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Asparagopsis taxiformis supplementation to mitigate enteric methane emissions in dairy cows—Effects on performance and metabolism

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ABSTRACT

Methane emissions from ruminant digestion contribute significantly to global anthropogenic greenhouse gas emissions. Members of the phylum Rhodophyta (red algae), particularly *Asparagopsis* sp., have shown promising results in reducing methane emissions in ruminants, due to their high content of halogenated methane analog compounds. However, knowledge is lacking regarding the effects of red algae on animal performance and metabolism. This study investigated the effects of dairy cow diet supplementation with *Asparagopsis taxiformis* on enteric methane performance, metabolism of bromine and iodine, and health status of the cows. Thirty lactating Nordic Red dairy cows fed a TMR were blocked according to parity and DIM, and randomly assigned to 1 of 3 diets: a control diet with no *A. taxiformis* (CON), a diet with 0.15% *A. taxiformis* on an OM basis (L-AT), and a diet with 0.3% *A. taxiformis* on an OM basis (H-AT). The cows were fed the experimental diets continuously for 13 wk, beginning with a baseline week (wk 0), which served as covariate by week where all cows received the basal diet. Individual feed intake and milk yield were recorded automatically throughout the experiment. Milk composition was determined by collecting milk samples during each milking session on 2 consecutive days every experimental week. Enteric methane and hydrogen levels were measured continuously by the GreenFeed system. Feces grab samples were collected as spot samples from a subset of 6 cows per treatment after milking during sampling wk 0, 2, 4, 8, and 12. Urine spot samples were collected from the same subset of cows during the same weeks as fecal samples. One urine sample was taken per day on 2 consecutive days, and the samples were analyzed for wk 12. Rumen fluid was collected after morning milking using a stomach tube in wk 0, 2, 4, and 12. We observed a 30% reduction in methane production in the H-AT group, with a concomitant increase in hydrogen production by 383%. However, the interaction between treatment and week showed that the AT effect on methane reduction began to diminish by wk 9 of the experiment. In the L-AT group, methane was reduced by 7.6% and hydrogen production was increased by 70%. However, DMI was 7% lower and ECM yield was 2% lower in the H-AT group compared with the other 2 groups. Total concentration of volatile fatty acids in rumen fluid was lower in the H-AT group compared with CON, with a reduction in acetate concentration and an increase in propionate, butyrate, and valerate in the H-AT group. Bromine concentration was 5-fold higher, and iodine concentration was 9-fold higher in milk from the H-AT group compared with CON. Bromine concentration in feces and urine samples from H-AT cows was approximately 4-fold and 9-fold higher, respectively, than in samples from CON cows. Metabolic profiling revealed a reduction in cholesterol levels and a decrease in the ferric-reducing ability of plasma in the H-AT treatment group compared with CON, as well as an increase in plasma magnesium concentration in the H-AT group. In conclusion, using 0.3% *A. taxiformis* as an additive in dairy cow feed rations can mitigate enteric methane emissions, but this reduction was observed only during the first 8 wk of the experiment, with no effect on methane emissions from wk 9 to 12. Additionally, it may have negative effects on DMI and ECM yield. Further long-term studies on red algae as methane inhibitor is needed to examine its sustained inhibitory effects over time and its effect on various metabolic processes. The effects appear to decline after wk 8 and influence several metabolic mechanisms.

Key words: *Asparagopsis taxiformis*, bromine, environmental impact, iodine, seaweed supplementation

INTRODUCTION

Several strategies to reduce enteric methane (CH_4) emissions from ruminants have been examined globally.

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

Table 1. Chemical composition of dietary ingredients (g/kg DM unless otherwise stated; n = 5)[1]

Item	Grass silage (n = 6)	SD	Concentrate[2] Komplett 180	SD	Concentrate[3] Komplett 180 GF	SD	Concentrate[4] ExPro	SD
DM, g/kg	250	12.0	872	2.45	871	3.40	892	2.40
ME, MJ/kg DM	11.9	0.07	13.4	ND	13.4	ND	15.5	ND
Composition								
Ash	80.6	1.66	61.3	4.55	59.8	0.65	74.3	0.72
CP	185	4.86	187	3.63	187	4.26	343	3.05
NDF	530	10.1	218	4.32	226	20.10	293	73.28
iNDF	72.8	3.0	74.6	6.94	ND	ND	99	3.37
Starch	ND	ND	309	6.38	311	6.69	23.4	8
pH[5]	3.8	0.01	ND	ND	ND	ND	ND	ND
NH_3-N,[5] % DM	3.4	0.32	ND	ND	ND	ND	ND	ND

[1]iNDF = indigestible NDF; ND = not determined.

[2]Commercial concentrate, Komplett Norm 180 (Lantmännen), added to the TMR.

[3]Commercial concentrate, Komplett Norm 180 (Lantmännen), added to the GreenFeed (GF) system.

[4]Heat-treated rapeseed meal, added to the TMR. ExPro, AAK, Karlshamn, Sweden.

[5]Fermentation quality for silage.

The livestock sector is responsible for ~80% of annual carbon dioxide equivalent emissions related to anthropogenic CH_4, with ~90% of these emissions resulting from enteric fermentation in ruminants, particularly cattle and sheep (Reisinger et al., 2021). In this context, dietary supplementation with seaweed, particularly *Asparagopsis taxiformis* (**AT**) and *Asparagopsis armata*, has been tested as a CH_4 mitigation strategy. These species of algae are known for their high content of halogenated analog compounds, such as bromoform and di-bromochloro-methane (Paul et al., 2006), which are able to block the last step of the methanogenesis in the rumen (Machado et al., 2016).

Several short-term trials have demonstrated varying mitigation effects of *Asparagopsis* sp. on enteric CH_4 depending on dosage, seaweed storage conditions, and basal diet (Roque et al., 2019, 2021; Kinley et al., 2020; Stefenoni et al., 2021; Krizsan et al., 2023). A crossover study by Stefenoni et al. (2021) observed a reduction in enteric CH_4 emissions of 65% in vivo when adding AT to the diet of lactating dairy cows, whereas Roque et al. (2021) observed a reduction of 98% when adding AT to the diet of steers, with both studies utilizing an AT inclusion level of 0.5% of OM. However, supplementation with AT has also been shown to reduce voluntary feed intake and potentially damage the rumen wall in sheep and dairy cattle (Li et al., 2018; Muizelaar et al., 2021). Further, there is evidence of transfer of metabolites such as bromoform to milk, feces, and urine of lactating dairy cows (Muizelaar et al., 2021; Stefenoni et al., 2021). Red algae are known to accumulate iodine and bromine (Vinogradov, 1953), and the relationship between dietary intake of these elements and their presence in dairy cows shows a dose-response pattern (Stefenoni et al., 2021; Krizsan et al., 2023). Both iodine and bromine residuals play crucial roles in public health, as inadequate iodine levels can impair thyroxin synthesis in humans and animals (Leung and Braverman, 2014), whereas even small doses of bromine can affect inhibition processes in the nervous system (Saenko et al., 1978). Against this background, continuous, long-term studies are needed to acquire a comprehensive understanding of the overall effect of *Asparagopsis* sp. supplementation of ruminant diets. Most of the studies cited above use a high level of AT (0.5% of OM) in vivo. However, given the potential negative effects on metabolism and the digestive process in cows, which could influence productivity and animal health, it would be beneficial to delve deeper into the long-term effects and assess the effect of reducing the proportion of AT in the diet. The optimal dosage will be that which achieves a significant reduction in methane emissions while minimizing adverse effects. Our main aims of this study were therefore to examine the effect of long-term AT supplementation on methane emissions and production parameters in dairy cows, check for presence of bromine and iodine residues in milk, urine, and feces, and assess cow health through analysis of metabolic blood parameters. We hypothesize that long-term AT supplementation in dairy cows will lead to detectable residues of bromine and iodine in milk, urine, and feces and altered metabolic responses, indicating risk of impaired health in the cows.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

