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Rumen fermentation and microbial population in lactating dairy cows receiving diets containing oilseeds rich in C-18 fatty acids

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Abstract

Sixteen Holstein rumen-cannulated primiparous milking dairy cows were fed a control diet (CN) based on maize silage and soyabean meal during a 4-week period before the start of a 21-d experiment with oilseeds containing high concentration of linoleic acid (Linola™) or linolenic acid (NuLin™). Thereafter, four cows received *ad libitum* one of each of four dietary treatments comprising of CN, Linola (LN), NuLin (NL) and LN/NL (50/50 % combination). Each LN, NL and LN/NL treatment contained 6 % oil of DM. Rumen digesta samples were collected on days 6, 11, 16 and 21 and milk samples on days 13, 15 and 17. There were no effects ($P>0{\cdot}05$) of the oilseeds on pH and concentrations of NH_3-N and total volatile fatty acids, while the acetate:propionate ratio was decreased ($P<0{\cdot}05$). The oilseeds also decreased ($P<0{\cdot}05$) protozoa and increased ($P<0{\cdot}1$) total cellulolytic bacteria in rumen fluid, especially when containing high dietary linoleic acid ($P<0{\cdot}05$). The milk protein concentration was increased ($P<0{\cdot}1$) by the dietary linoleic acid, which produced most beneficial results. It was concluded that supplements of linoleic acid in diets of ruminants might contribute to better digestion of dietary fibre and increased quality of milk.

Key words: Dairy cows: Linoleic acid: Linolenic acid: Rumen fermentation: Rumen microbial population

The negative effects of the presence of ciliate protozoal species in the rumen on the efficiency of the ruminal microbial synthesis of protein and on the duodenal flow of non-NH_3-N components are well established[1–4]. These effects result in the lower dietary N utilisation by ruminants[5,6] and increase the dietary protein requirement by about 30 %[7]. As ciliate protozoa are not considered by many to be essential for the well being of the host ruminant, numerous chemical and physical methods have been applied experimentally over the last few decades to eliminate ciliate protozoa from the rumen (defaunation), but none of the methods was practical for use in ruminant production[8,9]. Also, a fauna-free sheep flock was established by a protozoa-free breeding programme[10,11] and maintained with normal growth and without protozoa re-infection for several generations (over 20 years).

The dietary lipids, including C-18 fatty acids, are toxic to rumen ciliate protozoa[12–14] and may result in either a total elimination (defaunation) or in a significant reduction of the rumen protozoal population (reduced fauna)[15]. Because the large vestibuliferid and cellulolytic protozoal species are highly susceptible to lipids, more than the small protozoal species, reduced fauna usually contains only *Entodinium* sp.[15]. All, fauna-free, defaunation and reduced fauna contribute to increased efficiency of the ruminal microbial synthesis of protein[4,16–18]. Decreased protozoa numbers due to dietary lipids have been reported earlier in several studies[19]. But, dietary lipids can also reduce the rumen population of cellulolytic bacteria[20], especially due to their content of mid-chain fatty acids that are toxic to such bacteria[21], resulting in an undesirable lower digestibility of dietary fibre[22]. Such lower digestibility is due to both decreased population of cellulolytic bacteria and to the elimination of cellulolytic protozoal species from the rumen[15]. In spite of the total elimination of rumen ciliate protozoa and associated loss of the protozoal cellulolytic activity[23] due to dietary supplements of a high-linoleic acid (C-18 : 2) variety of sunflower seeds in a concentrate diet, higher digestion of the dietary fibre was achieved in lambs[7]; this was accompanied with a lower dietary protein requirement. It was, therefore, hypothesised that the increased dietary concentration of linoleic acid had positive effects on some cellulolytic species of rumen bacteria. The hypothesis was tested in the present experiment with the rumen-cannulated

Abbreviations: CN, control diet; Linola™, linoleic acid; LN, linoleic acid; NuLin™, linolenic acid; NL, linolenic acid.

