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Original Paper

The Effect of Microalga *Chlorella vulgaris* and Cyanobacteria *Spirulina platensis* Supplementation on Rumen Fermentation in Cattle

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Microalgae are a promising protein source for ruminants, yet their *in vivo* effects on rumen fermentation are variable. We evaluated dietary *Chlorella vulgaris* (CHL), *Spirulina platensis* (SPIR), and a 50 : 50 mixture (MIX) in four ruminally cannulated Simmental cows using a 4 × 4 Latin square. Cows received a control diet (CON) or CON supplemented with CHL (100 g/d), SPIR (100 g/d), or MIX (50 g CHL + 50 g SPIR/d). Rumen fluid collected three hours post-feeding was analyzed for pH, volatile fatty acids (VFA), ammonia (NH_3–N), total nitrogen (N_{tot}), and total protozoal ciliates (TPC). Treatments affected most fermentation endpoints. Relative to CON, CHL lowered pH (6.17 vs. 6.37; $P < 0.001$) and increased propionate (19.24 vs. 16.78–17.40 mmol/L; $P = 0.001$), indicating a more glucogenic profile. MIX increased acetate (78.43 vs. 69.02 mmol/L; $P = 0.002$), the acetate: propionate ratio (4.82 vs. 3.99–4.38; $P < 0.001$), isovalerate (1.09 vs. 0.75–0.86 mmol/L; $P < 0.001$), NH_3–N (2.83 vs. 1.69–2.34 mmol/L; $P < 0.001$), N_{tot} (0.57 vs. 0.46–0.51 g/kg; $P = 0.01$), and TPC (35.02 vs. 26.43–29.04 $\times 10^4$/mL; $P = 0.005$). SPIR responses were intermediate and generally closer to CON. At practical inclusion rates, *C. vulgaris* favored propionate production with a modest decline in pH. In contrast, the CHL+SPIR mixture shifted fermentation toward higher acetate, branched-chain VFA, and deamination products, alongside greater protozoal abundance. Thus, single-species versus mixed microalgae should be selected based on production goals: CHL for glucogenic energy supply, and MIX for acetate-oriented fermentation, acknowledging potential impacts on rumen nitrogen efficiency.

Keywords: rumen, volatile fatty acid, feed supplement, *Chlorella vulgaris*, cyanobacteria

1 Introduction

Microalgae, such as *Chlorella* spp. and *Spirulina* (*Arthrospira*) spp., are rich in protein, pigments, vitamins, and bioactive compounds and are being explored as alternative or complementary protein sources for ruminants. Contemporary reviews highlight their potential to influence rumen fermentation, microbial ecology, and product quality. However, responses are heterogeneous and may depend on factors such as species, dose, processing, and the diet background (e.g., forage-to-concentrate ratio) (Saadaoui et al., 2021; Zhu et al., 2024).

In vivo data in cattle remain limited; for instance, *Chlorella* spp. supplementation has changed rumen fatty acid profiles in cattle fed low-quality forages, and *Spirulina* spp. has affected rumen development and VFA in small ruminants; however, applying these findings across different species, diets, and dosages remains uncertain (Costa et al., 2020; Wang et al., 2023).

We therefore tested whether practical amounts of *C. vulgaris* and *S. platensis* – alone or in combination – alter rumen fermentation endpoints in cannulated dairy cows fed diets formulated to meet the requirements.

2 Material and Methods

2.1 *Experimental Design and the Animals' Diet*

Four ruminally cannulated Simmental cows (6.0 ±0.83 yr of age; 521 ±9.5 kg BW) were used as experimental animals. Cows were housed in individual pens (6.7 m^2) at the experimental facility of Agrovyzkum Rapotin (Czech Republic) and had access only to their assigned ration.

