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Digestion in defaunated and refaunated sheep fed soybean oil hydrolysate or crushed toasted soybeans

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Abstract

The effect of soybean oil hydrolysate (SOH : 70g per day) or an equivalent amount of lipids administered as crushed toasted soybeans (TSB) on rumen- and post-ruminal digestion in sheep was investigated. Defaunation increased the molar percentage of propionate in the rumen, while butyrate decreased. SOH caused a similar effect in both the defaunated and refaunated rumen, while the effect on acetate proportions was variable. Protozoal counts were lower after feeding SOH. Crushed toasted soybeans had a minor effect on rumen fermentation pattern. Rumen digestibility of organic matter (OM) was decreased by both defaunation and SOH feeding, with a concommitant shift in digestion to the lower intestinal tract. Total tract digestibility was not affected. Both treatments increased non ammonia N (NAN) flows at the duodenum, but this was only statistically significant with defaunation. Total tract digestion of N remained almost constant during the whole experiment. In the case of defaunation, more microbial protein (MN) reached the duodenum. Except for the TSB period, total lipid leaving the rumen equalled intake. Total tract digestibility of total lipid was much higher with SOH and TSB. Defaunation almost doubled microbial growth efficiency (E) and this value tended to increase by SOH feeding. The decrease of protozoal count or even elimination of protozoa after lipid feeding could not entirely explain the change in rumen metabolism, as additional changes in defaunated sheep were shown.

Keywords; sheep, rumen digestion, digesta flow, soybean oil hydrolysate, defaunation, protozoa

Introduction

Feeding lipid supplements to ruminants increases the energy density of the ration. Consequently, energy intake can be increased , which is useful for high productive animals, requiring high energy input (Palmquist, 1988). Fats have an interesting feeding value (energy/cost) and are highly digestible (Andrews & Lewis, 1970 a,b). Unfortunately, higher amounts of lipids can influence rumen fermentation in a negative way. Decreased fibre digestion and a smaller acetate/propionate ratio can result in milk-fat depression (Palmquist, 1984). Long-chain fatty acids (FA) are known to inhibit methanogenic bacteria (Prins et al. 1972) with associated effects on propiona-

te and acetate proportions (Demeyer et al., 1969).In most cases, fat supplements decreased protozoal numbers or even eliminated ciliates in the rumen (refs: see Van Nevel & Demeyer, 1988), with subsequent effects on rumen fermentation pattern and fibre degradation (Jouany et al., 1988; Demeyer, 1989). So, the overall effect of lipid supplements involves both changes in bacterial and protozoal activities.

In order to investigate the importance of decreasing numbers of protozoa in the overall effect of lipids, we studied the effect of soybean oil hydrolysate (SOH) on rumen fermentation in both defaunated and refaunated sheep. A similar experimental set-up was earlier reported by Broudiscou et al. (1990). Soybean oil hydrolysate was used instead of soybean oil to exclude difficulties in interpretation because of eventual differences in lipolysis. Rumen lipolysis may indeed be affected by ration composition (Latham et al., 1972; Demeyer et al., 1972; Gerson et al., 1985; Abel et al., 1990), and possibly by absence of protozoa. The effect of the fatty acids on post-ruminal digestion was also studied. In a last and complementary experimental period, an equivalent amount of lipids was fed as crushed toasted soybeans (TSB).

Materials and methods

Animals and Rations

Three wethers were cannulated in rumen, proximal duodenum (prior to the biliary and pancreatic duct) and terminal ileum. During four experimental periods (P1-P4) the animals were fed 1kg of a pelleted feed (Feed 1) and 200g of hay, in six equal portions, supplemented with 35g of SOH (Oleofina, Ertvelde, Belgium) infused in the rumen twice daily during P2 and P3. During P5, an adjusted pelleted feed (Feed 2) was fed (600g/d) while an equivalent amount of fat was fed as TSB (200g twice daily). By changing the composition of Feed 2, it was attempted to equalize the intake of feed components during P3 and P5 (Table 2). Water was available at all time and consumption was measured. Between periods, animals were adapted to new experimental conditions during at least twelve days. The composition of the pelleted feeds, chemical analysis, experimental design and intake of feed components is shown in Tables 1, 2 and 3, respectively. SOH was free of glycerol, contained 200 ppm of butylated hydroxytoluene and was kept at –20 °C under N_2 until feeding.

