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Effects of dietary sunflower seeds on rumen protozoa and growth of lambs

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Linoleic acid-rich sunflower-seed supplements (SSS) were used in two experiments (experiment 1, high-concentrate diets; experiment 2, high-forage diets) to study effects on rumen protozoa and the growth of lambs. Both experiments consisted of four treatments, two with a low-protein diet (120 g/kg) and two with a high-protein diet (160 g/kg). For both diets, one treatment was without (control) and one with the SSS (140 g/kg dietary DM). The lambs were fed *ad libitum* for 70 and 140 d in experiments 1 and 2, respectively. Thereafter, the digestibility of organic matter (OM), acid-detergent fibre and neutral-detergent fibre were determined for each diet with four lambs, and then all lambs were slaughtered and rumen fluid samples were collected and analysed. The results showed substantial decreases ($P<0{\cdot}001$) or total elimination of protozoa in the rumen fluid of the SSS-receiving lambs. In the first experiment the SSS also decreased ($P<0{\cdot}05$) feed intake, but an increase in average daily gain ($P<0{\cdot}06$) resulted in an improved ($P<0{\cdot}05$) feed:gain ratio. Also, the SSS increased ($P<0{\cdot}05$) the digestibility of fibre. In the second experiment the SSS decreased ($P<0{\cdot}05$) the OM digestibility, feed intake and growth of lambs. It was concluded that the use of sunflower-seed supplementation in high-concentrate diets of ruminants reduces rumen fauna, resulting in savings on dietary protein supplements and an increased digestion of feed.

Sunflower seeds: Rumen ciliate protozoa: Lamb growth

The microbial populations in the rumen consist mainly of bacteria, protozoa and fungi, and are involved in the digestion of feed in the rumen. Rumen bacteria provide the largest proportion of non-NH_3-N entering the small intestine of the host ruminant (Robinson *et al.* 1996). Rumen ciliate protozoa are predators of rumen bacteria (Williams & Coleman, 1992), but themselves contribute only a small proportion (10–15 %; Robinson *et al.* 1996) of non-NH_3-N for utilisation by the host. Overall, rumen protozoa decrease the intestinal flow of amino acids (AA), mainly those of bacterial origin, by 23–30 % (Ivan *et al.* 2000*a*). This is a sizable amount that in most cases must be replaced as dietary protein to ensure that the protein requirement of the animal is met. Elimination of protozoa from the rumen (defaunation) results in an increased growth rate in young ruminants fed diets high in energy and low in rumen undegradable protein (Bird & Leng, 1978, 1984; Bird *et al.* 1979). Defaunation is presently not practical due to the unavailability of a suitable defaunating agent (Hegarty, 1999). However, a considerable reduction in rumen fauna may also be beneficial as it increases milk yield and the protein:fat ratio in dairy cows (Moate, 1989). Sunflower-seed oil was found to be an effective dietary supplement that produces a massive reduction in the rumen protozoa population (Ivan *et al.* 2001). However, the extracted oil is relatively expensive for the purpose of dietary supplementation to ruminants. Therefore, we tested crushed sunflower seeds as a dietary antiprotozoal component (Ivan *et al.* 2003) and obtained results similar to those with a dietary sunflower-seed-oil supplement (Ivan *et al.* 2001). We hypothesised that a reduction in the rumen protozoa population should increase the rumen microbial synthesis of protein and, proportionally, reduce the requirement for dietary protein to sustain a similar growth in lambs. The objective of the present work was to test this hypothesis in two experiments with lambs, utilising sunflower-seed supplements (SSS) in concentrate- and forage-based diets. To measure the effects of the reduced protozoa populations on the dietary protein requirement, two, low (120 g/kg) and high (160 g/kg), dietary concentrations of protein were used in each experiment.