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Water was provided *ad libitum*, and diets were based on the farm's standard forage-based ration, formulated to meet maintenance energy requirements and to reflect the relatively low crude-protein concentrations typical of local low-input systems, which are slightly below NRC (2016)D recommendations for non-lactating cows. Microalgae were evaluated as a potential supplemental source of high-quality protein under these conditions. All animal procedures were reviewed and approved by the Ministry of Agriculture of the Czech Republic (protocol No. 14608/2021-MZE-18134).

Cows were assigned to four dietary treatments in a 4 × 4 Latin square design (four cows × four periods). The treatments included: a control diet (CON; basal diet only); the basal diet plus lyophilized *Chlorella vulgaris* (CHL; 100 g per cow daily; 6.23 g per kg of dry matter, DM); the basal diet plus lyophilized *Spirulina platensis* (SPIR; 100 g per cow daily; 6.02 g per kg of DM); and a 50:50 mixture (MIX) of *Chlorella vulgaris* and *Spirulina platensis* (50 g of each per cow daily; 3.03 g per kg of DM for each component). Each period lasted 14 days, including a 10-day adaptation phase. Cows were fed individually twice a day at 0600 and 1600 hours, receiving 60% and 40% of the daily allotment, respectively.

The proportions of feed ingredients and their chemical compositions are shown in Tables 1 and 2, respectively.

2.2 Sampling, Data Collection, and Chemical Analyses

Chopped meadow hay, maize silage, and granulated feed mixture were sampled at the start and at the end of the experiment to determine DM and nutrient composition. The chemical compositions of all diets (Table 2) were analyzed for dry matter, crude protein, crude fat, crude fiber, starch, and ash according to the EC Commission Regulation (2009). The neutral detergent fiber (NDF) concentration was determined using an ANKOM A200 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) according to the manufacturer's methodology, which is based on the methods described by Van Soest et al. (1991).

Rumen fluid samples were collected from each cow twice per period (on days 7 and 14), yielding 32 samples In total (4 cows × 4 diets × 2 sampling days). Sampling

Table 1 Ingredients composition of the experimental diets

	Diets[1]			
Ingredients, % of DM	CON	CHL	SPIR	MIX
Meadow hay	67.32	72.17	70.60	72.50
Maize silage	16.84	17.47	16.73	17.55
GFM[2]	15.84	9.45	11.8	9.49
Chlorella[3]	0.00	0.91	0.00	0.23
Spirulina[4]	0.00	0.00	0.87	0.23
Total	100	100	100	100

1 – Diets: CON – control diet without *C. vulgaris* or *S. platensis* supplementation; CHL – diet contains 2.76 g of lyophilized *C. vulgaris*/kg of dry matter; SPIR – diet contains 8.22 g of lyophilized *S. platensis*/kg of dry matter; MIX – diet contains 16.3 g of lyophilized *C. vulgaris*/kg of dry matter and 16.3 g of lyophilized *S. platensis*/kg of dry matter; 2 – GFM – granulated feed mixture BIOSTAN (Biokron, s.r.o., Czech Republic), (quantity per kg of product): barley (15%), oat mill feed (65%), wheat (15%), malt flour (10%), sunflower expellers (5%), extracted soybean meal (3%); 3 – lyophilized microalga *Chlorella vulgaris* (Centre ALGATECH, Institute of Microbiology, Czech Academy of Sciences, Czech Republic); 4 – lyophilized microalga *Spirulina platensis* (Centre ALGATECH, Institute of Microbiology, Czech Academy of Sciences, Czech Republic)

Table 2 Chemical characteristics of primary feed ingredients

	Ingredients				
Nutrient composition, % of DM	meadow hay	maize silage	GFM	*C. vulgaris*	*S. platensis*
Dry matter	89.98	91.58	88.00	92.11	89.57
Crude protein	4.15	6.93	11.41	50.13	41.95
Ash	7.06	3.01	8.47	4.16	5.91
Crude fiber	30.96	14.43	5.62	0.91	0.04
Fat	1.55	2.74	2.68	1.78	0.87
Starch	0.00	38.07	38.73	5.78	25.4
NDF	56.74	31.30	19.47	9.73	1.12

