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SHORT COMMUNICATION

Effect of freshwater microalgae *Nannochloropsis limnetica* on the rumen fermentation *in vitro*

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ABSTRACT. It was hypothesised that *Nannochloropsis limnetica* due to the specific chemical composition and fatty acids profile, may positively affect rumen fermentation. To confirm this hypothesis the batch culture experiment was conducted to evaluate *N. limnetica* supplemented at 0, 2, 4 and 6% of the substrate dry matter (DM) on both fermentation and fatty acid proportion in the ruminal culture. It was found that microalgae *N. limnetica* contain ($g \cdot kg^{-1}$ DM): crude protein 238, Ca 48.7, Na 31.8 and unsaturated fatty acids ($51.7\ g \cdot 100\ g^{-1}$ fatty acids). Moreover, leucine and lysine were the most abundant essential amino acids in the analysed microalgae. The total bacteria count was negatively affected if *N. limnetica* algae were supplemented at more than 4%. So, the research hypothesis that microalgae *N. limnetica* may affect rumen fermentation was confirmed, mainly by increasing propionic acid concentration without changes in the total volatile fatty acids concentration. However, a high dose (6%) decreased rumen bacteria count. Further research under commercial farm conditions should be conducted to confirm the usefulness of freshwater microalgae *N. limnetica* as a feed additive for ruminants.

Introduction

Algae have been found to be a valuable feed source for poultry, fish and pigs (Kotrbáček et al., 2015). However, there is no comprehensive research on the use of algae in ruminant nutrition and their effect on rumen functions (da Silva et al., 2016). There is also lack of research on specific freshwater microalgae *Nannochloropsis limnetica* as potential feed additive, especially for ruminants. The main advantage of the microalgae, compared to common algae is the ability to produce highly valuable molecules such as *n*-3 fatty acids (FA), especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Spolaore et al., 2006). The FA composition of the freshwater microalgae species *N. limnetica* is similar to that of marine species, however, the total amount of FA is higher than in marine species (Krienitz et al., 2000). In the study of Costa et al. (2016) whole or post-lipid extraction microalgae were used as a potential crude protein supplement for cattle.

Feeds or feed additives containing PUFA may change rumen fermentation, including decreasing methane production (Moate et al., 2013). So, in the present study it was hypothesised that *N. limnetica* with its specific chemical composition and FA profile may positively affect rumen fermentation. Thus, the purpose of this *in vitro* study was to investigate the dose effect of freshwater microalgae *N. limnetica* on rumen fermentation and FA proportion in the ruminal culture.

Material and methods

Collection and preparation of microalgae samples

N. limnetica was isolated from Wadi El-Rayan upper lake, El-Fayioum governorate (Egypt). Isolation and purification of *N. limnetica* was performed using streaking plate method (Stein, 1973) with BG-11 agar medium prepared at the Laboratory of Marine Toxicology, National Research Centre (Egypt) according to Allen (1973). *N. limnetica* was then cultivated in 0.5-l Erlenmeyer flasks using an Environ-shaker incubator (MP-7552, cv-cc power supply, hsiHefer, San Francisco), lighted with fluorescent lamps on a 12-h photoperiod (light intensity 440 W · m^{-2} and temperature 30 ± 2 °C). An initial 10% (v/v) inoculum of *N. limnetica* was used and the yielded mass was obtained through propagation on a modified BG-11 medium (Rippka et al., 1979). After cultivation, *N. limnetica* was centrifuged for 10 min at a 1750 rfc. The obtained pellets were washed three times with demineralized water, resuspended, and centrifuged one more time. The remaining algae pellets were frozen at −20 °C, freeze-dried and ground to a fine powder.