Materials and methods

Experiment 1

Fifty-six 14-week-old Canadian Arcott lambs were sheared, weighed and then divided according to weight and sex into four groups of fourteen animals each (seven males and seven females). The four dietary treatments were: (1) low-protein control (LPC); (2) low-protein diet with SSS

Abbreviations: AA, amino acids; ADG, average daily gain; ADF, acid-detergent fibre; HPC, high-protein control; HPS, high-protein diet with sunflower-seed supplement; LPC, low-protein control; LPS, low-protein diet with sunflower-seed supplement; NDF, neutral-detergent fibre; OM, organic matter; SSS, sunflower-seed supplement; VFA, volatile fatty acids.

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(LPS); (3) high-protein control (HPC); (4) high-protein diet with SSS (HPS). The experimental diets were based on rolled barley grain and are presented in Table 1. Crushed sunflower seeds (high-linoleic acid (65·10 g/kg total oil) variety 6150) were supplemented at 140 g/kg dietary DM, providing sunflower-seed oil in the amount of 60 g/kg DM. Barley straw was used to balance the diets for approximate equal fibre content, while variable amounts of soyabean meal provided the desired protein concentration in the diets. Each diet was mixed as a single batch and pelleted. The lambs were offered feed *ad libitum* once daily (08.30 hours). Feed refusals were sampled, accumulated in a plastic bag for each lamb and stored in a freezer. A small quantity of each diet was taken at the time of feeding to form composite samples.

The lambs were housed in individual pens and had free access to feed and drinking water. Both the initial and final individual weighing was each performed on two consecutive days, after water and feed were withdrawn overnight. The average of the two consecutive weights, at both the beginning and the end of the experiment, was considered to be the start or finish body weight of each lamb. The lambs were also weighed individually every 14 d and records of feed intake were kept for each lamb throughout the 70 d experiment. After the last weighing, the lambs were sheared. Before the total shearing of each lamb a sample of wool (40 to 50 g) was sheared from the hip area. The processing of wool samples and of the total fleece was as described previously (Ivan *et al.* 1992). One lamb (treatment LPS) was removed during the experiment due to health complications. The experimental protocol was approved by the local animal care committee and followed the guidelines of the Canadian Council on Animal Care (1993).

After completion of the shearing of all lambs, four male lambs of similar body weight from each treatment were placed in metabolism cages and fed 85 % of the latest feed intake. They were fitted with harnesses and after 5 d of constant feed intake total faeces were collected for 5 d. Faeces were collected into plastic bags and 10 % of the daily output of each lamb was accumulated as a sample and frozen. The rest of the experimental lambs continued to receive the same diet *ad libitum* after the final weighing, and all lambs were harvested thereafter at a commercial slaughter facility.

Total rumen contents were removed, weighed, mixed and sampled. One half of each sample was strained through a single layer of cheesecloth for the enumeration of protozoa and pH was measured immediately. The second half was strained through two layers of cheesecloth for the determination of NH_3-N and volatile fatty acids (VFA).

Ciliate protozoa were counted using a Neubauer Improved Bright-Line counting cell (0·1 mm depth; Hausser Scientific, Horsham, PA, USA) in rumen fluid samples preserved with a methyl green–formalin–saline solution (Ogimoto & Imai, 1981). Each sample was counted twice, and if the CV of the two counts was greater than 10 % the counts were repeated.

A subsample (10 ml) of rumen fluid was combined with 2 ml of 25 % (w/v) meta-phosphoric acid before freezing for the analysis of VFA and NH_3-N. Later, the samples were thawed and centrifuged at 20 000 ***g*** for 10 min, then analysed for VFA using a Varian Star 3400 CX gas chromatograph (Varian Associates, Palo Alto, CA, USA) equipped with an 8200 autosampler and a fused silica column (DB-FFAP, 15 m × 0·25 mm internal diameter; J & W Scientific, Folsom, CA, USA). NH_3-N was analysed