The study was conducted at the Swedish University of Agricultural Sciences, Röbäcksdalen Livestock Research Center, Umeå, Sweden (N63°45′; E20°17′) from January to April 2022. All animals were cared for according to the rules and guidelines proposed by the

Table 2. Intake and production parameters in dairy cows fed the control diet (CON, 0% AT) and diets containing 0.15% (L-AT) or 0.3% (H-AT) *Asparagopsis taxiformis* (AT) on an OM basis[1]

	Treatment				*P*-value		
Item per cow and day	CON	L-AT	H-AT	SEM	Treatment	Week	Treatment × Week
Intake							
Total DMI, kg	24.9[a]	24.7[a]	23.1[b]	0.35	<0.001	<0.001	0.09
Silage DMI, kg	12.4[a]	12.2[a]	11.4[b]	0.18	<0.001	<0.001	0.04
Concentrate DMI, kg	12.4[a]	12.3[a]	11.6[b]	0.17	<0.001	<0.001	0.23
CP, kg	4.6[a]	4.8[a]	4.3[b]	0.19	<0.001	<0.001	<0.001
Starch, kg	3.3[a]	3.4[a]	2.9[b]	0.15	0.02	<0.001	<0.001
NDF, kg	9.4[a]	9.7[a]	8.8[b]	0.38	<0.001	<0.001	<0.001
iNDF,[2] g/kg NDF	1.7	1.8	1.5	0.08	0.12	<0.01	0.05
Milk production							
Milk,[3] kg	37.1	37.6	36.2	0.43	0.48	<0.001	0.41
Milk,[4] kg	37.3[a]	37.7[a]	35.7[b]	0.50	0.03	<0.001	0.21
ECM, kg	39.2[a]	40.8[a]	38.3[b]	0.55	0.01	0.46	0.17
Fat, g/kg milk	45.0[a]	45.4[a]	42.9[b]	0.07	0.009	0.02	0.10
Protein, g/kg milk	37.2	36.9	35.9	0.07	0.64	<0.001	0.78
Lactose, g/kg milk	47.2	48.1	47.1	0.03	0.16	<0.001	0.60
ECM/DMI, kg/kg	1.60	1.63	1.64	0.50	0.13	0.52	0.13

[a,b]Different superscripts within a row indicate a significant difference ($P \leq 0.05$).

[1]Least squares means and SEM (n = 360).

[2]Analysis performed only for wk 0, 4, and 12; iNDF, indigestible NDF.

[3]Milk yield as mean for the entire experimental period.

[4]Milk yield as mean for the milk sampling weeks of the experiment.

Swedish University of Agricultural Sciences Animal Care and Use Committee and the National Animal Research Authority (Dnr: A6-2021).

We carried out a continuous study running for 13 experimental weeks, including an initial wk 0, where all cows were fed the basal diet, which served as covariate in the statistical analyses. Two weeks before the experiment, the cows were adapted to the basal diet, assigned feed bins, and introduced to the GreenFeed methane measurement system. In total, 9 primiparous and 21 multiparous Nordic Red dairy cows were recruited. At the start of the experiment, these cows were 61 ± 25.3 (mean ± SD) DIM, with average daily milk yield of 32.7 ± 8.7 (mean ± SD) kg of ECM, parity was 2.4 ± 1.36 (mean ± SD). Based on parity and DIM, we assigned the 30 animals to 10 blocks and randomly assigned each cow in the block to 1 of 3 dietary treatment groups. We randomly selected 6 of the blocks (i.e., 6 cows per treatment) as subgroups for sampling of feces, urine, blood, and rumen fluid. The 3 treatments comprised different inclusion levels of AT in the TMR: a control group with no AT (**CON**), a group with 0.15% AT on an OM basis (**L-AT**), and a group with 0.3% AT on an OM basis (**H-AT**). Accidently, the H-AT cows received a double dose of AT during the first week, and corrective measures were implemented immediately, and the correct dose was administered starting from d 1 of wk 2 of the experiment. To mitigate any effects of the initial incorrect dosage, the experimental period was extended by 4 wk (from 8 to 12).

The cows were housed in an insulated freestall barn equipped with an automatic feed intake recording system (Insentec RIC system bins, B.V., Marknesse, the Netherlands) and had free access to fresh water and salt licks. The stalls were bedded with sawdust on rubber mattresses. The cows were milked twice daily in a parlor, starting at 0500 and 1600 h. Milk yield at each milking was recorded using a gravimetric milk recorder (SAC Swing-over, S. A. Christensen and Co. Ltd., Kolding, Denmark).

The diets were composed of feeds commonly used in Swedish dairy production, fed as TMR ad libitum (Table 1). The ration was calculated according to the Nordic feed evaluation system (**NorFor**; Volden, 2011) to cover the energy and protein requirements for the cows based on the average ECM yield at the start of the experiment. The TMR had a forage:concentrate ratio of 50:50 on DM basis; 50% grass/clover silage, 47% commercial concentrate that contained 37% barley, 28% rapeseed meal, 12% corn, 7% sugar beet fiber, 5% oat husks, 4% distillers grains, 2% wheat bran, 2% molasses, 2% vegetable fat, 2% minerals and vitamins (Komplett Norm 180, Lantmännen, Malmö, Sweden), and 3% pure rapeseed meal (ExPro, AAK, Karlshamn, Sweden), plus an additional 100 g of mineral mixture (Effekt intensiv, Lantmännen, Malmö, Sweden) per cow and day. Iodine was excluded from the mineral mixture for the cows that received AT, due to high content of iodine in AT. The grass silage was harvested from a primary-growth perennial ley

Table 3. Apparent digestibility of the control diet (CON; 0% AT) and of the dairy cow diet containing 0.3% *Asparagopsis taxiformis* (AT) on an OM basis (H-AT) in experimental wk 4 and 12[1]

	Treatment			*P*-value		
Item	CON	H-AT	SEM	Treatment	Week	Treatment × Week
Digestibility, g/kg						
DM	745	734	6.1	0.08	0.63	0.08
OM	758	749	6.0	0.14	0.70	0.04
CP	732	739	8.0	0.43	0.74	0.35
NDF	713	657	20.7	0.04	0.32	0.24

[1]Least squares means and SEM (n = 24).

dominated by timothy (*Phleum pratense*), with <25% red clover (*Trifolium pratense*), and ensiled in bunker silos. The TMR mix was the same for all cows but differed in the amount of AT included. The AT supplement was added as such directly into the mixer wagon (Nolan A/S, Viborg, Denmark) through an automatic hand-made system, which consisted of a custom-designed apparatus equipped with the capability to automatically introduce different amounts of algae based on the AT inclusion level assigned to the treatment groups. In this system, AT was placed in small plastic cups, which were then positioned on an electric roller situated above the TMR mixer and linked to the automatic feeding system. When the mixer was activated, the cups were moved along the roller and emptied into the mixer. The TMR was delivered to the cows 3 times daily (at 0500, 1300, and 1800 h) by an automatic feeding wagon (Mullerup Smart Feeder M2000, Ullerslev, Denmark). The AT used was harvested at the gametophyte life cycle stage in the Azores, Portugal (N38°31′45″; W28°37′09″), during 2020 by the company SeaExpert (Feteira, Ilha Do Faial, Portugal). The algal biomass was collected as described by Stefenoni et al. (2021) and shipped by truck to the company European Freeze Dry A/S (Kirke Hyllinge, Denmark) for freeze-drying. It was then stored in hermetic plastic bags and sent frozen to the Swedish University of Agricultural Sciences. The AT bags were stored in a dark room at −20°C until the start of the experiment. During the experimental period, the AT bags were kept in a dark room at 0°C until use. To reduce the risk of variation between bags when feeding the cows, AT from 3 different bags was milled in a grain mill (Golia 4V, NOVITAL, Italy) once per day, pooled in vacuum bags, and stored at 4°C until feed mixing.