Chemical composition of *N. limnetica*

The dry matter (DM), ash, crude protein (CP) and ether extract (EE) were determined in *N. limnetica* powder according to the AOAC International procedures (1995). Crude fibre (CF) was determined using Fibertec^{TM} 2010 Auto Fibre Analysis System (FOSS Analytical, Hilleroed, Denmark) as described by Van Soest et al. (1991). For macro and trace elements analysis, 1 g sample of algae was dried at 550 °C (STN 46 9072) and dissolved in 10 ml of hot HCl (1:3). The concentrations of Ca, Mg, Na, K, Fe and Zn were determined using atomic absorption spectrophotometry (AAS Solar 9000, Unicam, Cambridge, UK). The amino acid content of *N. limnetica* was determined by ion-exchange chromatography on automatic amino acid analyser AAA 400 (Ingos, Prague, Czech Republic) after hydrolysis with 6 M HCl in a sealed vacuum ampoule at 110 °C for 24 h. Methionine and cysteine were determined in pre-oxidized samples as methionine sulfone and cysteic acid, respectively. The FA profile of *N. limnetica* was determined by gas chromatography (GC; 456-GC, Bruker, Billerica, MA, USA) equipped with flame ionization detector and a 100-m fused-silica capillary column (0.25 mm i.d.) coated with 0.25 μm Agilent HP (Chrompack CP7420). Briefly, 3 ml of 2 N NaOH was added to 100 mg of feed. The borontifluoride (Fluka – Sigma-Aldrich, St. Louis, MO, USA) was used to convert extracted FA into fatty acid methyl esters (FAME). The details are described in Szczechowiak et al. (2016). The FA were expressed as g · 100 g^{-1} FA.

In vitro dry matter and crude protein digestibility of *N. limnetica*

Daisy incubator system (ANKOM II Daisy Incubator; ANKOM Technology, Macedon, NY, USA) was used to measure microbial-enzymatic *in vitro* dry matter (IVDMD) and crude protein digestibility (IVCPD).

The analysis was conducted at the National Agricultural and Food Centre (Slovak Republic) and adopted from the modified *in vitro* procedure by Gargallo et al. (2006). Rumen fluid was collected from two ruminally cannulated Holstein-Fresian cows before the morning feeding with diet consisting of 70% forage (lucerne hay and maize silage) and 30% concentrate (wheat meal:barley meal (1:1); mineral and vitamin feed additive).

The algal powder (0.5 g per bag) was transferred to 10 filter bags (F57, pore dimension 50 μm, ANKOM Technology, Macedon, NY, USA). Microbial digestion of *N. limnetica* powder was initiated in a 2-l jar. The fermentation culture fluid was prepared by mixing 400 ml of the rumen fluid and the buffer solution (McDougall, 1948) in a 1:4 ratio.

The collected rumen fluid was filtered through 4 layers of cheesecloth into a 5-l flask pre-heated to 39 °C. The incubation jar was flushed with CO_2 gas for 30 s and secured by lid to maintain the anaerobic condition. After 18 h of incubation at 39 °C under constant rotation, all bags were transferred from rumen fluid buffer to pepsin-pancreatic solution jar, which contained pepsin and pancreatic enzymes. Enzymatic digestion was initiated in a solution of 0.1 M HCl (adjusted to pH 1.9 using 10 M NaOH) and 2 g · 2 l^{-1} pepsin at 39 °C for 1 h. Then, filter bags were thoroughly rinsed with distilled water

and transferred to another 2-l jar containing a solution of 0.5 M KH_2PO_4 (adjusted to pH 7.75 using 10 M NaOH), 0.1 g · 2 l^{-1} thymol and 6 g · 2 l^{-1} pancreatin, and incubated at 39 °C for 24 h. After the incubation, the bags were rinsed using distilled water and dried at 105 °C until constant weight was achieved. In dry sample and total N was than determined using Kjeldahl method. DM and CP disappearance was calculated by the difference.

In vitro batch culture experiment

Batch fermentation culture experiment was conducted according to El-Sherbiny et al. (2016) to evaluate the effect of *N. limnetica* on rumen fermentation parameters and microbial population count. This experiment was performed at the Department of Animal Nutrition and Feed Management, Poznań University of Life Sciences (Poland).