Table 1. Composition of the experimental diets

	Experiment 1				Experiment 2			
	LPC	LPS	HPC	HPS	LPC	LPS	HPC	HPS
Ingredient (g/kg DM)								
Maize silage					520·0	520·0	520·0	520·0
Maize grain (rolled)					411·8	291·8	277·8	156·8
Barley grain (rolled)	692·8	694·8	571·8	574·8				
Barley straw	202·0	112·0	202·0	110·0				
Soyabean meal	57·0	5·0	180·0	127·0	20·0		154·0	135·0
Molasses (beet)	20·0	20·0	20·0	20·0	20·0	20·0	20·0	20·0
Sunflower seeds (crushed)		140·0		140·0		140·0		140·0
Dicalcium phosphate	4·0	4·0	4·0	4·0	4·0	4·0	4·0	4·0
Limestone	13·0	13·0	13·0	13·0	13·0	13·0	13·0	13·0
Trace mineral mix*	6·0	6·0	6·0	6·0	6·0	6·0	6·0	6·0
Vitamins A, D and E†	0·2	0·2	0·2	0·2	0·2	0·2	0·2	0·2
Zeolite	5·0	5·0	5·0	5·0	5·0	5·0	5·0	5·0
Chemical analysis (g/kg DM)								
Crude protein	134	126	173	169	123	116	170	159
Acid-detergent fibre	158	165	163	160	79	130	87	154
Neutral-detergent fibre	345	398	350	350	200	313	223	336
Digestible energy (calculated MJ/kg DM)	13·8	15·5	13·8	15·5	13·8	15·1	13·8	15·1

LPC, low-protein control; LPS, low-protein diet with sunflower-seed supplement; HPC, high-protein control; HPS, high-protein diet with sunflower-seed supplement.

* Contained (g/kg): NaCl, 930·1; Dynamate, 50 (IMC-Agro Feed Ingredients Ltd, Oakville, Ontario, Canada; content (g/kg): S, 200; K, 180; Mg, 110; Fe, 1000 mg); $ZnSO_4.H_2O$, 9·2; $MnSO_4.4H_2O$, 8·3; $CuSO_4.5H_2O$, 1·3; ethylenediamine dihydroiodide, 0·14; Na_2SeO_3, 0·03; $CoSO_4.6H_2O$, 0·05).

† Contained (per g vitamin mix): retinol, 3 mg; cholecalciferol, 25 μg; α-tocopherol, 67 μg.

in the same samples by the phenol-hypochlorite procedure (Broderick & Kang, 1980).

Feed and faecal samples were oven-dried at 55°C to constant weight and then ground in a Wiley Mill to pass through a 1 mm screen and analysed for protein (N × 6·25) (Association of Official Analytical Chemists, 1984). Concentrations of acid-detergent fibre (ADF) and neutral-detergent fibre (NDF) were determined as described by van Soest *et al.* (1991), while organic matter (OM) was calculated as the DM loss during ashing at 550°C. The digestibility of OM, ADF or NDF was calculated as the percentage disappearance of the nutrient between the daily amount fed and the amount excreted in the faeces.

Experiment 2

The experimental procedure was essentially the same as that for experiment 1 with minor exceptions. There were twelve lambs (six males and six females) per treatment and these were fed high-forage diets based on maize silage and maize grain (Table 1). Variable amounts of soyabean meal provided the desired protein concentration in the diets. A premix of rolled maize grain, soyabean meal, molasses, crushed sunflower seeds (for LPS and HPS), dicalcium phosphate, limestone, minerals, vitamins and zeolite was prepared for each diet and pelleted. The pellets were mixed with maize silage daily in a Data Ranger (American Calan Inc., Northwood, NH, USA). A small quantity of each diet was taken at the time of mixing to form composite samples. Two lambs, one receiving the HPC treatment and one the HPS, were removed during the experiment due to health complications. The experiment lasted 140 d.

