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Effects on rumen microbiome and milk quality of dairy cows supplemented the macroalgae *Asparagopsis taxiformis* in a grass silage-based diet

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Introduction

The globally increasing cattle population contribute to the total anthropogenic greenhouse gas emissions. Methane (CH₄) emission from cattle is part of a cycle, but contributes to global heating because of the much greater heating effect of CH₄ compared to carbon dioxide. However, when CH₄ composes to carbon dioxide in the atmosphere it suggestively does not contribute to further increase because it will be bound to vegetation in near future. This implies that reduced CH₄ emissions will lead to a drop in average global temperature (Sterner and Johansson, 2017). The red seaweed *Asparagopsis taxiformis* (AT) has been shown to be a strong natural inhibitor of ruminal CH₄ formation (Machado et al., 2016). Stefenoni et al. (2021) decreased CH₄ emissions in lactating dairy cows by 80% by inclusion of AT at 0.5% of DM intake. Important factors to consider when using alga supplemented feed is the rumen microbiome and the milk quality, which were not assessed in the previous studies. The objective of this study was thus to assess these parameters combined with level of CH₄ reduction and feed digestibility after the addition of AT to diets of dairy cows.

Material and methods

Six Nordic Red cows at 122 ± 13.7 (mean ± SD) days in milk, parity 2.7 ± 0.52 and producing 36 ± 2.5 kg milk/d at the start of the trial were blocked by milk yield, and assigned to an extra period Latin square change-over design (Lucas, 1957) comprising two dietary treatments. The dietary treatments were either a diet consisting of grass silage and a commercial concentrate mixture (50:50) not supplemented or supplemented with 0.5% of *Asparagopsis taxiformis* (AT) on OM intake basis. Recordings of CH₄ and hydrogen production with the GreenFeed system (C-Lock Inc., Rapid City, SD, USA), feed intake and milk yield were made the last week of every 3 week period. Milk samples were collected and subjected to sensory, composition and milk fatty acid analysis. Diet digestibility was measured by faecal spot sampling with indigestible NDF as an internal marker. Rumen fluid samples were collected for microbiome and volatile fatty acid composition analysis. Milk samples, rumen fluid and faecal samples were also analyzed for bromoform content, and milk samples additionally iodine and bromine content.

Bacterial library preparation and sequencing was performed by Novogene with total DNA extracted using TIAN amp Stool DNA Kit. For archaeal library, DNA was extracted with the FastDNA™ Spin kit for soil (MP Biomedicals, Irvine, USA) according to Singh (2020). Archaeal amplicon sequencing was performed by the SNP&SEQ Platform. Illumina MiSeq sequencing with v3 chemistry and paired end reads (2x 300 bp) was used for both bacterial and archaeal amplicon libraries.

Experimental data were subjected to analysis of variance using the GLM procedure in SAS (SAS Inc. 2002-2003, Release 9.4 SAS Inst. Inc., Cary, NC, USA) by applying a model correcting for effect of period, cow within square and experimental dietary treatment. Potential carry-over effects on all traits were evaluated by including a residual effect in the model described above. There were no significant ($P \geq 0.22$) residual effects for any traits evaluated, and the effect was not included in the final analysis.

Results and Discussion

AT supplementation decreased CH₄ production on average by 60%, dry matter intake by 2.8 kg/d, and ECM by 1.9 kg/d compared to cows fed the non-supplemented control diet (Table 1). The decrease in ECM was caused by lower milk fat yield for cows fed AT, and was in agreement with the shift in rumen fermentation from acetate to propionate. Except for the observed effect on milk fat yield, these results are in agreement with a previous study investigating an AT supplemented diet fed to dairy cows (Stefenoni et al. 2021). Moreover, also in line with Stefenoni et al. (2021), the present study observed an increase in milk iodine and bromine, and no change in bromoform in milk. Sensory test results did not reveal any quality changes and there were only minor changes in milk fatty acid composition when diets were supplemented with AT (results not presented).