Rumen fluid was collected before the morning feeding from 3 ruminally cannulated Polish Holstein-Friesian dairy cows (mean weight 680 ± 30 kg). The cows were fitted with ruminal cannula by surgical procedure approved by the guidelines of Local Ethical Commission. The rumen fluid was mixed and squeezed through a 4-layered cheesecloth into a Schott Duran® bottle (l) with an O_2-free headspace and immediately transported to laboratory at 39 °C where it was used as a source of inoculum. A mixture of meadow hay and barley meal (60:40 ratio) was used as a substrate. The *N. limnetica* algae were supplemented at the following levels: 0, 2, 4 and 6% on a DM basis. Treatments were as follows: control (substrate without *N. limnetica* algae), control + 2% *N. limnetica* of DM, control + 4% *N. limnetica* of DM, and control + 6% *N. limnetica* algae of DM. Each treatment was tested in 4 replicates accompanied by blank vessels (no substrate). The incubation vessels included the following: 4 vessels for each tested treatment, 4 control vessels without any supplements and 4 vessels as blanks (without substrate). Milled substrate (400 mg) was added to the 100 ml incubation vessels. Each vessel was filled with 40 ml of the incubation medium (mg: K_2HPO_4 292, KH_2PO_4 240, $(NH_4)_2SO_4$ 480, NaCl 480, $MgSO_4 \cdot 7H_2O$ 100, $CaCl_2 \cdot 2H_2O$ 64, Na_2CO_3 4, cysteine hydrochloride 600) per 1 l of double-distilled water (ddH_2O) and dispensed anaerobically in the 1:4 (v/v) ratio. The treatments were incubated at 39 °C for 48 h and the experiment was repeated once in 2 consecutive batch experiments.

After 48 h of incubation, the pH of the rumen fluid was measured (pH-meter CP-104, ELMETRON, Zabrze, Poland). Overall quantity of methane gas was determined according to Makkar and McSweeney (2005). Quantitative analysis of ammonia concentration was carried out by a modified Nessler's method (Szczechowiak et al., 2016). The volatile fatty acids (VFA) were determined by GC (GC Varian CP 3380, Sugarland, TX, USA) according to Szczechowiak et al. (2016) with some modifications. The measurements of FA in the rumen fluid were carried out as described by Szczechowiak et al. (2016), but initially 3 ml of 2 M NaOH were added to 2500 mg of rumen fluid. The methane concentration was also analysed after 48 h of incubation. A 500 µl gas was sampled from the gastight syringe (GASTIGHT® Syringes, Hamilton Robotics, Bonaduz, Switzerland) into SRI310 GC (Alltech, PA, USA) equipped with a thermal conductivity detector (TCD) and Carboxen-1000 column (matrix 60/80, 4.6 m × 2.1 mm, Supelco – Sigma-Aldrich, St. Louis, MO, USA). After 48 h of incubation, both substrates with or without *N. limnetica* and their subsequent residues were dried at 70 °C and subsequently analysed for DM and organic matter (OM) digestibility.

For protozoa count determination, the content of the serum flasks after incubation were mixed properly and 1-ml samples were mixed with 6 ml of 4% formaldehyde. Whereas for the total number of bacteria determination, 20 µl of sample was added to 6980 µl of Hayem solution. The protozoa and bacteria numbers were determined microscopically (light microscope Zeiss, type Primo Star no. 5, Jena, Germany). The protozoa were counted in the drop of rumen fluid with the defined volume (100 µl), with the division of the *Holotricha* and *Entodiniomorpha* groups. Whereas the bacteria were determined with Thoma chamber (0.02 mm depth, Blau Brand, Wertheim, Germany).

Statistical analysis

The results of this study were subjected to one-way analysis of variance using PROG-GLM of SAS software (version 9.3; SAS Institute Inc., Cary, NC, USA). The differences between means were then verified by Duncan's post hoc test. Differences were considered significant at $P \leq 0.05$.

Results and discussion

The aim of this study was to evaluate the potential use of *N. limnetica* algae in ruminant diets either as a source of nutrients, or as a dietary additive modulating rumen fermentation parameters. *N. limnetica* (Table 1) is a rich source of CP (238 g · kg^{-1} DM), minerals (g · kg^{-1} DM: Ca 48.7, Na 31.8) and unsaturated FA (51.7 g · 100 g^{-1} FA).

Table 1. Chemical composition, mineral content and fatty acid proportions in meadow hay, barley and *Nannochloropsis limnetica*

Indices	Meadow hay	Barley	*N. limnetica*
Chemical composition[1], g · kg^{-1} DM			
OM	942	975	717
ash	58.2	24.2	283
CP	121	121	238
EE	17.2	21.9	41.0
CF	247	54.2	30.2
Mineral content, g · 100 g^{-1} DM			
Ca	0.54	0.08	4.87
K	2.42	0.52	0.66
Na	0.43	0.01	3.18
Mg	5.37	1.61	0.71
Zn	2.68	3.92	0.49
Fe	52.5	14.5	0.44
Fatty acid proportion, g · 100 g^{-1} FA			
C14:0	3.01	0.79	3.34
C16:0	25.1	21.4	30.6
C18:0	4.56	1.67	6.06
C18:1*c*9	6.33	10.7	10.7
C18:1*c*11	0.65	0.64	3.83
C18:2*c*9*c*12	19.9	58.5	7.30
C18:3*c*9*c*12*c*15	37.3	6.05	5.78
C20:5*n*3	nd[7]	nd	0.55
C22:5*n*3	nd	nd	1.16
C22:6*n*3	nd	nd	1.02
others[2]	3.15	0.25	29.7
SFA[3]	33.7	23.9	48.3
UFA[4]	66.3	76.1	51.7
MUFA[5]	8.98	13.3	36.2
PUFA[6]	57.3	62.8	15.4
n-6	21.4	58.6	11.9
n-3	37.4	6.15	10.1