Sampling and Analyses

Diet and Feed Ingredients. Samples of silage were collected daily on 5 consecutive days per week. Samples of concentrate were collected 2 times a week. The DM content of the feed was assessed by drying 2 times a week, and feed proportions were adjusted relative to the changes in DM.

Daily silage samples were pooled into 1 sample for a 14-d period and then dried at 60°C for 48 h and milled to 1-mm size for chemical composition analysis. Additional samples from wk 0, 4, and 12 were milled to 2-mm size, using a cutter mill (SM 300, Retsch GmbH, Haan, Germany), for determination of indigestible NDF (**iNDF**). Separate samples of fresh silage were stored at −20°C for analysis of fermentation quality. Concentrate samples from the silos and the GreenFeed unit were pooled to 1 sample over a 14-d period and stored at −20°C until further analysis.

Chemical analyses were performed at the laboratory at the Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, Umeå, Sweden. Silage DM content was determined following the method described by Åkerlind et al. (2011), whereas concentrate DM was determined through overnight drying at 103°C (EC No. 152/2009). Starch content was analyzed according to Larsson and Bengtsson (1983). Ash content in all feeds was evaluated by ignition at 550°C for 3 h (EC No. 152/2009), whereas CP was analyzed for nitrogen (N) in an automated Kjeldahl procedure (Kjeltec 8400 Analyser unit and 8460 sampler unit, Foss, Hillerød, Denmark). Feed NDF content was analyzed following the procedure of Chai and Udén (1998), after applying a heat-stable amylase and excluding residual ash (see Danielsson et al., 2017, for details). Organic matter digestibility in vitro was determined following Bertilsson and Murphy (2003) and Volden (2011), from which ME in silage was calculated according to Lindgren et al. (1983). The ME content in concentrate was calculated based on feed table values from the Swedish Board of Agriculture (SJVFS, 2011).

In silage, pH and ammonia-N (NH_3-N) were evaluated. Analysis of ammonia-N was carried out using a flow injection analyzer technique (FIA star 5010 Analyzer, 5017 samples and 5032 controller, Tecator; Broderick and Kang, 1980). The concentrations of iNDF in silage, in concentrate, and in feces from 6 cows in CON and 6

Table 4. Methane (CH_4) and hydrogen (H_2) production during the entire experimental period in dairy cows fed the control diet (CON, 0% AT) and diets containing 0.15% (L-AT) or 0.3% (H-AT) *Asparagopsis taxiformis* (AT) on an OM basis[1]

	Treatment				*P*-value		
Per cow	CON	L-AT	H-AT	SEM	Treatment	Week	Treatment × Week
CH_4, g/d	433[a]	401[a]	281[b]	15.5	<0.001	<0.001	<0.001
CH_4, g/kg DMI	17.4[a]	16.4[a]	11.5[b]	0.70	<0.001	<0.001	<0.001
CH_4, g/kg milk	11.7[a]	10.7[a]	7.2[b]	0.40	<0.001	<0.001	<0.001
CH_4, g/kg ECM	10.9[a]	9.6[a]	6.6[b]	0.37	<0.001	<0.001	0.0006
H_2, g/d	1.2[b]	2.5[b]	5.8[a]	0.32	<0.001	<0.001	<0.001

[a,b]Different superscripts within a row indicate a significant difference ($P \leq 0.05$).

[1]Least squares means and SEM (n = 360).

cows in H-AT were determined in situ as described by Krizsan et al. (2015).

Five grams (approximately) of pooled AT was sampled weekly and stored at −20°C until analysis. The bromine content and iodine content in pooled AT were analyzed at Eurofins Food and Feed Testing, Lidköping, Sweden (DS EN 15111:2007), whereas the bromoform content was analyzed at Scantox, Mölndal, Sweden (Method/Ref 1635; BioventureHub, Mölndal, Sweden).

Gas Measurements. Enteric emissions of CH_4 and hydrogen (H_2) were recorded during the whole experiment by a GreenFeed emission monitoring system (C-Lock Inc., Rapid City, SD), as described by Huhtanen et al. (2015). Gas calibration (N_2 and mixture of CH_4, O_2, and CO_2) was performed following the procedures described in Krizsan et al. (2023). The cows were fed a commercial concentrate (Komplett Norm 180, Lantmännen, Malmö, Sweden) in the GreenFeed. The average number of drops per day was 19.8 ± 7.5 (mean ± SD) for the CON group, 21.4 ± 6.8 (mean ± SD) for L-AT, and 24.4 ± 3.7 (mean ± SD) for H-AT. The average number of visits per day was 8 ± 1.3 (mean ± SD) for the CON group, 8 ± 2.2 (mean ± SD) for L-AT, and 9 ± 1.3 (mean ± SD) for H-AT, during the experimental period.

Rumen Fluid. Rumen fluid was collected from the same subset of cows during wk 0, 2, 4, and 12. Collection was performed after morning milking using a stomach tube (Ruminator) as described by Geishauser and Gitzel (1996). To minimize saliva contamination, the first portion of rumen fluid (~500 mL) was discarded. Then ~500 mL was collected, filtered through 2-layer cheesecloth, and aliquots were transferred to one 50-mL Falcon tube (Falcon Conical Centrifuge Tube, Corning Inc., Corning, NY) and four 2-mL SafeSeal microtubes (Sarstedt Inc., Nümbrecht, Germany). All samples were stored at −20°C until analysis of VFA at the Department of Molecular Sciences SLU, Uppsala, Sweden. Rumen fluid samples for VFA analysis were prepared by pipetting 700 µL of sample into a microcentrifuge tube, adding 70 µL of H_2SO_4 (5 *M*), mixing, and centrifuging at 14,000 × *g* for 15 min at 20°C (room temperature). The supernatant was then filtered through a 0.2-µm syringe filter into an HPLC glass vial. Volatile fatty acids were analyzed by HPLC (Shimadzu 2050 Series HPLC, Shimadzu Corporation, Kyoto, Japan), where samples were separated on an ion exclusion column (Rezex ROA-Organic Acid H^+, 300 × 7.80 mm, Phenomenex) and detected by a UV detector at wavelength 210 nm. The mobile phase used was 5 n*M* H_2SO_4 with a flow rate of 0.6 mL/min.

Milk Production and Composition. Milk samples were collected from all 30 cows at each milking on 2 consecutive days biweekly, and stored in small cups with preservative (bronopol) at 4°C until analysis. The samples were sent to the laboratory at the Department of Applied Animal Science and Welfare (SLU, Uppsala, Sweden) for analysis of fat, protein, and lactose, which was performed using infrared spectroscopy (MilkoScan FT120, Foss, Hillerød, Denmark). Additional milk samples were collected during wk 0, 4, and 12 for analysis of iodine and bromine. These samples were pooled by cow and week and stored at −20°C until further analysis at Eurofins Food and Feed Testing, Lidköping, Sweden (DS EN 15111m: 2007).