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lactating dairy cows. In addition, effects of the dietary linolenic acid (C-18 : 3) and the combination of linoleic and linolenic acids were also tested. The results are discussed herein and show decreases in the rumen protozoal population and increases in the total population of measured cellulolytic species of rumen bacteria due to the dietary supplements of two oilseeds high in C-18 : 2 (Linola™) or C-18 : 3 (NuLin™) fatty acids; these oilseeds are varieties of flaxseed.

Materials and methods

Sixteen Holstein rumen-cannulated primiparous milking dairy cows were used in the present experiment. The cows weighed 695 (SEM 35) kg and were housed in tie stalls in a dairy barn. During a 4-week period before the start of the 21-d experiment a control diet (CN; Table 1) was given to all cows. Thereafter, the cows were randomly assigned to four equal treatment groups. One group of four cows continued to receive the CN diet throughout the experiment (treatment CN). On the morning of day 1, cows in each remaining three groups were given one of the additional three diets (Table 1) containing high C-18 : 2 Linola (treatment LN), high C-18 : 3 NuLin (treatment NL) or 50/50 % combination of Linola and NuLin (treatment LN/NL). Each of the three diets (LN, NL and LN/NL) contained 5 % of C-18 fatty acids (6 % of oil) of the dietary DM. Fatty acid composition of the oilseeds used is summarised in Table 2. All total mixed diets were prepared each morning and a small quantity was taken and accumulated as a sample. The cows were milked daily at 07.00 and 19.00 hours. There were no health or feed intake problems with any cow during the experiment. The cows were cared for according to the guidelines of the Canadian Council on Animal Care[24] and the experimental protocol was reviewed and approved by the Institutional Animal Protection Committee. All cows were given diets daily at 08.00 hours for *ad libitum* intake (6 % orts) throughout the experiment. Feed and refusals were collected and sampled daily, and individual feed intakes were recorded throughout the experiment. Fresh drinking-water was available continuously.

Rumen digesta samples were collected on days 6, 11, 16 and 21 of the experiment, 2 h after the morning feeding. Rumen contents (600 ml) were obtained through the cannulas from different parts of the rumen of each cow using a 100 ml syringe attached to a plastic tube. The pH of the rumen contents was measured immediately. Approximately 200 ml of the rumen contents were then strained through one layer of cheesecloth for enumeration of protozoa. A measure of 5 ml of the filtrate was preserved with 5 ml of methylgreen-formalin-saline solution[25] and ciliate protozoa were counted using a Neubauer Improved Bright-Line counting cell (0·1 mm depth; Hausser Scientific). Each sample was counted twice, and if the CV of the two counts was greater than 10 % the counts were repeated. Another sample of approximately 200 ml of the rumen contents was strained through four layers of cheesecloth and 10 ml of rumen fluid was combined with 2 ml of 25 % (w/v) *meta*-phosphoric acid and analysed for volatile fatty acids and NH_3-N, as described previously[18].

The rest of the samples of each rumen contents was used for real-time PCR to quantify total bacteria[26], cellulolytic bacteria[27] and methanogenic archaea[28]. The DNA from rumen fluid samples and from pure cultures was extracted in duplicate by physical disruption using a repeated bead beating plus column purification for bacteria[29], methanogens[29] and cellulolytic bacteria[30]. Standard curves for the absolute quantification of total bacteria were obtained using DNA from *Fibrobacter succinogenes* S85, *Ruminococcus albus* AR67 and *Ruminococcus flavefaciens* Y1. For cellulolytic bacteria, three closely related strains were used, *F. succinogenes* GC5, *R. albus* 7 and *R. flavefaciens* C52. All bacterial strains

Table 1. Composition of the experimental diets

Dietary treatment...	Control	Linola	NuLin	Linola/NuLin
Ingredients (g/kg DM)				
Hay	68	68	68	69
Maize silage	438	412	407	411
Sugarbeet pulp	54	55	55	54
Maize grain	168	112	113	112
Soyabean meal	167	134	138	137
Barley grain	36	36	36	36
Concentrate*	37	38	38	37
Vitamin–mineral supplement†	32	28	28	28
Linola™ (crashed)	0	117	0	58
NuLin™ (crashed)	0	0	117	58
Chemical analysis (g/kg DM)				
Crude protein	179	192	192	167
Acid-detergent fibre	159	156	151	174
Neutral-detergent fibre	269	267	254	288