[1] chemical composition: OM – organic matter, CP – crude protein, EE – ether extract, CF – crude fibre; [2] others – sum of: C6, C8, C10, C10:1, C12, C14:1, C15, C15:1, C17, C17:1, *c*12C18:1, C19, C20, C20:1*n*9, C20:3*n*3, C20:4*n*6, C22, C24, C24:1; [3] SFA – sum of saturated fatty acids (C6, C8, C10, C12, C14, C15, C16, C17, C18, C19, C20, C22, C24); [4] UFA – sum of unsaturated fatty acids (C10:1, C14:1, C15:1, C16:1, C17:1, *c*9C18:1, *c*11C18:1, *c*12C18:1, *c*9*c*12C18:2, *c*9*c*15C18:2, *c*9*c*12*c*15C18:3, C20:1*n*9, C20:3*n*3, C20:4*n*6, C20:5*n*3, C22:1*n*9, C22:2, C22:5*n*3, C22:6*n*3, C24:1); [5] MUFA – sum of monounsaturated fatty acids (C10:1, C14:1, C16:1, C17:1, *c*9C18:1, *c*11C18:1, *c*12C18:1, C20:1*n*9, C22:1*n*9, C24:1); [6] PUFA – sum of polyunsaturated fatty acids (*c*9*c*12C18:2, *c*9*c*15C18:2, *c*9*c*12*c*15C18:3, C20:3*n*3, C20:4*n*6, C20:5*n*3, C22:2, C22:5*n*3, C22:6*n*3); [7] nd – non detected

Moreover, leucine and lysine are the most abundant essential amino acids (Table 2). The isolated *N. limnetica* was characterized by a low crude fibre content which was lower than that reported in seawater *Nannochloropsis* spp. *N. limnetica* and *N. oculata* (Rebolloso-Fuentes et al., 2001). Rebolloso-Fuentes et al. (2001) reported that other *Nannochloropsis* spp. that are grown in sterilized seawater have relatively high (18.4%) total lipids

Table 2. Amino acids proportion in *Nannochloropsis limnetica*

Indices	%
Essential AA[1]	
histidine	2.14
arginine	5.16
threonine	3.72
valine	5.85
methionine	1.69
isoleucine	4.63
leucine	9.16
phenylalanine	5.10
lysine	5.27
total essential AA	42.7
Non-essential AA	
aspartic	7.33
glutamic	16.7
serine	4.26
glycine	8.68
proline	8.17
alanine	7.72
tyrosine	2.97
cystine	1.45
total non-essential AA	57.3
Total AA	100
EAA/N-EAA	0.74

[1] AA – amino acids

content. In the study of Ma et al. (2014) *N. granulata* attained the highest lipid content around 60.35%, while *N. salina* showed the lowest – around 36.95% and *N. limnetica* – medium around 41.17%, which was relatively higher in comparison to the isolated in this study freshwater *N. limnetica,* that accumulated approximately 4% total lipids. *N. limnetica* in this study was relatively well digested since IVDMD and IVCPD were 62.96 and 73.56%, respectively.

The pH value increased significantly with 2 and 6% of *N. limnetica* supplementation in comparison to the control (Table 3). Ammonia concentration decreased at 2% of *N. limnetica* supplementation, and no effect was found at 4 and 6% additions. The total bacteria count was affected by the 2 and 4% levels of *N. limnetica* supplementation; however, it significantly decreased at 6% supplementation. The results of the batch culture study clearly indicate that total bacteria count was negatively affected when *N. limnetica* algae were supplemented at more than 4%. Probably too much PUFA inhibited bacterial activity particularly during the lag-phase of microbial development, making bacteria more sensitive to the presence of UFA at the beginning of the incubation (Maczulak et al., 1981).