Feces and Urine. Grab samples of feces (~300 g) were collected as spot samples (Mehtiö et al., 2016) from the subset of 6 cows per treatment in the parlor immediately after milking at 0500 and 1600 h on 2 consecutive days in sampling wk 0, 2, 4, 8, and 12. Feces samples were stored at −20°C, pooled by cow and week to obtain representative samples, dried in a forced-air oven at 60°C for 48 to 72 h, and ground using a mortar to pass through a 2.5-mm sieve. For iNDF analysis, samples from wk 0, 4, and 12 were analyzed at the Department of Applied Animal Science and Welfare (SLU, Umeå, Sweden). Additionally, small fresh (before drying) subsamples of feces collected during wk 12 were stored at −20°C for bromine analysis on the same subsets of 6 cows per treatment. Urine was sampled by stimulating the area below the vulva on the same subset of cows, with 1 sample per day taken on 2 consecutive days in wk 0, 2, 4, 8, and 12, collected in the morning in the freestalls in the barn. Urine samples were collected in plastic cups, filtered into

Table 5. Effect of the control diet (CON, 0% AT) and the diet containing 0.3% (H-AT) *Asparagopsis taxiformis* (AT) on an OM basis on VFA concentration in rumen fluid from lactating dairy cows in experimental wk 2, 4, and 12[1]

	Treatment			*P*-value		
Item	CON	H-AT	SEM	Treatment	Week	Treatment × Week
Total VFA, mmol/L	110	92.8	6.62	0.01	0.43	0.22
Molar proportion, mmol/100 mol						
Acetate	60.3	55.4	1.21	<0.001	<0.001	<0.001
Propionate	23.5	24.3	1.15	0.73	<0.001	<0.001
Butyrate	12.2	13.0	0.60	<0.01	<0.01	<0.01
Isobutyrate	1.8	1.7	0.30	0.99	0.37	0.17
Valerate	1.7	3.3	0.62	<0.01	0.09	0.01
Isovalerate	0.9	1.1	1.17	0.38	0.12	<0.01
Acetate/propionate, mmol/L	2.6	2.5	0.16	0.63	<0.01	<0.01

[1]Least squares means and SEM (n = 54).

a funnel with 2-mm sieve, and pooled within cow and week. The pooled urine samples were stored at −20°C. The urine samples from the CON and H-AT groups collected at wk 12 were subsequently analyzed for bromine concentration at Eurofins Food and Feed Testing, Lidköping, Sweden (DS EN 15111m: 2007).

Metabolic Profile Assessment. Blood samples were collected during morning milking from the same 6 cows/treatment, during wk 0, 4, and 12 of the experiment. Sampling was performed using evacuated tubes (13 × 75 mm BD Vacutainer, NJ) containing lithium heparin as an anticoagulant. The tubes were centrifuged for 15 min at 3,500 × *g* at 4°C (centrifuge model 48 R Rotina Hettich, Germany). Plasma was then divided into 3 aliquots and stored in 2-mL Eppendorf tubes (Eppendorf AG, Hamburg, Germany) at −20°C until further analysis at the Department of Animal Sciences, Food and Nutrition, Faculty of Agriculture, Food and Environmental Science, Universitá Cattolica Del Sacro Cuore, Piacenza, Italy. The concentrations of glucose, urea, zinc, aspartate aminotransferase (**AST**), gamma-glutamyl transferase (**GGT**), total protein, haptoglobin, ceruloplasmin, albumin, cholesterol, and globulin were determined by a clinical auto-analyzer (ILAB-650, Instrumentation Laboratory, Lexington, MA). Serum amyloid α (**SAA**) were determined by a commercial kit (SAA TP-802, Tridelta D.L., Ireland) and reactive oxygen metabolites (**ROMt**), paraoxonase, according to Mezzetti et al. (2019), whereas the ferric-reducing ability of plasma (**FRAP**) was determined as described by Premi et al. (2021).

Calculations and Considerations

Concentrations of milk constituents were calculated as the weighted mean of morning and afternoon milk yields. Daily ECM yield was calculated according to Sjaunja et al. (1990). Feed efficiency was determined as the ratio of ECM yield to DMI. Dietary chemical components and feeding values were estimated based on ingredient proportions and their corresponding values.

Total-tract apparent DM digestibility and OM, CP, and NDF digestibility were calculated with equations taken from Guinguina et al. (2021), using iNDF as an internal marker in both feeds and feces. Daily fecal DM output was calculated using NorFor (Volden, 2011), with iNDF used as an internal marker for these calculations. Daily nutrient excretion in feces, including OM, NDF, and CP, was determined by multiplying fecal nutrient concentration by daily fecal DM output.

Because CH_4 emissions did not differ between the CON and L-AT groups, analyses on urine, feces, blood, and rumen fluid were only performed on samples from the CON and H-AT subgroups of cows to save financial resources.

Statistical Analysis

Statistical analyses were conducted using the MIXED procedure in RStudio Team (4.2.1, RStudio: Integrated Development for R, RStudio, PBC, Boston, MA). The analyses involved estimating treatment effects on feed intake, milk yield, CH_4 and H_2 production, and digestibility. The statistical model included fixed effects of treatment and experimental week, and random effect of block. Autoregressive correlation structure of order AR (1) was applied to account for the temporal autocorrelation within each cow, ensuring that the correlation between measurements decreased as the time interval between them increased. Week was considered as repeated measurement. During analysis, nonsignificant parameters were removed. The model used was

$$Y_{ijkl} = \mu + T_i + B_j + W_k + P_l + (TW)_{ik} + \varepsilon_{ijkl}, \quad [1]$$

where Y_{ijkl} is the dependent variable, μ is the mean of all observations, T_i is the effect of treatment, B_j is the effect

of block, W_k is the effect of week, P_l is the pretreatment week (0, which was used as a covariate), $(TW)_{ik}$ is the interaction between treatment and week, and ε_{ijkl} is the random residual error.

A slightly different statistical model, without the covariate, was used for milk composition, bromine and iodine content in the milk, metabolic profile, ECM, and feed efficiency parameters

$$Y_{ijk} = \mu + T_i + B_j + W_k + (TW)_{ik} + \varepsilon_{ijk}, \quad [2]$$

where the variables have the same meanings as in Equation [1].

To calculate LSM, the LSMEANS/DIFF option was used. Statistical differences between treatments were determined using Tukey adjustment with a significance level of $P < 0.05$. Denominator df were obtained by the Kenward-Roger method. Welch 2-sample *t*-test was used to test the null hypothesis (H_0) that the mean bromine concentration in both feces and urine from CON and H-AT cows did not differ. The equation was as follows:

$$H_0: \overline{x_1} = \overline{x_2};\ H_a: \overline{x_1} \neq \overline{x_2}, \quad [3]$$

$$t = \frac{\overline{x}_1 - \overline{x}_2}{\sqrt{\frac{s_1^2}{n^1} + \frac{s_2^2}{n^2}}},$$

where $\overline{x}_1$ and $\overline{x}_2$ are the sample means of group 1 and 2, s_1^2 and s_2^2 are sample variance of the 2 groups, and n^1 and n^2 are sample sizes of the 2 groups.

RESULTS

DMI and Digestibility

The grass silage was of high nutritional and hygienic quality (Table 1). The cows in the H-AT group had lower daily total DMI than cows in the other 2 groups ($P < 0.01$; Table 2). The NDF digestibility was reduced by 8% in the H-AT group compared with CON. Overall, there were no effect of treatment on OM digestibility, but an interaction between treatment and week was shown ($P = 0.04$; Table 3) in wk 12, with lower OM digestibility in the H-AT group compared with CON ($P = 0.03$), with estimated means of 744 and 762 g/kg, respectively.