Linola, linoleic acid; NuLin, linolenic acid.
* Contained (g/kg DM): canola meal, 200; maize gluten meal, 300; soyabean meal, 200; brewer's maize, 300.
† Contained (g/kg): Ca, 140; P, 50; Mg, 39; Na, 100; S, 12; K, 14; NaCl, 75; (mg/kg) Zn, 1760; I, 85; Co, 31; Mn, 2850; Cu, 354; Fe, 2154; Se, 15; (mmol/kg) vitamin A, 4041; vitamin D_3, 5·34; vitamin E, 22 311.

Table 2. Chemical composition of oilseeds

Oilseed...	Linola™		NuLin™	
	Composition	% of lipids	Composition	% of lipids
Proximal				
DM (g/kg)	940		941	
Crude protein (g/kg DM)	244		222	
Lipids (g/kg DM)	479		513	
Acid-detergent fibre (g/kg DM)	86		118	
Neutral-detergent fibre (g/kg DM)	138		123	
Fatty acids (g/kg DM)				
C-14 : 0	0·25	0·05	0·18	0·03
C-15 : 0	0·06	0·01	0·07	0·01
C-16 : 0	30·60	6·39	22·41	4·37
C-16 : 1	0·47	0·10	0·26	0·05
C-18 : 0	15·02	3·13	11·81	2·30
C-18 : 1	82·40	17·20	59·31	11·56
C-18 : 2	335·00	69·94	64·10	12·49
C-18 : 3	8·80	1·84	346·00	67·45
C-20 : 4	0·09	0·02	0·12	0·02
Total C-18	441·31	92·11	481·34	93·80

Linola™, linoleic acid; NuLin™, linolenic acid.

were grown overnight in a rumen fluid-based medium in culture at 39°C, as reported previously[31].

For total bacteria, cultures were centrifuged and the cells were re-suspended in clarified rumen fluid. The bacterial cell densities were determined by microscopic enumeration using a Helber counting chamber (Weber Scientific Instruments) at 400 × magnification. The DNA was then extracted from a known number of cells and used in a 5 log dilution series (at a range of 1×10^6 to 1×10^{10} cells). Bacterial universal real-time PCR primers 1114F and 1221R were used to quantify total bacteria[26]. Real-time PCR amplifications were performed on an icycler (Bio-Rad Laboratories Private Limited) in a 25 μl volume containing the following reagents: 1·0 μl template DNA (10 ng), 400 nM (final concentration) of each primer, 12·5 μl iQ SYBR Green supermix (Bio-Rad) and 9·5 μl double distilled water. Real-time PCR amplification was initiated by a hot start at 95°C for 15 min, followed by forty cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 60 s. A final melting curve analysis was carried out by continuously monitoring fluorescence between 60 and 95°C, with 0·5°C increments every 10 s. Analyses were performed in triplicate with correlation coefficients >0·995.

For cellulolytic bacteria, the number of cells in fresh overnight cultures was determined by microscopic enumeration using a Petroff-Hausser counting chamber at 400 × magnification and dilution in the Norris-Powell solution[32], as described previously[33]. Counts were repeated four times for each bacterial group. Real-time PCR amplifications of each bacterial species were carried out on an ABI 7500 Fast Real-Time PCR System (PE Applied Biosystems) in a 10 μl total volume containing: 5 μl of TaqMan Universal PCR Master Mix (PE Applied Biosystems); 2 μl of diluted DNA; forward primers; reverse primers; and probes at 300, 300 and 200 nM for *R. flavefaciens*, 900, 900 and 200 nM for *R. albus*, 300, 300 and 200 nM for *F. succinogenes*. Selected primer sets and probes were described previously[27]. Analyses were performed in triplicate. The cycling parameters were 95°C for 20 s, followed by forty cycles of 95°C for 3 s and 60°C for 30 s. Real-time TaqMan assays were calibrated by the cycle threshold method[34]. The standard curves for each primer/probe set were linear (correlation coefficients >0·993) and used in a 5 log dilution series. Amplification efficiencies, calculated from the slopes, ranged from 88 to 95 %. A standard curve was made for each analysis run.

