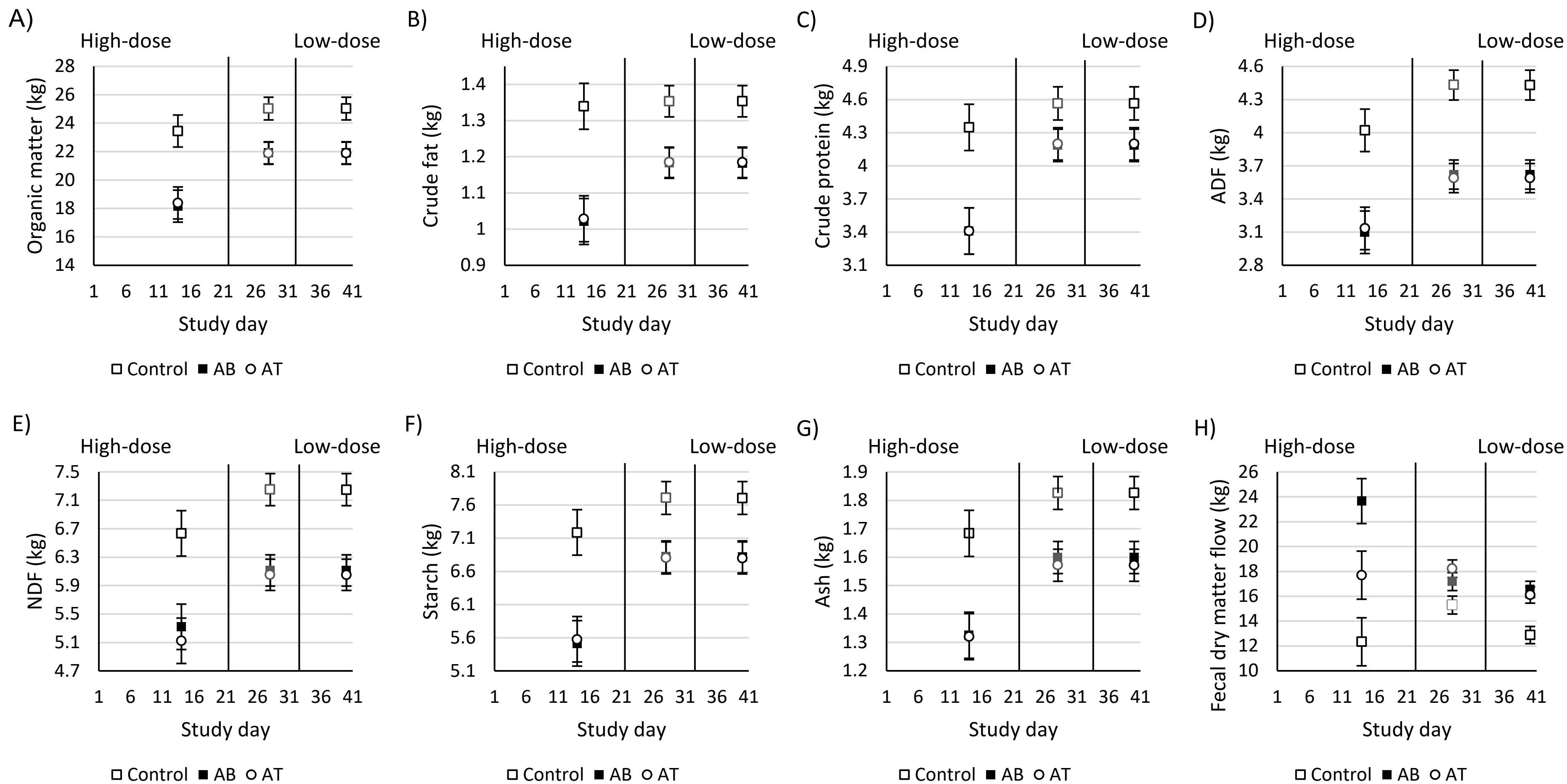


Figure 17. Nutrients digestibility LSM and SEM for cows assigned to control, Alga Bioscience (AB) and Asparogopsis Taxiformis (AT) by day on study as estimated by models described in Table 8. The middle part of each figure represents the washout period.