Gas Emissions and Rumen Fermentation

The results revealed a reduction of CH_4 production gram per day in the H-AT group compared with the other 2 groups ($P < 0.01$). Additionally, CH_4 yield, measured as gram per kilogram of DMI, and emission intensity per kilogram milk and ECM were lower in the H-AT group compared with the other groups (Table 4). Significantly, during the initial week of the experiment, the H-AT group showed a substantial reduction in CH_4 emission compared with the other groups, indicating a treatment × week interaction ($P < 0.001$), as illustrated in Figure 2. This reduction initially diminished but then stabilized into a more consistent trend by wk 6. However, no differences in methane emissions were observed from wk 9 to 12. The CH_4 mitigation effect caused by AT was reinforced by a concomitant increase in H_2 production. Indeed, cows in the H-AT group showed the highest H_2 production, which was greater compared with both CON and L-AT ($P < 0.01$) and a treatment × week interaction, due to the higher inclusion rate of AT the first week, as shown in Table 4.

Cows in the H-AT treatment demonstrated lower total VFA concentration in rumen fluid compared with CON cows ($P = 0.05$; Table 5). Additionally, the concentration of acetate was lower in the H-AT group compared with CON ($P < 0.0001$), whereas the concentrations of butyrate and valerate were significantly higher in H-AT compared with CON ($P < 0.01$). Additionally, there was a treatment-by-week interaction effect for propionate, with the H-AT group showing a higher concentration in wk 2 compared with CON ($P < 0.05$), but no significant differences were observed between treatments in wk 4 or 12.

Milk Yield, Milk Composition, and Feed Efficiency

Overall, milk yield recorded over the whole study period did not differ between the treatment groups. Milk yield in the sampling weeks for milk composition was lower in the H-AT group than in the other groups ($P = 0.03$). Additionally, the H-AT group showed lower ECM yield and milk fat concentration compared with the other groups ($P = 0.01$). Our results showed no differences between the treatment groups in terms of feed efficiency during the sampling week (Table 2).

Bromine, Iodine, and Bromoform Content

The bromine concentration in the AT material added to the TMR was 59 ± 5.9 g/kg (mean ± SD), the iodine concentration was 5.9 ± 0.45 mg/kg, and the bromoform concentration was 6.44 ± 0.55 mg/kg. Throughout the experiment, the bromoform content in pooled AT samples was measured, showing a reduction over time by 23% (Figure 1). Figure 2 shows (A) enteric methane (CH_4) and (B) hydrogen (H_2) production (g/kg DMI) in cows fed the control diet (CON, 0% AT on an OM basis), L-AT (0.15% AT on an OM basis), and H-AT (0.3% AT on an OM basis).

Table 6. Bromine and iodine concentrations in milk from cows fed the control diet (CON, 0% AT) and the diet containing 0.3% AT (H-AT) on an OM basis during experimental wk 4 and 12[1]

	Treatment			*P*-value		
Item	CON	H-AT	SEM	Treatment	Week	Treatment × Week
Bromine, mg/kg milk	3.9	20.8	1.51	<0.001	<0.001	<0.001
Iodine, mg/kg milk	0.1	0.9	0.83	<0.001	<0.001	<0.001

[1]Least squares means and SEM (n = 24).

In milk, both bromine and iodine were higher in samples from the H-AT group compared with CON (Table 6). The concentrations of bromine and iodine in the milk were determined on 3 occasions (wk 0, 4, and 12) and showed an increase with time and an interaction between treatment and week ($P < 0.001$; Figure 3). A decrease in the concentrations of both bromine (5.2%) and bromoform (16.9%) was observed in the AT from wk 4 to wk 12. Furthermore, the concentration of bromine in milk during this same period showed a 25.2% reduction. The mean bromine concentration in feces samples during the final week of the experiment was 28.5 ± 3.01 mg/kg, whereas that in urine samples was 29.7 ± 5.00 mg/kg. The corresponding values for the CON group were 6.1 ± 0.67 mg/kg in feces and 3.3 ± 0.58 mg/kg in urine. The *t*-tests results revealed differences between the 2 groups for both feces (t [5.5] = −7.24, $P < 0.001$) and urine (t [5.1] = −5.17, $P < 0.01$; Figure 4).

Metabolic Profile

The concentrations of plasma parameters used as biomarkers of energy, protein, mineral metabolism, antioxidants, and inflammation are shown in Table 7. Inclusion of AT resulted in lower cholesterol levels and FRAP levels in plasma from H-AT cows compared with CON cows ($P < 0.001$). Magnesium concentration was slightly higher in plasma samples from the H-AT group compared with CON (mean ± SEM: 1.02 ± 0.02 mmol/L vs. 0.97 ± 0.02 mmol/L, respectively; $P = 0.03$).

DISCUSSION

Our study sought to address knowledge gaps regarding the potential physiological side effects and long-term effects of supplementing dairy cow diets with AT to mitigate enteric CH_4 emissions. We confirmed that AT supplementation effectively reduces enteric CH_4 production in ruminants, consistent with previous findings (Roque et al., 2021; Stefenoni et al., 2021; Krizsan et al., 2023). However, our results also showed that the efficacy of AT diminished over time, likely due to a reduction in bromoform content in AT, similar to what was observed in Stefenoni et al. (2021). By extending the experimental period to 13 wk and conducting a thorough evaluation of animal productivity and potential residues in biological fluids, our research offers novel insights into the long-term efficacy of AT in reducing methane emissions, as well as the metabolism of bromoform and its residue excretion.

DMI and Digestibility

In our study, an inclusion level of the L-AT group did not affect DMI compared with CON, whereas an inclusion level of H-AT decreased DMI by 7% compared with CON. Similarly, Eikanger et al. (2024) demonstrated in a 5-wk continuous experiment that an inclusion level of 0.25% AT on an OM basis resulted in a 16.2% reduction in DMI compared with the control group and a 12.7%

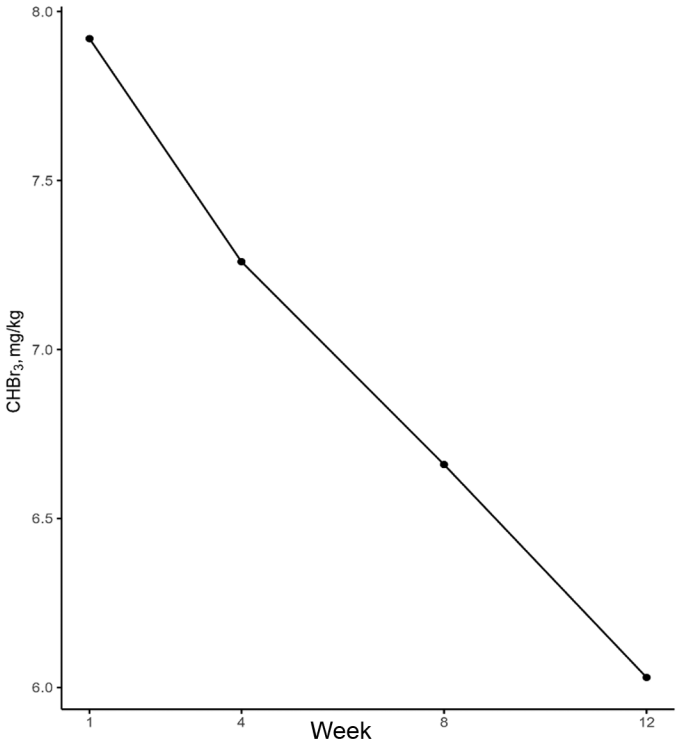

Figure 1. Concentration of bromoform ($CHBr_3$) in *Asparagopsis taxiformis* (AT) pooled samples collected at wk 1, 4, 8, and 12 (n = 4).