NDF – neutral detergent fiber; GFM – granulated feed mixture BIOSTAN; DM – dry matter was performed three hours after the morning feeding to standardize postprandial fermentation conditions. Samples were collected via a rumen cannula with a probe connected to a vacuum pump and sent to the laboratory for further analysis. Tests of rumen fluid included the measurement of pH, physical characteristics, concentration of total nitrogen (N_{tot}), nitrogenous compounds (NC) and ammonia (NH_3–N), determination of the total number of protozoal ciliates (TPC), the molar proportions of volatile fatty acid (VFA), and the total amount of VFA. The pH was measured immediately after sample collection using a portable pH meter (EUTECH CyberScan PC510 pH/Conductivity Bench Meter, USA). Twenty milliliters of ruminal fluid aliquots were stored at -20 °C for subsequent analysis of VFA, N_{tot}, NC, and NH_3–N. Samples for rumen protozoa analysis were preserved in 1ml of 10% formaldehyde solution, stained with Brilliant Green Dye, and allowed to stand overnight. The density of rumen protozoa per mL was determined using a Bürker counting chamber on a fluorescent optical microscope (INTRACO FL200, Czech Republic) at 40× magnification, following the procedure described by Dehority (2004).

Volatile fatty acids were determined by gas chromatography (Agilent 6820 Gas Chromatograph System; Agilent Technologies, Santa Clara, USA) using the method described by Filipek and Dvorak (2009). The Kjeldahl method was used to determine the amount of nitrogenous compounds and ammonia, as defined by Chen et al. (1987).

2.3 Statistical Analysis

The software Statistica 14.0.0. (TIBCO, USA) was used to perform the statistical analysis. Data were analyzed using a two-way ANOVA with diet, sampling day (days 7 and 14), and their interaction as fixed effects, using cow as a random effect If statistical significance was observed, post-hoc comparison analysis was performed using Tukey's test. $P < 0.05$ was considered statistically significant, and $0.05 \leq P < 0.10$ was considered as a trend. The normality of the distribution of variables was assessed using the Shapiro-Wilk test, and all variables were found to be normally distributed.

3 Results and Discussion

The effect of dietary *Chlorella* and *Spirulina* on rumen fermentation is shown in Table 3.

Dietary treatment significantly affected several fermentation parameters (Table 3). Ruminal pH was lowest with *Chlorella* supplementation (6.17) and highest in the CON group (6.37; $P < 0.001$). Total VFA concentrations increased with dietary *Chlorella* (107.97 mmol/L) and combined CHL and SPIR (106.35 mmol/L) compared to CON (95.20 mmol/L; $P < 0.001$), whereas SPIR (102.11 mmol/L) was intermediate. Acetate concentrations were most significant with *Chlorella* and *Spirulina* co-supplementation (78.43 mmol/L), exceeding CON (69.02 mmol/L; $P = 0.002$), while CHL and SPIR were not different from either. Dietary *C. vulgaris* increased propionate (19.24 mmol/L), exceeding all other

Table 3 The impact of *Chlorella vulgaris* and *Spirulina platensis* supplementation on rumen fermentation parameters. Least squares means (LSMs) and standard error of the mean (SEM), $n = 32$