Statistical analysis

For the protozoa counts in experiment 1, all of the animals in one treatment (LPS) had zero counts and large variations were observed for the other treatments. ANOVA could have been performed using log-transformed data with the treatment with all zeros omitted. However, it was decided to convert the protozoa counts to binary data (present, absent) for each animal and use 'Proc Genmod' (SAS Institute, Inc., 1999) to perform an analysis using a binomial generalised linear model with a logit link. Contrast statements were used to evaluate differences among estimates that were of interest when an effect was significant. 'Proc Mixed' (SAS Institute, Inc., 1999) was used to analyse the other dependent variables as a completely randomised design (Steel & Torrie, 1980) with sunflower-seed treatment, dietary protein, and their interaction in the model as fixed effects. Main effect and interaction means were evaluated for significance using Fisher's protected least significant difference test and were considered significant if $P<0·05$. The evaluation of interactions was important in the present study since it was expected that the SSS treatment would produce a response for the low- but not for the high-protein treatments for some of the dependent variables.

Results

Growth performance

In experiment 1, the numerically highest average daily gain (ADG; 319 g) was achieved with the LPS and lowest (276 g) with the LPC diet (Table 2). The ADG with the HPC diet and the HPS was similar to that with the LPS. Although the main effect of the SSS was not significant, it was of interest to compare the least square means, which showed statistical differences between the LPC diet and the LPS at $P=0·061$. This was associated with decreases in the average daily DM intake ($P<0·01$) and the feed:gain ratio ($P<0·001$) due to the SSS. The ratio was highest (5·2) with the LPC diet and lowest (4·2) with the LPS, while due to the SSS the average daily DM intake decreased by 9 %. High dietary protein increased ($P<0·01$) the total weight of clean fleece, but the other effects were not significant.

In experiment 2, there were higher dietary concentrations of fibre (Table 1) in the SSS-containing diets (LPS, HPS) than in the control diets (LPC, HPC). The SSS decreased ($P<0·001$) ADG from 240 g for the control to 191 g for the supplemented lambs (Table 2). This was associated with the decrease ($P<0·001$) in average daily DM intake due to the SSS, without affecting ($P>0·05$) the feed:gain ratio. The total weight of clean fleece was decreased ($P<0·05$) by the SSS and increased ($P<0·01$) by dietary protein.

Digestibility

In experiment 1, the digestibility of OM was affected ($P<0·05$) by the SSS × protein interaction (Table 3). The digestibility was not affected by the SSS with the low-protein diet (LPC *v.* LPS), but the OM digestibility increased ($P<0·05$) from 72·1 to 75·8 % when the SSS was included in the high-protein diet (HPC *v.* HPS). Both the SSS and high dietary protein significantly increased the digestibility of ADF and NDF.

In experiment 2, the SSS decreased ($P<0·05$) the percentage digestibility of OM from 75·8 to 72·8. The supplement had no significant effects on the digestibility of ADF and NDF.

Protozoa and fermentation

The number of protozoa, pH, and NH_3-N and VFA concentrations in the rumen fluid of lambs killed at the end of both experiments are summarised in Table 4. In experiment 1, the logit analysis for protozoa indicated a significant interaction ($P=0·01$), and the contrast statements showed that the odds ratio for LPS was significantly lower than the other three odds ratios, which were similar. NH_3-N concentrations were not affected by any factor or interaction, while pH ($P<0·01$) and VFA concentration ($P<0·05$) were both affected by the SSS × protein interaction. The pH increased ($P<0·05$) due to the SSS with the low-protein diet (LPC *v.* LPS), but decreased ($P<0·05$) due to the supplement with the high-protein diet (HPC *v.* HPS). The effect of interaction on the concentration of VFA was reversed; the concentration decreased ($P<0·05$) due

Table 2. Growth and feed efficiency in lambs fed concentrate- (experiment 1) or forage- (experiment 2) based diets with a low (120 g/kg) or high (160 g/kg) protein content and without (control) or with a sunflower-seed supplement (SSS)†
(Mean values with their standard errors)