Table 1 Effect of supplementation of *Asparagopsis taxiformis* (AT) compared to a grass-silage based diet (Control) on ruminal production of methane (CH₄) and hydrogen (H₂), dry matter intake (DMI), energy corrected milk (ECM), milk quality parameters and digestion

Item ¹	Control	AT	SEM	P-value
CH ₄ , g/d	383	154	30.2	<0.01
CH ₄ , g/kg DMI	19.0	9.2	1.47	<0.01
CH ₄ , g/kg ECM	11.6	5.1	0.79	<0.01
H ₂ , g/d	0.9	5.0	0.44	<0.01
DMI, kg/d	20.3	17.5	0.42	<0.01
Organic matter digestibility, g/kg	764	760	4.0	0.51
Neutral detergent fibre digestibility, g/kg	656	646	4.8	0.17
ECM, kg/d	33.3	31.4	0.58	0.05
Milk fat, g/d	1399	1280	32.8	0.03
Milk bromine, mg/L	5.1	43.2	2.70	<0.01
Milk iodine, µg/L	139	2105	146.2	<0.01
Milk bromoform, µg/kg	4.09	4.92	0.712	0.44
Rumen acetate, mmol/mol	62.9	55.2	0.83	<0.01
Rumen propionate, mmol/mol	20.4	25.9	0.68	<0.01
Rumen butyrate, mmol/mol	12.0	14.2	0.28	<0.01

The archaeal community was mainly represented by two different phyla (Figure 1a). The relative abundance of Methanobacteriota was lower ($P=0.05$) in diets with AT than in Control (29.9 vs. 58.8 %, respectively). Methanobrevibacter is dominating in Methanobacteriota and has been linked to ruminal production of CH₄. The relative abundance of Thermoplasmata was numerically higher ($P=0.08$) in diets with AT than in Control (66.2 vs. 40.3%, respectively). Thermoplasmata was mainly represented by a genus belonging to the family

Methanomethylphilaceae. Not much is known about this archaeal group but members within this family use methyl groups, instead of H₂, for CH₄ production and these methylotrophs have been associated with reduced CH₄ emissions from the rumen (Poulsen et al., 2013). The bacterial population was represented by 44 different phyla, with 17 phyla found across all samples (Figure 1b).

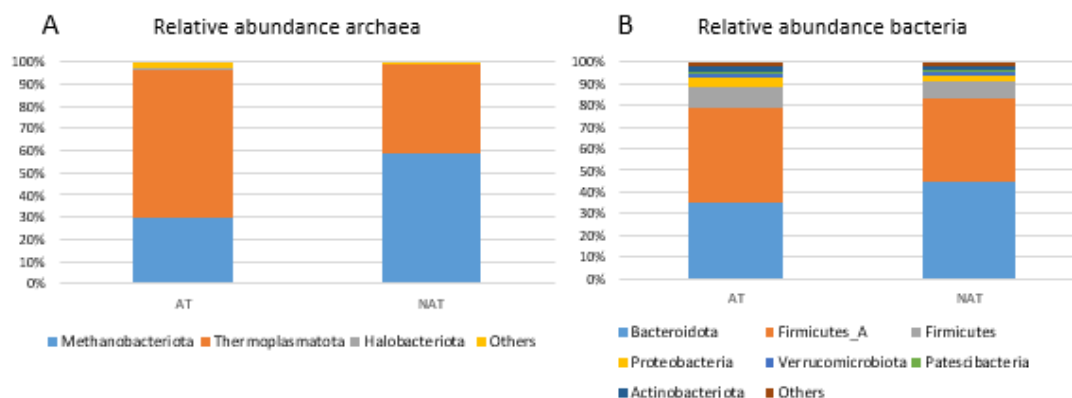


Figure 1. Relative abundance of all sequences at phyla level for archaea (A) and bacteria (B) in cows fed diets with inclusion of *Asparagopsis taxiformis* (AT) or without (Control).

Conclusions

Milk energy output was decreased by 5.7% and CH₄ production by 60 % when cows were fed AT. The AT treatment had a tendency to effect the archaeal population with a decrease in relative abundance of Methanobacteriota. The most prominent change in milk quality were the increases in bromine and iodine when the diets were supplemented with AT.

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