For methanogens, external standards were prepared using a mixture of pure cultures of *Methanobrevibacter ruminantium* M1 and *M. smithii* PS[28] and ranged from $1{\cdot}0 \times 10^3$ to $1{\cdot}0 \times 10^7$ cells. Real-time PCR amplification was performed using the methanogen methyl coenzyme-M reductase (mcrA) primers mcrA-F and mcrA-R[28]. Real-time PCR amplifications were carried out with the Bio-Rad Icycler in a 25 ml volume containing the same reagents and under the same PCR protocol as the total bacteria real-time conditions.

The oilseed, refusals (for the determination of DM only) and feed samples were ground to pass through a 1-mm sieve (Wiley Mill model 4; Thomas Scientific) prior to the determination of DM, organic matter, diethyl ether extract, neutral-detergent fibre, acid-detergent fibre and fatty acids. Analytical DM was determined by drying samples at 135°C in an oven for 2 h, followed by hot weighing. The organic matter content was calculated as the difference between 100 and the percentage ash[35]. The determination of fibre fractions, neutral-detergent fibre and acid-detergent fibre, without sodium sulphide and expressed free of ash, was based on the procedure of Van Soest *et al.*[36]. The diethyl ether extract and Kjeldahl N were determined according to the procedure of the Association of Official Analytical Chemists[35]. Methylation of fatty acids in oilseed samples was conducted according to Folch *et al.*[37] and methyl esters of fatty acids were separated and quantified using a gas chromatograph (Agilent 6890; Agilent Technologies) equipped with a flame ionisation detector.

Daily milk samples were collected from the two daily milking sessions, combined proportionally to the milk produced on days 13, 15 and 17 of the experiment and analysed for fat, protein and lactose by IR spectroscopy (Bently 2000; Bently Instrument, Inc.).

The present experiment was conducted to obtain practical, uncomplicated results and their interpretation on the main effects of the dietary fatty acids used on the microbial population in the rumen and milk composition. It was not the objective of the experiment to determine interactions. Therefore, the results on individual parameters obtained from the individual chemical analysis of samplings at different days during the experiment were averaged for each cow. The data were statistically analysed as a completely randomised design with cows nested in treatments. To account for the possibility of unequal variances among treatments, the MIXED procedure from SAS (SAS Institute, Inc.)[38] was used to analyse the data with a variance component and a banded covariance structure, and the best covariance structure was selected for the final analysis for each dependent variable. Least square means were calculated for each treatment, and multiple comparisons were conducted using Tukey's adjustment to evaluate differences among treatment means. Differences were declared significant at $P<0·05$ and trends were discussed at $P<0·1$.

Results

Rumen fermentation

There were no differences ($P>0·05$) among the treatments in feed consumption, rumen pH and concentrations of NH_3-N in rumen fluid (Table 3). The concentration of lactic acid was higher ($P<0·05$) for the treatment LN/NL than for the treatments CN and LN, but not different ($P>0·05$) from the treatment NL; the concentration was not different ($P>0·05$) among the treatments CN, LN and NL. The concentration of total volatile fatty acids in the rumen fluid was not affected ($P>0·05$) by the treatments, but there were some differences in the molar proportion of individual fatty acids among the treatments. Thus, the proportion of acetate was lower ($P<0·05$) for the treatment NL than for the treatment CN, but the other differences among the treatments were not significant. The proportions of propionate and isovalerate were lower ($P<0·05$) for the treatment CN than for the other treatments (LN, NL and LN/NL), but the differences among the treatments LN, NL and LN/NL were not significant. The ratio of acetate:propionate was higher ($P<0·05$) for the treatment CN than for the other treatments (LN, NL and LN/NL), but the other differences among the treatments were not significant. There was a trend for the concentration of valerate to be higher ($P<0·1$) for the treatment NL than for the treatment CN, but the means for the other treatments (LN, NL and LN/NL) were similar ($P>0·1$). There were no significant differences among the treatments for the molar proportions of isobutyrate, butyrate and caproate.