	Average start weight (kg)		Average finish weight (kg)		Average daily gain (g)		Average daily DM intake (g)		Feed:gain ratio (g/g)		Total weight of fleece (g)	
Treatment	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Experiment 1 (high-concentrate diet; 70 d)												
Main effects												
SSS												
Control					284	10	1463[a]	30	5·2[a]	0·2	509	23
Supplemented					305	9	1335[b]	30	4·4[b]	0·1	518	15
Protein												
Low					293	11	1389	34	4·7	0·2	476[a]	16
High					296	8	1412	31	4·8	0·1	549[b]	20
Interaction												
Low-protein control	25·4	1·0	44·7	1·6	276	16	1423	52	5·2	0·3	457	17
Low-protein diet + SSS	25·6	1·1	47·9	1·5	319*	14	1352	43	4·2	0·1	497	27
High-protein control	25·4	0·9	45·9	0·9	293	12	1504	30	5·1	0·2	561	38
High-protein diet + SSS	25·8	0·9	46·7	1·3	299	12	1320	42	4·4	0·1	537	14
Effects (*P*)												
SSS					0·123		0·004		0·001		0·751	
Protein					0·879		0·567		0·926		0·007	
Interaction					0·249		0·188		0·663		0·222	
Experiment 2 (high-forage diet; 140 d)												
Main effects												
SSS												
Control					240[a]	10	1104[a]	43	4·6	0·1	673[a]	27
Supplemented					191[b]	7	892[b]	32	4·7	0·1	587[b]	33
Protein												
Low					210	8	993	40	4·7	0·1	571[a]	29
High					222	11	1003	48	4·5	0·1	694[b]	29
Interaction												
Low-protein control	18·5	1·5	50·1	3·1	226	14	1078	64	4·8	0·1	611	39
Low-protein diet + SSS	18·4	1·4	45·5	1·7	194	6	908	37	4·7	0·2	531	41
High-protein control	18·8	1·5	54·5	2·9	255	13	1132	57	4·4	0·1	741	27
High-protein diet + SSS	17·9	1·6	44·4	2·8	189	13	874	54	4·6	0·2	648	48
Effects (*P*)												
SSS					0·001		0·001		0·778		0·034	
Protein					0·312		0·860		0·315		0·003	
Interaction					0·165		0·419		0·345		0·876	

[a,b] Mean values in a column, for SSS or protein, with unlike superscript letters were significantly different ($P<0·05$).
* Mean value was statistically different from the low-protein control ($P=0·06$).
† For details of diets and procedures, see Table 1 and p. 303.

to the SSS with the low-protein diet (LPC *v.* LPS), but tended ($P>0·05$) to increase with the high-protein diet (HPC *v.* HPS).

In experiment 2, the number of protozoa/ml rumen fluid decreased ($P<0·001$) from 730 000 to 60 000 due to the SSS, but was not affected by the dietary protein ($P>0·05$). The pH was increased ($P<0·05$) by the supplement and decreased ($P<0·01$) by high dietary protein. Both NH_3-N ($P<0·001$) and VFA ($P<0·05$) concentrations were increased by high dietary protein, while the other effects were not significant.

Discussion

The presence of ciliate protozoa in the rumen of sheep decreases the flow of AA from the stomach to the intestinal tract for absorption and utilisation by up to 30 % (Ivan *et al.* 1991, 2000*a*,*b*). Most of such a decrease must be compensated for in the form of an increased dietary protein concentration to ensure that the N requirement is met to achieve the optimal productivity of the animal. In the present study, the difference in protein concentration between the low- and high-protein diets was, therefore, set at approximately 30 % (120 *v.* 160 g/kg). Actual differences after chemical analysis of the experimental diets were 29 % and over. Since concentrated energy sources such as fats and oils reduce protozoa (Clemens *et al.* 1974; Machmüller *et al.* 2000) and inhibit cellulolytic rumen bacteria (Henderson, 1973), no attempt was made to make the diets in the two experiments isoenergetic.