Diets[1]	*P*-values						
Items	CON	CHL	SPIR	MIX	SEM	D	D x SD
pH	6.37^{a}	6.17^{b}	6.35^{ab}	6.28^{ab}	0.05	<0.001	0.016	0.005
total VFA, mmol/L	95.20^{a}	107.97^{b}	102.11^{ab}	106.35^{b}	1.46	<0.001	0.046	0.32
Acetic acid, mmol/L	69.02^{a}	74.52^{ab}	73.27^{ab}	78.43^{b}	1.71	0.002	0.42	0.16
Propionic acid, mmol/L	17.40^{a}	19.24^{b}	16.88^{a}	16.78^{a}	0.46	0.001	0.30	0.009
Butyric acid, mmol/L	10.43^{ab}	10.56^{b}	9.62^{a}	10.43^{ab}	0.23	0.019	0.009	0.09
Isovaleric acid, mmol/L	0.75^{a}	0.86^{a}	0.80^{a}	1.09^{b}	0.04	< 0.001	0.17	0.07
A : P ratio	4.05^{a}	3.99^{a}	4.38^{ab}	4.82^{b}	0.15	< 0.001	0.39	0.008
NH_3, mmol/L	1.69^{a}	2.12^{ab}	2.34^{bc}	2.83^{c}	0.16	< 0.001	0.05	0.58
N_{tot}, g/kg	0.48^{a}	0.51^{ab}	0.46^{a}	0.57^{b}	0.02	0.01	0.02	0.10
TPC, $x10^4$/ml	26.43^{a}	29.04^{a}	28.81^{a}	35.02^{b}	1.39	0.005	0.51	0.83

1 – Diets: CON – control diet without *C. vulgaris* or *S. platensis* supplementation; CHL – diet contains 2.76 g of lyophilized *C. vulgaris*/kg of dry matter; SPIR – diet contains 8.22 g of lyophilized *S. platensis*/kg of dry matter; MIX – diet contains 16.3 g of lyophilized *C. vulgaris*/kg of dry matter and 16.3 g of lyophilized *S. platensis*/kg of dry matter; D – diet effect; SD – sampling day (days 7 and 14) effect; D × SD = diet × sampling day interaction; TPC – total protozoa count

treatments (16.78–17.40 mmol/L; $P = 0.001$). Butyrate was higher in CHL (10.56 mmol/L) than SPIR (9.62 mmol/L; $P = 0.019$), with CON and MIX intermediate. *Chlorella* and *Spirulina* co-supplementation also increased rumen isovalerate (1.09 mmol/L) compared to other treatments (0.75–0.86 mmol/L; $P < 0.001$). The acetate: propionate (A : P) ratio was greater with MIX (4.82) than with CHL and CON (3.99–4.05; $P < 0.001$); SPIR was intermediate (4.38). Rumen ammonia increased linearly across treatments from CON to MIX ($1.69 < 2.12 < 2.34 < 2.83$ mmol/L; $P < 0.001$), and total nitrogen was greater with the MIX diet (0.57 g/kg) than with the CON and SPIR diets (0.48–0.46 g/kg; $P = 0.01$). Total protozoal counts were also elevated in MIX ($35. \times 10^4$/mL), compared with all other diets ($26.43–29.04 \times 10^4$/mL; $P = 0.005$). Diet × day interactions were mainly non-significant, although interactions were detected for propionate and A : P ratio ($P < 0.05$).

The observed fermentation responses to microalgal supplementation were diet-specific and modest at the tested inclusion levels. Supplementation with *Chlorella vulgaris* (CHL) lowered ruminal pH and increased propionate concentration, indicating a shift toward a more glucogenic fermentation profile. Similar shifts have been reported in goats supplemented with *Spirulina* and yeast (Emara Rabee et al., 2025), as well as in dairy cows fed microalgae-rich diets that modulated VFA profiles toward higher propionate levels (Dewanckele et al., 2018). The slight pH reduction observed here likely reflects increased fermentation activity without risk of acidosis. Enhanced propionate supports hepatic gluconeogenesis, which may benefit early-lactation cows under negative energy balance (Overton & Waldron, 2004).