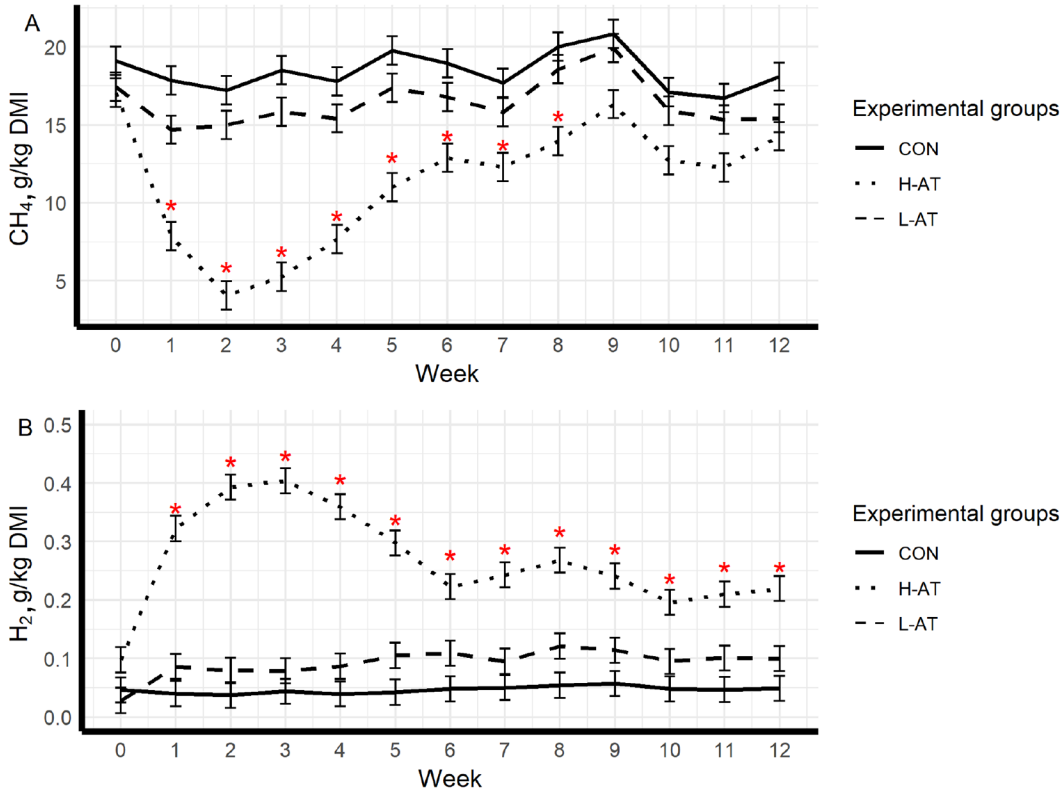

Figure 2. (A) Enteric methane (CH_4) production and (B) enteric hydrogen (H_2) production (both g/kg DMI) in cows fed the control diet (CON, 0% AT on OM basis), low additive treatment diet (0.15% AT on an OM basis; L-AT), and high additive treatment diet (0.3% AT on an OM basis; H-AT). Least squares means per treatment group and week are shown, with error bars representing the SEM. Each data point represents observations from 10 cows per treatment over 12 wk (n = 360). Statistical significance ($P < 0.001$) of the difference between CON and H-AT is indicated with an asterisk (*).

reduction compared with an inclusion level of 0.12% AT on an OM basis. Additionally, Krizsan et al. (2023) observed in a short-term changeover trial, a decline in DMI in cows fed a diet supplemented with 0.5% AT on an OM basis, with a reduction of 13.8% compared with the control group.

Despite a high SEM (20.7), our data clearly demonstrate a reduction in NDF digestibility by 8% in the H-AT group compared with CON. This finding contradicts previous short-term observations reported by Stefenoni et al. (2021) and Krizsan et al. (2023).The extended duration of our study suggests that additional factors or interactions may contribute to the observed differences in NDF digestibility between the CON and H-AT groups. This underscores the importance of considering study duration and potential time-dependent effects in such experiments.

Gas Emissions and Rumen Fermentation

Methane yield was 30% lower for the H-AT group compared with CON (Table 4). A similar trend was observed by Stefenoni et al. (2021) when cows were fed AT at an inclusion level of 0.5% (OM basis), compared with 0.3% in our study. When expressed as gram of CH_4 per kilogram of DMI, the CON and L-AT groups showed similar levels of emissions over time, whereas H-AT showed a marked decrease in CH_4 emissions in wk 1 and then a gradual increase over time until the level stabilized at around 30% lower emissions compared with CON and L-AT from wk 6. However, no mitigating effect of AT was observed from wk 9. The rapid decrease in CH_4 production in H-AT cows in wk 1 was caused by the accidently higher (double) dose of AT than originally intended. Corrective measures were taken instantly, and the correct dose was distributed from d 1 in wk 2 of the experiment. To offset any effects of the incorrect dosage, the experimental period was extended by 4 weeks, from a planned 8 wk to 12 wk, to ensure time for the rumen microbiota to re-establish. Methane yield (g of CH_4/kg of DMI) was 34% lower in the H-AT group compared with CON, similar to what was observed in other studies (Roque et al., 2021; Stefenoni et al., 2021; Krizsan et al., 2023). We observed a decline in CH_4 in-

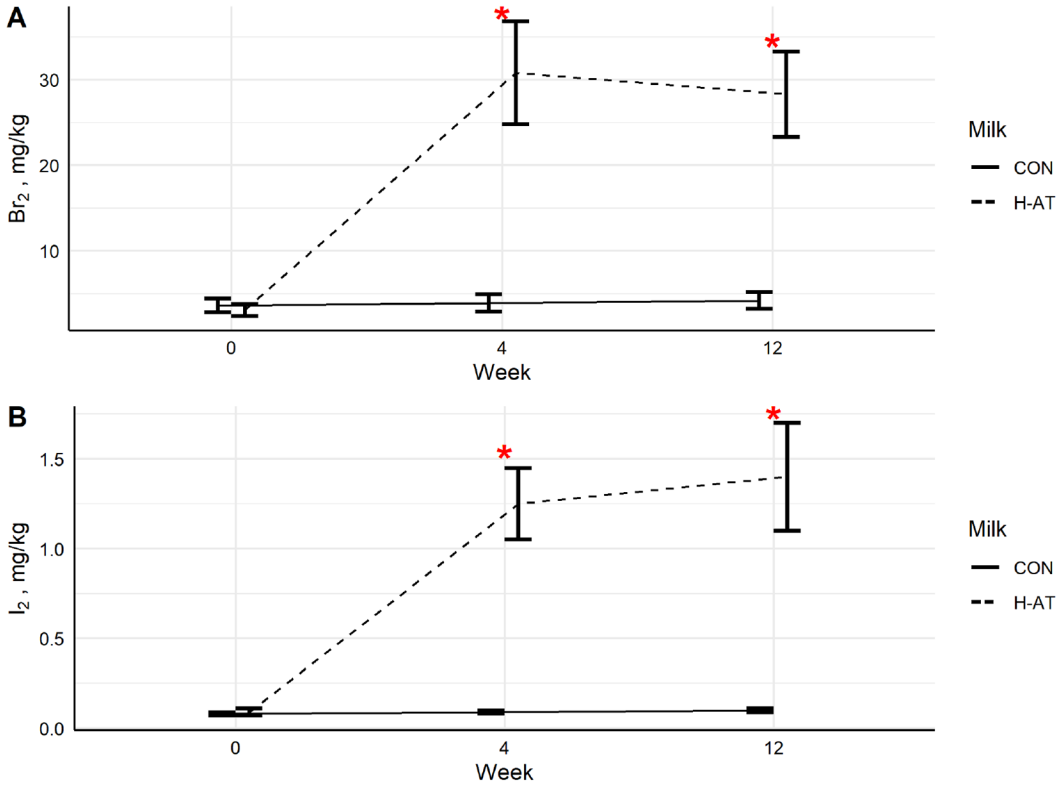

Figure 3. Concentration of (A) bromine (Br_2) and (B) iodine (I_2) in milk samples taken at wk 0, 4, and 12 from cows fed the control diet (CON, 0% AT) and the diet containing 0.3% AT (H-AT) on an OM basis. Least squares means per group and week (n = 36). Error bars represent the SD within each group. A significant ($P < 0.001$) difference between CON and H-AT is indicated with an asterisk (*).