Use of the SSS in the concentrate diets with equal fibre content in experiment 1 increased ADG in the lambs fed the LPS compared with the LPC diet. It is interesting to note that the ADG in the lambs receiving the LPS was even numerically higher than in those receiving the high-protein diets (HPC and HPS). It is obvious that the difference is not a response to the slightly higher energy content in the LPS as compared with the LPC diet. This is because there was an almost identical difference in energy content between the two high-protein diets (HPC *v.* HPS), while

Table 3. Percentage digestibility of organic matter (OM), acid-detergent fibre (ADF) and neutral-detergent fibre (NDF) in lambs fed concentrate- (experiment 1) or forage- (experiment 2) based diets with a low (120 g/kg) or high (160 g/kg) protein content and without (control) or with a sunflower-seed supplement (SSS)*

(Mean values with their standard errors)

	OM		ADF		NDF	
	Mean	SE	Mean	SE	Mean	SE
Experiment 1 (high-concentrate diet; 70 d)						
Main effects						
SSS						
Control	71·2	0·57	22·9[a]	1·23	36·8[a]	1·04
Supplemented	73·1	1·17	30·7[b]	2·00	46·3[b]	1·69
Protein						
Low	70·3	0·30	24·0[a]	1·22	39·2[a]	1·40
High	73·9	0·93	29·5[b]	2·51	43·9[b]	2·63
Interaction						
Low-protein control	70·2[a]	0·22	21·3	1·04	35·8	0·91
Low-protein diet + SSS	70·3[a]	0·61	26·8	0·91	42·7	0·74
High-protein control	72·1[a]	0·95	24·4	2·10	37·8	1·89
High-protein diet + SSS	75·8[b]	1·03	34·5	2·80	50·0	1·96
Effects (*P*)						
SSS	0·029		0·001		0·001	
Protein	0·001		0·014		0·008	
Interaction	0·037		0·239		0·095	
Experiment 2 (high-forage diet; 140 d)						
Main effects						
SSS						
Control	75·8[a]	1·17	40·0	3·00	46·3	2·21
Supplemented	72·8[b]	0·68	38·0	1·41	41·1	1·56
Protein						
Low	73·0	1·13	35·8	2·46	41·6	2·22
High	75·5	0·85	42·2	1·55	45·8	1·74
Interaction						
Low-protein control	73·9	1·97	35·3	5·01	43·5	4·02
Low-protein diet + SSS	72·2	1·24	36·4	1·72	39·8	2·09
High-protein control	77·6	0·36	44·7	1·58	49·0	1·26
High-protein diet + SSS	73·4	0·57	39·7	2·13	42·5	2·38
Effects (*P*)						
SSS	0·031		0·520		0·077	
Protein	0·060		0·054		0·145	
Interaction	0·325		0·328		0·621	

[a,b] Mean values in a column, for SSS, protein or interaction, with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures, see Table 1 and p. 303.

the ADG for the two high-protein diets were almost the same. This is a clear evidence of the beneficial effect of the SSS when used in a low-protein diet. There was a high variation in ADG among individual lambs. Undoubtedly, the level of the significance of the differences between the LPC diet and the LPS would increase with a higher number of lambs per group. Also, as expected, there was no benefit of the addition of the SSS to the high-protein diet, which decreased the level of probability of the main effect of the SSS on ADG. The superiority of the SSS is further demonstrated by the improved feed:gain ratio, as a result of the lower DM intake required to produce greater growth performance. Since there were no protozoa present in the rumen of the lambs fed the LPS, it would be expected that there was a higher yield of bacterial protein in the rumen of these lambs. Such expectation is due to the removal of the protozoal predatory activity (Demeyer & van Nevel, 1979), resulting in lower rumen concentrations of NH_3-N and a higher intestinal flow of AA (Veira *et al.* 1983, 1984; Kayouli *et al.* 1986; Ivan *et al.* 1991). Consequently, because wool growth is positively affected by the increased intestinal supply of protein, especially sulfur AA (Reis & Schinckel, 1961; Bird & Leng, 1984; Ivan *et al.* 1992), there should be more wool growth in the lambs fed the LPS than in the lambs fed the LPC diet. However, the results show no effects of the SSS on wool growth in experiment 1.