Notably, *C. vulgaris* did not increase NH_3–N or total rumen nitrogen (N_{tot}), indicating no change in proteolysis or deamination at this inclusion rate. This finding aligns with those of Wanapat et al. (2024), who reported unchanged NH_3–N levels in beef cattle supplemented with seaweed, and with Machado et al. (2016), who emphasized the dose-dependent effects of red algae on nitrogen metabolism. The unaltered protozoal count in CHL also suggests limited stimulation of protozoa-associated proteolysis or bacterial turnover (Rabee et al., 2022). Thus, *C. vulgaris* may enhance fermentation without compromising nitrogen efficiency.

In contrast, the MIX diet (50 : 50 *Chlorella vulgaris* and *Spirulina platensis*) caused broader changes in rumen fermentation: increased total VFA, acetate, isovalerate, NH_3–N, and protozoal counts. Elevated isovalerate suggests greater deamination of branched-chain amino acids, possibly indicating higher proteolytic activity or microbial turnover (Mitchell et al., 2023). Similar boosts in branched-chain VFA and NH_3–N have been observed in small ruminants supplemented with high-protein microalgae (Bikker et al., 2020). The rise in protozoal counts in MIX may be due to more available degradable nitrogen or stimulatory compounds like peptides and fatty acids present in *Spirulina* (Podgórska-Kryszczuk, 2024). Although protozoa help digest fiber, they also prey on bacteria, which could decrease net microbial protein flow and nitrogen retention (Firkins, 1996; Yu et al., 2024).

These findings support prior *in vitro* observations, where the inclusion of microalgae increased acetate production and NH_3–N (Sucu, 2019), although *in vivo* responses remain variable. For example, Roque et al. (2019) observed methane inhibition with Asparagopsis spp. but noted inconsistent effects on VFA and NH_3–N, highlighting species-specific differences in algal bioactivity.

The lack of NH_3–N increases with CHL, but its rise in MIX further illustrates how algal composition and interactions determine outcomes. The protein quality, cell wall digestibility, and presence of bioactives (e.g., pigments, polyphenols) differ between *Chlorella* and *Spirulina* (Lobo et al., 2024), which may explain the non-additive or synergistic effects seen with the MIX.

Spirulina platensis dietary responses were mostly intermediate, suggesting limited impact at the tested dose or potential antagonism when not combined with the alga. Unlike *C. vulgaris*, *Spirulina* did not significantly alter any nitrogen or protozoal parameters despite its high protein content. This may reflect differences in cell wall composition and digestibility, as *Spirulina* has a more open, more digestible structure than the thick-walled *Chlorella* (Lobo et al., 2024). Processing methods, such as cell rupture or enzymatic treatment, may be necessary to enhance bioavailability and unlock the full fermentative potential (Demarco et al., 2022; Mekuto, 2024).

3 Conclusions

Under the conditions of this study, daily supplementation with lyophilized *Chlorella vulgaris*, *Spirulina platensis*, or a 50:50 mixture of the two affected rumen fermentation parameters in a treatment-specific manner. *C. vulgaris* increased propionate and lowered pH without raising NH_3–N or protozoal counts, indicating a glucogenic fermentation profile with limited nitrogen disruption. In contrast, the combination of *C. vulgaris* and *S. platensis* (MIX diet) increased acetate, branched-chain VFA, ammonia, total nitrogen, and protozoa, suggesting a shift toward fibrolytic and proteolytic fermentation, potentially with reduced nitrogen retention. These findings indicate that dietary *C. vulgaris* may support energy partitioning without impairing nitrogen metabolism, while the MIX may promote fiber fermentation at the expense of nitrogen efficiency. Future research should explore higher inclusion levels, more extended adaptation periods, and production outcomes such as microbial protein synthesis, nitrogen excretion, and milk composition to optimize the use of microalgae in ruminant diets.

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Conflict of Interest

The author declares that they have no conflict of interest.

Author Contributions

Svetlana Malyugina: conceptualization, data curation, formal analysis, investigation, methodology, supervision, visualization, writing – original draft, writing – review & editing.

AI and AI-Assisted Technologies Use Declaration

No generative AI tools/AI-assisted technologies were used during the preparation of the manuscript.

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