Rumen microbial populations

The concentration of total ciliate protozoa in rumen fluid was different ($P<0·05$) for each CN, LN and NL treatments. However, for the treatment LN/NL, the concentration was lower ($P<0·05$) than for the treatments CN and NL, but similar ($P>0·05$) to the treatment LN; numerically, the protozoa concentration ranked CN > NL > LN > LN/NL (Table 4). Relative to the treatment CN, the treatment LN/NL reduced the number of protozoal cells in rumen fluid by 93 %, the treatment LN by 87 % and the treatment NL by 70 %. The concentration of total methanogenic archaea and total rumen bacteria were not affected ($P>0·05$) by the dietary treatments. However, there were major effects of the treatments on the total and individual species of cellulolytic bacteria. Thus, the concentration

Table 3. Feed intake and effects of individual and combination of dietary oilseeds rich in C-18 : 2 (Linola) and C-18 : 3 (NuLin) on fermentation in the rumen of milking cows

(Mean values with their standard errors)

Dietary treatment…	Control	Linola	NuLin	Linola/NuLin	SEM
Average daily DM intake (kg/cow)	23·2	23·3	22·8	22·4	1·2
Rumen pH*	5·9	6·1	6·0	5·9	0·1
NH_3-N (mg/100 ml)*	21·4	21·0	22·2	19·8	1·1
Lactic acid (mg/100 ml)*	1·7^{a}	2·1^{a}	4·3^{a,b}	6·2^{b}	0·7
Volatile fatty acids*					
Total concentration (mg/100 ml)	97·3	90·0	100·5	93·4	2·8
Molar proportion (mol/100 mol)					
Acetate	50·9^{a}	48·0^{a,b}	46·4^{b}	46·7^{a,b}	0·7
Propionate	22·4^{a}	26·6^{b}	28·0^{b}	29·1^{b}	0·9
Isobutyrate	2·2	2·2	2·2	2·0	0·1
Butyrate	19·2	17·0	16·0	16·1	0·6
Isovalerate	2·0^{a}	2·6^{b}	3·1^{b}	2·6^{b}	0·1
Valerate	2·5^{A}	3·0^{A,B}	3·5^{B}	2·9^{A,B}	0·2
Caproate	0·8	0·6	0·8	0·6	0·1
Acetate:propionate	2·3^{a}	1·8^{b}	1·7^{b}	1·6^{b}	0·1

^{a,b} Mean values within a row with unlike superscript lower case letters were significantly different ($P<0·05$).
^{A,B} Mean values within a row with unlike superscript capital letters were significantly different ($P<0·1$).
* Average from individually analysed samples taken on days 6, 11, 16 and 21.

British Journal of Nutrition

Table 4. Effects of individual and combination of dietary oilseeds rich in C-18 : 2 (Linola) and C-18 : 3 (NuLin) on the concentrations of ciliate protozoa in rumen fluid (cell no/ml), and of methanogenic archaea and bacteria in rumen contents (cell no/g wet weight) of milking cows*
(Mean values with their standard errors and percentages)

Dietary treatment…	Control	Linola		NuLin		Linola/NuLin		SEM
		Mean	% difference from control	Mean	% difference from control	Mean	% difference from control	
Total ciliate protozoa $\times 10^3$	620^{a}	78^{b}	−87	184^{c}	−70	45^{b}	−93	60
Total methanogenic archaea $\times 10^5$	272	325	+19	177	−35	229	−16	34
Total rumen bacteria $\times 10^8$	851	1031	+21	783	−8	827	−3	174
Cellulolytic bacterial species $\times 10^6$								
Fibrobacter succinogenes	$0{\cdot}5^{a}$	$0{\cdot}02^{b}$	−96	$0{\cdot}03^{b}$	−95	$0{\cdot}02^{b}$	−96	0·00
Ruminococcus flavefaciens	$225^{a,A}$	$385^{b,B}$	+71	$305^{a,b,A,B}$	+35	$322^{a,b,B}$	+43	23
Ruminococcus albus	$4{\cdot}0^{A}$	$8{\cdot}4^{B}$	+107	$8{\cdot}7^{B}$	+116	$8{\cdot}2^{B}$	+103	0·8
Total cellulolytic bacteria†	$229^{a,A}$	$394^{b,B}$	+72	$314^{a,b,A,B}$	+37	$330^{a,b,B}$	+44	23