tensity (g of CH_4/kg of ECM), with a 40% reduction in H-AT cows compared with CON. Similarly, Stefenoni et al. (2021) observed a 26% reduction in CH_4 intensity from dairy cows at an AT inclusion level of 0.5% of DM compared with a control diet, although the effect diminished over time. It seems that the reduction of the effect of AT was due to a reduction in the concentration of bromoform in AT during storage (Figure 1). The reduction effect in CH_4 in the H-AT group from wk 4 to 12 decreased by 42.2%; however, from wk 9 to 12, there was no interaction effect between treatment and week. This pattern may be compared with the 16.9% reduction in bromoform concentration in AT over the same period. This discrepancy may indicate a potential adaptation of rumen microbes to bromoform, potentially reducing its long-term effectiveness in CH_4 reduction, warranting further investigation. As highlighted by Kinley et al. (2020) and Roque et al. (2019), achieving reductions in CH_4 emissions requires a certain concentration of bromoform. These studies indicate that when bromoform levels are below a critical threshold, the effectiveness of CH_4 mitigation may be diminished. Specifically, Roque et al. (2019) found that a low inclusion rate of 12.1 mg of bromoform/kg of DMI fed to dairy cows resulted in a 20% methane reduction, whereas Kinley et al. (2020) observed an inclusion of 11.8 mg of bromoform/kg of DMI in beef steers leading to a 38% methane reduction. These results, as noted by Alvarez-Hess et al. (2024), suggest a minimum bromoform concentration of approximately 12 mg bromoform/kg DMI in both beef and dairy cattle TMR systems is needed for substantial CH_4 reduction, reinforcing the nonlinear response to lower bromoform levels. In our study, bromoform concentrations in L-AT were below 12 mg bromoform/kg DMI, whereas for H-AT, the levels were 20.7 mg bromoform/kg DMI at wk 4, decreasing to 16.8 mg bromoform/kg DMI by wk 12. Although the bromoform concentration in H-AT exceeded 12 mg/kg DMI by the end of this study, its inhibitory effect on CH_4 was reduced.

Early work by Chalupa (1977) showed that inclusion of halogenated compounds as feed additives for ruminants appears to have repercussions. It has been established that supplementation of dairy cow diets with halogenated compounds or other methanogen-inhibiting compounds, such as 3-nitroxypropanol, leads to an increase in metabolic H_2 concentration in the rumen (Hristov et al., 2015; Roque et al., 2019; Stefenoni et al., 2021). This increase, in turn, is associated with impaired microbial

production of vitamin B_{12} and ineffective metabolism of increased proportions of propionate and butyrate in rumen fluid caused by AT supplementation (Chalupa, 1977). In line with these findings, we observed an almost 5-fold increase in enteric H_2 concentration in the H-AT group compared with CON, and the elevated concentration of H_2 in H-AT, compared with CON, remained consistent across all weeks. This increase underscores the relevance of previous research demonstrating similar effects of methanogen-inhibiting compounds on rumen metabolism. Such findings support our observations and highlight the potential metabolic implications of the additive treatment in our study.

Methane inhibition in the H-AT group was accompanied by a decrease in total VFA concentration in rumen fluid, a reduction in acetate concentration, and an increase in propionate, butyrate, and valerate concentrations. Similar results have been reported by Stefenoni et al. (2021) and Krizsan et al. (2023), indicating a shift in fermentation pattern as a result of inhibited methanogenesis in the rumen. This shift may be associated with alterations in microbial metabolic activity.

The lack of effect of AT supplementation on CH_4 and H_2 production in the L-AT group indicates that the effect of AT as a CH_4-mitigating additive is dose-dependent, necessitating further investigations on the minimum level of addition required to achieve an inhibitory effect.

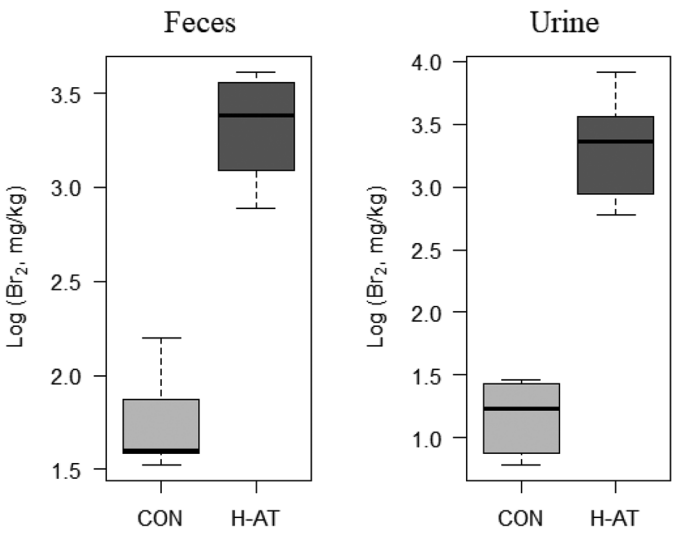

Figure 4. Box plot distribution of bromine concentration (Br_2, mg/kg; $P < 0.001$) in feces and urine samples collected in experimental wk 12 from cows fed the control diet (CON, 0% AT) and cows fed the diet containing 0.3% AT (H-AT) on an OM basis. Logarithmic values (n = 12). The line inside the box indicates the median concentration of bromine. The box represents the interquartile range (IQR) from the 25th to the 75th percentile of the data. The whiskers extend to the smallest and largest values within 1.5 times the IQR from the box.

Lactational Performance: Milk Yield and Composition

The H-AT group in our study had 4.7% lower fat concentration in milk compared with CON. This may be attributable to a shift in rumen fermentation pattern (Palmquist et al., 1993), resulting in increased propionate and reduced acetate in the rumen rather than directly affecting milk composition in the H-AT group. We observed a 2% and 4% reduction in ECM yield and milk yield, respectively, in the H-AT group compared with CON, during the study period. Similarly, Stefenoni et al. (2021) reported comparable reductions in both milk and ECM yields. Additionally, Krizsan et al. (2023) reported reduced DMI and ECM yields with 0.5% AT on OM supplementation, although milk yield did not differ. This links the observed differences in nutrient availability for milk production primarily to DMI. Additionally, differences were observed in rumen molar proportions of specific VFA across groups, as discussed previously. Given that up to 70% of the energy supplied to ruminants is derived from VFA produced in the rumen (Bergman, 1990), the observed variations in ruminal fluid total VFA concentrations and DMI among dietary groups in our study likely influenced the achieved milk yield and ECM yield. These findings highlight the importance of conducting long-term studies to understand the enduring effects of dietary interventions, such as AT, on milk production. The lower production outcomes observed in the current study underscore potential limitations of AT as a mitigation strategy, particularly in contexts where sustaining or enhancing milk output is critical for economic viability and production efficiency.