The total VFA concentration (Table 3) seemed to be unaffected by the algae supplementation. However, significant increases ($P < 0.05$) in pro

Table 3. Effect of *Nannochloropsis limnetica* algae supplementation on rumen fermentation and microbial parameters after 48 h of incubation in batch culture system

Indices	Treatment[1]				SEM	*P*-value
	control	2%	4%	6%		
pH	6.42[c]	6.59[a]	6.45[bc]	6.50[b]	0.020	0.0004
N-NH_3, mmol · l^{-1}	18.2[a]	16.9[b]	17.7[ab]	18.4[a]	0.209	0.0231
Methane, mmol	12.2	13.1	13.4	13.4	0.288	0.4436
TGP[2], ml	133	131	130	128	0.873	0.3926
Total number of bacteria, $10^7 \cdot ml^{-1}$	14.1[a]	15.3[a]	15.7[a]	11.4[b]	0.583	0.0057
Holotricha count, $10^2 \cdot ml^{-1}$	29.8	18.7	16.3	25.7	2.129	0.0603
Entodiniomorpha count, $10^3 \cdot ml^{-1}$	33.2	33.3	28.2	40.7	2.292	0.3126
IVDMD[3], %	43.8	40.5	44.3	37.0	1.310	0.1644
IVOMD[4], %	53.7	53.1	59.6	50.8	1.658	0.3057
Volatile fatty acids (VFA), mM						
total VFA	70.9	71.9	71.6	73.6	1,832	0.0965
acetic (A)	40.3	40.2	40.6	38.8	1.017	0.1236
propionic (P)	11.7[b]	13.5[ab]	14.0[a]	14.8[a]	0.614	0.0423
butyric	11.7	11.7	10.5	11.3	0.409	0.1763
isobutyric	3.52	2.48	2.37	4.10	0.330	0.0612
isovaleric	2.34[b]	2.71[ab]	2.73[ab]	3.09[a]	0.167	0.0497
valeric	1.26[b]	1.34[ab]	1.32[ab]	1.58[a]	0.077	0.0482
A:P ratio	3.54[a]	3.06[ab]	3.15[ab]	2.67[b]	0.107	0.0327

[1] treatments: control (substrate without *N. limnetica* algae), control + 2% *N. limnetica* of DM, control + 4% *N. limnetica* of DM, and control + 6% *N. limnetica* algae of DM; [2] TGP – total gas production; [3] IVDMD – *in vitro* dry matter digestibility; [4] IVOMD – *in vitro* organic matter digestibility; [abc] – means with different superscripts within a row are significantly different at $P < 0.05$; SEM – standard error of means

Table 4. Rumen fluid fatty acids proportion affected by *Nannochloropsis limnetica* algae supplementation at different levels after 48 h of incubation using batch culture system

Fatty acids (FA)	Treatment[1]				SEM	*P*-value
	control	2%	4%	6%		
g · $100 g^{-1}$ of FA						
C10:0	0.26	0.34	0.46	0.48	0.038	0.1267
C12:0	1.02[b]	1.09[b]	1.35[a]	0.65[c]	0.065	0.0004
C14:0	1.75[b]	1.91[b]	2.32[a]	2.06[ab]	0.067	0.0100
C16:0	20.6[b]	21.2[a]	20.6[b]	20.6[b]	0.099	0.0356
C18:0	43.9[a]	42.5[a]	39.5[b]	39.8[b]	0.554	0.0028
C18:1*c*9	4.35	3.93	5.08	5.06	0.282	0.4011
C18:2*c*9*c*12	2.10	2.08	2.43	2.17	0.090	0.4148
C18:2*c*9*c*15	0.25	0.23	0.24	0.23	0.016	0.9970
C18:3*c*9*c*12*c*15	1.15	1.07	1.15	1.23	0.025	0.1962
C18:2*c*9*t*11	0.27[b]	0.48[a]	0.52[a]	0.41[a]	0.032	0.0240
C18:2*t*10*c*12	0.21[b]	0.28[ab]	0.32[ab]	0.39[a]	0.023	0.0372
C20:5*n*3	0.16[b]	0.14[b]	0.34[a]	0.54[a]	0.041	0.0004
SFA[2]	73.6[a]	73.6[a]	71.7[ab]	70.8[b]	0.426	0.0268
UFA[3]	26.4[b]	26.4[b]	28.3[ab]	29.2[a]	0.427	0.0268
MUFA[4]	20.0	19.6	20.8	21.5	0.327	0.1819
PUFA[5]	6.37[b]	6.76[b]	7.55[a]	7.70[a]	0.167	0.0024
n-6	4.09	4.17	4.54	4.41	0.110	0.4743
n-3	2.07[c]	2.13[bc]	2.48[ab]	2.79[a]	0.083	0.0018
n-6:*n*-3	2.01	2.00	1.82	1.61	0.079	0.1703
PUFA:SFA	0.08[b]	0.09[b]	0.10[a]	0.11[a]	0.003	0.0007