[a,b] Mean values within a row with unlike superscript lower case letters were significantly different ($P<0{\cdot}05$).
[A,B] Mean values within a row with unlike superscript capital letters were significantly different ($P<0{\cdot}1$).
* Average from individually analysed samples taken on days 6, 11, 16 and 21.
† Sum of *F. succinogenes*, *R. flavefaciens* and *R. albus*.

of *F. succinogenes* was higher ($P<0{\cdot}05$) for the treatment CN than for the other treatments (LN, NL and LN/NL), while the differences among the treatments LN, NL and LN/NL were not significant. The reduction in the concentration of *F. succinogenes* due to the treatments LN, NL and LN/NL was 95–96%. Contrary to the decreased concentrations of *F. succinogenes*, the dietary oilseed treatments LN, NL and LN/NL tended to increase ($P<0{\cdot}1$) the concentration of *R. albus* compared to the treatment CN; the increase amounted to 103–116%. There were no differences ($P>0{\cdot}1$) among the treatments LN, NL and LN/NL. The concentrations of *R. flavefaciens* and total cellulolytic bacteria were higher ($P<0{\cdot}05$) for the treatment LN than for the treatment CN, but there were no differences ($P>0{\cdot}05$) among the treatments CN, NL and LN/NL and among the treatments LN, NL and LN/NL. However, there was also a trend for the concentrations of *R. flavofaciens* and total cellulolytic bacteria being higher ($P<0{\cdot}1$) for the treatment LN/NL compared to the treatment CN. In comparison with the treatment CN, the concentrations of *R. flavofaciens* and total cellulolytic bacteria were increased, respectively, by 71 and 72% due to the treatment LN, by 43 and 44% due to the treatment LN/NL and by 35 and 37% due to the treatment NL.

Milk components

The concentrations of fat and lactose in milk samples were not affected ($P>0{\cdot}05$) by the dietary treatments (Table 5). However, there was a trend for increased ($P<0{\cdot}1$) concentration of protein for the treatments LN and NL compared to the treatment CN; there were no differences ($P>0{\cdot}1$) between the treatments CN and LN/NL and between LN and NL.

Discussion

We have previously discovered that the dietary supplement of linoleic acid (C-18 : 2) in the form of a high-C-18 : 2 variety of sunflower seeds increased the digestion of the dietary fibre and decreased the requirement of dietary protein in growing lambs[7]. We have postulated at the time that the decrease in the dietary protein requirement was due to the decreased

Table 5. Effects of individual and combination of dietary oilseeds rich in C-18 : 2 (Linola) and C-18 : 3 (NuLin) on the concentration (g/kg) of components in milk samples of cows*
(Mean values with their standard errors)

Dietary treatment…	Control	Linola	NuLin	Linola/NuLin	SEM
Fat	35·4	23·9	26·8	39·8	3·6
Protein	$34{\cdot}2^{a}$	$39{\cdot}8^{b}$	$37{\cdot}2^{b}$	$36{\cdot}5^{a,b}$	0·9
Lactose	43·7	46·2	43·7	45·0	1·0

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0{\cdot}1$).
* Average from individually analysed composite daily samples taken on days 13, 15 and 17.