The wool yield increased with the increased dietary protein in both of the present experiments. Therefore, it appears that in experiment 1 the lack of the expected increase in the clean fleece yield in the lambs fed the LPS to the level of the high-protein diets (HPC or HPS) was due to an insufficient intestinal supply of AA. Because wool growth is sensitive to the intestinal protein (AA) supply (Reis & Schinckel, 1961) and ADG is sensitive to the protein (AA):energy ratio (Leng, 1993), it is reasonable to suggest that the addition of the SSS to the low-protein diet increased the intestinal supply of AA and energy to the level required for the increased ADG. But, the intestinal supply of AA in the LPS-fed lambs was probably not high enough to support more wool growth, up to that obtained with the HPC diet or the HPS. This is further

Table 4. Protozoa numbers and fermentation parameters in rumen fluid taken after slaughter at the end of feeding trials with lambs fed concentrate- (experiment 1) or forage- (experiment 2) based diets with a low (120 g/kg) or high (160 g/kg) protein content and without (control) or with a sunflower-seed supplement (SSS)†

(Mean values with their standard errors)

	Protozoa* (number × 10^6/ml)		pH		NH_3-N (mg/100 ml)		Total VFA (mmol/l)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Experiment 1 (high-concentrate diet; 70 d)								
Main effects								
SSS								
Control	5·46	1·679	6·1	0·16	8·4	1·50	121	7·8
Supplemented	1·18	0·644	6·2	0·17	4·7	1·75	102	8·9
Protein								
Low	1·00	0·546	6·1	0·12	5·2	1·26	117	8·4
High	5·64	1·677	6·3	0·21	8·0	1·99	106	8·9
Interaction								
Low-protein control	1·99	0·98	5·8[a]	0·05	7·4	1·84	140[a]	4·8
Low-protein diet + SSS	0·00	0·00	6·4[bc]	0·18	2·9	1·33	94[b]	10·1
High-protein control	8·92	2·69	6·8[b]	0·06	9·5	2·46	101[b]	11·0
High-protein diet + SSS	2·36	1·15	6·1[ac]	0·30	6·6	3·22	110[ab]	14·7
Effects (*P*)								
SSS	0·001		0·832		0·124		0·094	
Protein	0·512		0·099		0·234		0·319	
Interaction	0·016		0·006		0·730		0·017	
Experiment 2 (high-forage diet; 140 d)								
Main effects								
SSS								
Control	0·73[a]	0·189	6·4[a]	0·09	4·2	0·73	94	7·4
Supplemented	0·06[b]	0·034	6·7[b]	0·10	4·0	0·99	79	5·8
Protein								
Low	0·30	0·128	6·7[a]	0·08	1·7[a]	0·30	75[a]	4·3
High	0·49	0·176	6·3[b]	0·11	6·8[b]	0·95	99[b]	8·1
Interaction								
Low-protein control	0·58	0·234	6·6	0·10	2·3	0·51	81	5·5
Low-protein diet + SSS	0·02	0·017	6·9	0·11	1·1	0·23	69	6·4
High-protein control	0·89	0·307	6·2	0·15	6·4	1·12	108	13·2
High-protein diet + SSS	0·10	0·068	6·5	0·15	7·2	1·58	90	9·3
Effects (*P*)								
SSS	0·001		0·040		0·854		0·093	
Protein	0·382		0·005		0·001		0·011	
Interaction	0·711		0·970		0·288		0·711	

[a,b,c] Mean values in a column, for SSS, protein or interaction, with unlike superscript letters were significantly different ($P<0·05$).

* The protozoa probabilities in experiment 1 come from the logit analysis of binary data (observed, not observed), but the counted means are shown in the Table for consistency.

† For details of diets and procedures, see Table 1 and p. 303.

supported by the higher digestibility of ADF and NDF due to the SSS in experiment 1. Increases in these digestibilities probably resulted in a higher supply of energy and in an improved available AA:energy ratio for the growth of the LPS-fed lambs.