Bromine and Iodine in Milk, Feces, and Urine Samples

There were higher levels of bromine and iodine residues in milk from the H-AT group compared with CON. The bromine concentration was approximately 7-fold higher, whereas the iodine concentration was approximately 13-fold higher, which is in line with findings in previous studies where dairy cows were fed 0.5% AT (Stefenoni et al., 2021; Krizsan et al., 2023). Additionally, Eikanger et al. (2024) reported an increase in iodine in milk samples from cows fed an inclusion level of 0.25% AT on an OM basis. However, the bromine and iodine concentrations in milk in our study were lower than observed by Krizsan et al. (2023), due to our lower inclusion level of AT.

In contrast, a decline in both bromine in the milk and bromoform content in AT was observed from wk 4 to 12. This may be explained by the degradation of bromoform content in AT during storage. We observed a linear trend in bromine and iodine concentrations in milk, which

Table 7. Plasma levels of different metabolic parameters in lactating dairy cows fed the control diet (CON, 0% AT) and the diet containing 0.3% AT on an OM basis (H-AT) during experimental wk 4 and 12[1]

	Treatment			*P*-value		
Parameter[2]	CON	H-AT	SEM	Treatment	Week	Treatment x Week
Glucose, mmol/L	4.04	4.15	0.1	0.30	0.56	0.12
Total cholesterol, mmol/L	8.35	7.01	0.4	<0.01	0.47	0.54
Total protein, g/L	78.0	80.9	1.8	0.11	0.06	0.50
Albumin, g/L	38.3	38.1	0.5	0.70	0.40	0.94
Globulin, g/L	39.7	42.8	1.1	0.05	0.01	0.42
Urea, mmol/L	5.75	5.40	0.3	0.83	0.42	0.005
Calcium, mmol/L	2.49	2.54	0.05	0.29	0.20	0.27
Magnesium, mmol/L	0.97	1.02	0.02	0.03	<0.01	0.01
Zinc, µmol/L	11.4	10.3	0.5	0.06	0.06	0.92
AST, U/L	92.3	91.4	6.6	0.36	0.04	0.36
GGT, U/L	27.1	29.7	3.4	0.45	<0.001	0.76
Haptoglobin, g/L	0.09	0.10	0.05	0.87	0.85	0.18
Ceruloplasmin, µmol/L	2.30	2.28	0.1	0.91	0.16	0.39
Paraoxonase, U/L	85.7	87.2	8.6	0.86	0.34	0.27
ROMt, mg of H_2O_2/100 mL	14.4	14.6	0.65	0.85	0.42	0.36
FRAP, µmol/L	191	175	3.51	<0.001	0.43	0.24
SAA, µg/mL	75.4	70.0	21.4	0.80	0.09	0.18

[1]Least squares means and SEM (n = 24).

[2]AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ROMt, reactive oxygen metabolites test; FRAP, ferric-reducing ability of plasma; SAA, serum amyloid A.

accumulated over time in the H-AT group (Figure 3). Knowledge of the accumulation rate is important when evaluating the metabolism of these compounds in cows, and further studies are required.

There are limited recommendations regarding daily bromine intake for human consumption. According to van Leeuwen et al. (1987), the acceptable daily dose of bromine is 0.4 mg/kg BW, which corresponds to 28 mg daily in an adult weighing 70 kg. The bromine concentration in milk samples from the H-AT group was 20.8 mg/kg, allowing for a maximum daily intake of 1.3 kg of milk per day, assuming no other dietary sources of bromine. Maximum daily iodine intake in humans varies with age, with a generally accepted range of 90 to 150 µg/d (EFSA, 2017) and a maximum upper level of 600 µg/d (Blomhoff et al., 2023). The concentration of iodine in milk from the H-AT group was 0.92 mg/kg, allowing for a daily milk consumption of 0.01 to 0.16 kg per person. For an individual, the recommended maximum daily milk intake would be 0.65 kg. Our analyses showed that during the final week of the experiment, the H-AT group excreted almost 5-fold higher average bromine concentrations in feces and 9-fold higher concentrations in urine compared with CON. This finding represents a significant and novel contribution to the current understanding of bromine excretion patterns in dairy cattle receiving an AT supplement to reduce enteric CH_4 emissions. The elevated bromine levels in both feces and urine suggest that a substantial portion of the bromine from the AT supplement is not metabolized but rather excreted by the cows. This finding suggests that the cows' digestive systems and metabolic pathways may not fully process or use bromine, resulting in its excretion. The high excretion rates indicate that bromine, in the form of bromoform, may have limited metabolic integration within the bovine system, with the compound or its metabolites being rapidly cleared from the body. These elevated excretion levels raise concerns about the long-term effects on animal health, particularly the potential toxicity of accumulated bromine in tissues, as well as environmental contamination through manure management practices.

Potential Alteration of Metabolic Profile

Metabolic profile assessment revealed an effect of AT on magnesium level in the H-AT group, although the levels recorded in both groups were within the normal range (Bertoni and Trevisi, 2013). A similar finding was made by Li et al. (2018) in sheep fed various concentrations of AT for 21 d. Red macroalgae species, such as AT, are rich in minerals (MacArtain et al., 2007), and could be beneficial in addressing magnesium deficiency issues in cattle.

In contrast to our findings, Li et al. (2018) observed an increase over time in blood cholesterol concentrations in sheep fed an AT supplement. In our study, blood cholesterol was lower in the H-AT group, although values in both CON and H-AT were higher than the normal range (Bertoni and Trevisi, 2013).

The FRAP level decreased in the H-AT treatment group compared with CON, suggesting that AT addition to the diet may have reduced the antioxidant capacity of blood plasma. Antioxidants play a crucial role in neu-

tralizing harmful free radicals in the body (Benzie and Strain, 1996), and the decrease in FRAP may indicate an imbalance between free radical production and the body's ability to neutralize these, potentially leading to increased oxidative stress. This increased oxidative stress can impair immune function, making cows more susceptible to infections and diseases. Additionally, it can negatively affect milk yield, quality, and reproductive performance, as well as hinder growth and overall development in young cattle (Sordillo and Aitken, 2009). Ongoing research into the specific antioxidant needs and effective supplementation strategies for cows, particularly when using AT, can lead to improved guidelines and practices for cattle management.

CONCLUSIONS

Supplementation of dairy cow diets with 0.3% AT on an OM basis reduced enteric CH_4 production by 30%, whereas a lower dose (0.15% OM) had no effect. However, from wk 9 to 12, no further reduction in CH_4 was observed, emphasizing the need for stable storage conditions to preserve bromoform in AT. This diminished effect may also indicate rumen microbiome adaptation over time. Cows on the 0.3% AT treatment showed reduced DMI, milk yield, and milk fat content, along with altered rumen fermentation. Elevated bromine and iodine concentrations in milk, and high bromine levels in feces and urine, suggest metabolic and excretion processes. Plasma FRAP values were lower in the 0.3% AT group, indicating increased oxidative stress. Positive outcomes included reduced total cholesterol and increased plasma magnesium. Further studies on algae storage and rumen microbiota adaptations are needed to understand metabolic changes and ensure cow health.

NOTES

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Nonstandard abbreviations used: AST = aspartate aminotransferase; AT = *Asparagopsis taxiformis*; CON = control group with no AT; FRAP = ferric-reducing ability of plasma; GF = GreenFeed; GGT = gamma-glutamyl transferase; H-AT = 0.3% AT on an OM basis; iNDF = indigestible NDF; L-AT = 0.15% AT on an OM basis; ND = not determined; NorFor = Nordic feed evaluation system; ROMt = reactive oxygen metabolites; SAA = serum amyloid α.

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