rumen protozoal population and the increase in the fibre digestion was probably due to an increase in the population of rumen cellulolytic bacteria. We suggested that the positive effect of the dietary linoleic acid on the rumen environment enhanced the growth of cellulolytic bacteria. The present results clearly confirm that the dietary unsaturated C-18 fatty acids, linoleic (C-18 : 2) and numerically linolenic (C-18 : 3), had positive effects on the total rumen cellulolytic bacteria, while notably decreasing the rumen protozoal population; the effects of the dietary linoleic acid appear to be superior to those of the linolenic acid and combination of both linoleic and linolenic acids. However, it should be noted that the rumen population of *F. succinogenes* was greatly reduced, while the populations of *R. flavefaciens* and *R. albus* were increased due to the dietary supplements of C-18 fatty acids in the present experiment. Henderson[21] showed that the growth of some strains of important rumen bacteria such as *Butyrivibrio* and *Ruminococcus* and the methanogen *Methanobacterium* are strongly inhibited by the presence of long-chain fatty acids. Such inhibition occurred in the present experiment of a relatively small Gram-negative *F. succinogenes*, but the growth of more important and larger *R. albus and R. flavefaciens* was greatly increased. It should be noted that the total rumen bacterial population was not affected by the dietary C-18 fatty acid supplements. Therefore, the increase in the total rumen population of the measured cellulolytic bacteria in the present experiment was at the expense of other bacterial populations.

Vegetable oil and oilseed supplements drastically reduced the rumen protozoal population and maintained low protozoa numbers during the duration of the supplementation in sheep[15,39]. This is extremely important because rumen protozoa are predators of bacteria and their consumption and digestion of bacteria is a wasteful process that contributes to the undesirable recycling of N in the rumen[40,41] and was found to decrease by 20–28 % the net amino acid supply for absorption in the intestinal tract of the host ruminant[42]. Complete removal of protozoa from the rumen (defaunation) is considered desirable for all situations in which the animal performance is limited by the availability of amino acids for absorption[43].

The total bacterial concentration in rumen fluid of cows receiving the two oilseeds was similar to the cows receiving the control diet. However, the rumen ciliate population in these cows was greatly reduced and it is well documented that such a reduction may increase the rumen bacterial population[44]. However, this was not the case in the present experiment and was contrary to our expectation.

The positive effect of the presently used dietary C-18 : 2 on the total population of the measured cellulolytic bacteria in the rumen appears to be direct. This is because a similar C-18 : 2-rich dietary sunflower seed supplement increased the digestion of dietary fibre in sheep; although the sheep used had large rumen protozoal populations before the start of the experiment, the populations drastically decreased to the point of sheep being almost free of protozoa, which would normally contribute to the rumen cellulosis[7]. To our knowledge, this is the first time from a nutritional point of view that both the protozoal and the cellulolytic bacterial populations were positively affected by a single dietary supplement; that is, the rumen protozoal population was notably reduced while the total measured cellulolytic bacterial population was notably increased. Although dietary fibre digestion was not measured in the present experiment, our previous results[7] clearly demonstrated a positive effect of the dietary sunflower seed variety high in C-18 : 2 on the digestion of dietary fibre in lambs fed high-concentrate diets. However, dietary oil originating from a normal (not high in C-18 : 2) variety of sunflower seeds did not affect *in vitro* gas production and growth and feed intake of sheep[15]. Similarly, there was no effect on the ruminal fibre digestion from the dietary linseed oil supplement in cows[45], but strong negative effects were obtained in sheep[46,47].

The results of the present experiment also indicated a positive effect of the dietary C-18 fatty acids, especially linoleic acid, on the quality of milk through the increased concentration of milk protein. The effect of dietary linoleic acid on milk production could not be assessed in the present metabolic-type experiment with rumen-cannulated cows; a production-type experiment with milking cows is required for such purpose. This is important, as reduced fauna increased milk yield by 13·5 % and milk protein by 13·3 %[48], while linoleic acid almost eliminated protozoa from the rumen[7]. The previous 13·3 % increase of milk protein[48] is in line with the 16·4 % increase due to the linoleic acid supplement in the present experiment. Furthermore, dietary linoleic acid resulted in 30 % reduction in the dietary protein requirement in growing lambs[7].

In conclusion, the above-mentioned evidence suggests that supplements of linoleic acid in diets of ruminants decrease the concentration of ciliate protozoa in the rumen while increasing the proportion of total cellulolytic bacteria in the rumen bacterial population. This might contribute to higher digestion of dietary fibre and increased quality of milk. However, production-type experiments are needed to confirm the possibility of such benefits from the dietary linoleic acid supplements and to determine the effect of the acid on total milk production.

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