[1] see Table 4; [2] SFA – sum of saturated fatty acids (C6, C8, C10, C12, C12*iso*, C12*aiso*, C13*iso*, C14, C14*iso*, C14*aiso*, C15, C15*iso*, C16, C16*iso*, C16*aiso*, C17, C17*iso*, C17*aiso*, C18, C19, C20, C22, C24); [3] UFA – sum of unsaturated fatty acids (C10:1, C14:1, C15:1, C16:1, C17:1, *t*5C18:1, *t*6-8C18:1, *t*9C8:1, *t*10C18:1, *t*11C18:1, *t*12C18:1, *t*15C18:1, *c*9C18:1, *c*11C18:1, *c*12C18:1, *c*13C18:1, *c*14C18:1, *c*15C18:1, *t*10*c*12C18:2, *c*9*t*12C18:2, *c*9*c*12C18:2, *c*9*c*15C18:2, *c*9*c*12*c*15C18:3, C20:1*n*9, C20:3*n*3, C20:4*n*6, C20:5*n*3, C22:1*n*9, C22:2, C22:5*n*3, C22:6*n*3, C24:1); [4] MUFA – sum of monounsaturated fatty acids (C10:1, C14:1, C15:1, C16:1, C17:1, *t*5C18:1, *t*6-8C18:1, *t*9C18:1, *t*10C18:1, *t*11C18:1, *t*12C18:1, *t*15C18:1, *c*9C18:1, *c*11C18:1, *c*12C18:1, *c*13C18:1, *c*14C18:1, *c*15C18:1, C20:1*n*9, C22:1*n*9, C24:1); [5] PUFA – sum of polyunsaturated fatty acids (*t*10*c*12C18:2, *c*9*t*12C18:2, *c*9*c*12C18:2, *c*9*c*15C18:2, *c*9*c*12*c*15C18:3, C20:3*n*3, C20:4*n*6, C20:5*n*3, C22:2, C22:5*n*3, C22:6*n*3); [abc] – means with different superscripts within a row are significantly different at $P < 0.05$; SEM – standard error of means

pionic, valeric and isovaleric acids were found in treatments with *N. limnetica* supplementation in comparison to the control. The proportion of palmitic acid (C16:0) was significantly increased by 2% level of supplementation, and stearic acid (C18:0) was decreased (P = 0.003) by the 4 and 6% levels of *N. limnetica* algae treatments (Table 4). An increase in eicosapentaenoic acid as well as *n-3* FA contents was also found when 4 and 6% of *N. limnetica* were added. These changes in FA proportion contributed to slightly lower (P = 0.027) saturated fatty acids (SFA) proportion at the 6% level of microalgae supplementation. Using *N. limnetica* microalgae at 6% on DM basis in this *in vitro* study led to an increase in UFA proportion and a decrease in SFA proportion. *N. limnetica* led to an increase in the *cis*-9, *trans*-11 C18:2 as well as polyunsaturated fatty acids (PUFA) and *n-3* fatty acids (Table 4). The results of the current study are consistent with those of Boeckaert et al. (2008), who reported that dietary supplementation of the algae reduced rumen SFA content while contents of monounsaturated fatty acid, PUFA, odd-, and branched-chain fatty acids increased. In the present study, the content of stearic acid was also decreased. This may suggest that the FA contents of *N. limnetica* affected bacteria involved in the rumen lipid metabolism (mainly in the biohydrogenation process).

Conclusions

Freshwater microalgae *Nannochloropsis limnetica* affected rumen fermentation, mainly by increasing propionic acid concentration. However, a high dose (6%) decreased rumen bacteria count. Further research under commercial farm conditions should be conducted to confirm the possibility of using the *N. limnetica* as feed additive for ruminants.

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