Contrary to experiment 1, the SSS produced a 19 % decrease in ADG in experiment 2. Since the feed:gain ratio was not affected, the decrease was most certainly due to the proportionally lower feed intake as a result of the higher fibre content and the lower digestibility of the SSS-containing diets compared with the control diets. The digestibility of OM was decreased by the SSS added to the high-forage diet, without significant changes in the digestibility of ADF and NDF (experiment 2). In contrast, the addition of the SSS to the concentrate diet (experiment 1) increased the digestibility of both ADF and NDF, while that of OM was not affected. This indicates a clear advantage of the SSS when used in high-concentrate diets, but not when used as part of the concentrate in high-forage diets.

It should be noted that in experiment 1 the pH increased and VFA concentration decreased, while protozoa disappeared when the SSS was added to the low-protein diet (LPC *v.* LPS). It has been suggested that protozoa exert a stabilising effect on pH, because their partial or total removal results in a lower and more variable rumen pH (Veira *et al.* 1983). Rumen fauna contains cellulolytic protozoa (Coleman, 1985) and some bacterial enzymes and growth are inhibited by a decline in pH (Blackburn & Hobsen, 1960; Russell *et al.* 1979), contributing to lower digestibility. Therefore, because protozoa disappeared from the rumen, a decline in the digestibility of feed due to the disappearance in the lambs fed the LPS and HPS in experiment 1 would be expected, but it did not appear. In contrast, the digestibility of fibre was increased in these lambs, probably due to improved rumen conditions provided by the SSS for the growth of cellulolytic bacteria.

The present results confirmed our previous observation of the antiprotozoal effects of the SSS (Ivan *et al.* 2003)

and its ability to reduce or completely eliminate rumen fauna. Depending on the diet used, such effects may increase the synthesis of microbial protein in the rumen and the efficiency of the dietary protein utilisation. This would lower substantially the protein requirement for the maximum growth of young ruminants. It would also lower the excretion and manure content of N. Since protozoa supply hydrogen to methanogens they contribute to the rumen production of methane by up to 25 % (U.S. Environmental Protection Agency, 1993). Therefore, in addition to the beneficial effects on production the SSS may also reduce the excretion of methane by ruminant livestock through the inhibition of methanogenesis (Whitelaw *et al.* 1984).

Use of the SSS in high-concentrate diets contributes to the dietary protein and effective fibre, and according to the present study, it increases the feed conversion efficiency and digestibility of fibre. Beneficial effects of a reduced fauna on milk production and quality has been reported previously (Moate, 1989), but the reduction of fauna was achieved by a chemical method (drenching with Alkanate 3SL3). Such a method is not practical for use in animal production. Also, most of the existing experimental defaunating techniques negatively affect rumen bacteria, especially cellulolytic species. There is at present no safe practical technique commercially available for the reduction or elimination of rumen fauna (Hegarty, 1999). Therefore, to the knowledge of the authors, the use of sunflower-seed oil (Ivan *et al.* 2001) or crushed sunflower seeds (Ivan *et al.* 2003; experiment 1) as an antiprotozoal dietary component in concentrate diets is the first safe and practical means for the beneficial reduction of protozoa populations in the rumen. The present study shows that the SSS increases the digestion of high-concentrate diets, probably partly through increased pH, which benefits cellulolytic bacteria (Blackburn & Hobsen, 1960; Russell *et al.* 1979) and partly through a reduced protozoa population that affects the numbers and species composition of the rumen bacteria population (Williams & Coleman, 1992).

It can be concluded from the results of the present study that, depending on the type of diet used, the antiprotozoal effects of sunflower-seed supplementation might be beneficial to ruminant production. The present study demonstrates for the first time that a reduced fauna may contribute to a higher efficiency of dietary protein utilisation and consequent savings on dietary protein supplements. Additional benefits of sunflower-seed supplementation might come from a reduced environmental impact of ruminant production.

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