Defaunation and refaunation

During P1 and P2, the sheep had no protozoa in the rumen, as regularly checked by direct microscopic observation of rumen fluid. Defaunation was done by administration of high amounts of SOH, mainly as described earlier (Broudiscou et al., 1990). When the animals refused feed for two or three days, the rumen was emptied and contents replaced by a lukewarm water extract of hay. When the rumen was not persistently protozoa-free, the whole treatment was repeated until complete and definitive defaunation. Then, P1 was started after weighing the animals (mean weight ± standard deviation : 45.5 ± 1.0kg). Sheep normally loose weight during defaunation, but these losses were rapidly gained back as the mean weight of the animals at

Table 1. Composition of the pelleted feed[3].

Ingredient (%)	Feed 1	Feed 2
Alfalfa	19.9	29.0
Flax chaff	27.9	40.6
Extracted soybean meal	28.1	–
Corn starch	16.0	19.7
Molasses (beet)	4.8	5.8
Dicalcium phosphate	1.9	2.8
Salt	0.7	1.0
Ground limestone	0.5	0.7
Trace minerals[1]	0.048	0.07
Rovimix[2]	0.15	0.22

[1] Composition : 1000g $FeSO_4$ 20%; 550g MnO_2 62%; 50g $CoSO_4$ 21%; 10g KI 76.4%; 500g ZnO 80%. [2] Rovimix, diluted with milo : 5000 IU Retinol/g and 1000 IU cholecalciferol/g. [3] Feed 1: 1000g per day in 6 equal portions. Feed 2: 600g per day in 4 equal portions and 400g of crushed toasted soybeans in 2 equal portions. Per day, 200g of hay were given in six equal portions. Detailed time schedule given in Table 3.

Table 2. Proximate analysis of components and daily intake during the experimental periods.

Feed component	Proximate analysis				Daily intake (g)			
	Feed 1	Feed 2	TSB[1]	Hay	P1[2]	P2-P3	P4	P5
Dry matter (g/kg)	896	902	931	911				
Org. matter (g/kg DM)	882	856	940	927	959.0	1029.0	959.0	981.5
Crude protein (g/kg DM)	180	75	365	107	180.2	180.2	180.2	196.0
Crude fat (g/kg DM)	27	31	223	32	30.0	100.0	30.0	105.5
Crude fibre (g/kg DM)	183	236	46	351	228.2	228.2	228.2	209.0
N-free extractives (g/kg DM)	492	514	306	437	520.6	520.6	520.6	471.0

[1] Crushed toasted soybeans. [2] P1 = defaunated period, control; P2 = defaunated period with SOH; P3 = refaunated period with SOH; P4 = refaunated period, control; P5 = refaunated period with TSB.

the end of the experiment was 58.2 ± 3.1kg.

After P2, refaunation of the rumen was done by administration during five days of 0.5l of rumen contents (daily), taken from a donor sheep with normal faunated rumen and fed the same ration.

After P3, during which the animals had a normal rumen fauna but received SOH, refaunation procedure was repeated in order to abolish the effect of the fatty acids, to restore rapidly normal conditions in the rumen and to avoid carry-over effects. Period 4 was indeed the control period : faunated rumen on basal ration.

Table 3. Experimental scheme and ration fed.

Rumen	Period	Ration[1]
Defaunated	P1	167g of Feed 1 and 33g of hay, six times daily
Defaunated	P2	167g of Feed 1 and 33g of hay, six times daily, but 35g of SOH[2] at 8h and 20h
Refaunated	P3	same as P2
Refaunated	P4	same as P1
Refaunated	P5	8h: 200g of TSB[3] and 33g of hay 12h: 150g of Feed 2 and 33g of hay 16h: 150g of Feed 2 and 33g of hay 20h: 200g of TSB and 33g of hay 24h: 150g of Feed 2 and 33g of hay 4h: 150g of Feed 2 and 33g of hay

[1] Feed was given at: 8, 12, 16, 20, 24 and 4h. [2] SOH = Soybean oil hydrolysate; fatty acid composition: C16:0 ; 10.1%, C18:0 : 2.7%, C18:1 : 24.5%, C18:2 : 53.3%, C18:3 : 9.2%; C22:0 : 0.2%. [3] TSB = crushed toasted soybeans.

Markers

For determining the flow of digesta in the different segments of the gastrointestinal tract, the double marker method of Faichney (1975) was used, with PEG 4000 and Rutheniumphenanthroline as marker of liquid and solid phase, respectively. Markers were dissolved in water and continuously infused in the rumen (240ml or ca. 13g of PEG and 5.9mg of Ru per 24h), after a priming dose of 240ml. Rutheniumphenanthroline was prepared as described earlier (Tan et al., 1971). Cytosine was used as marker for microbial nitrogen entering the small intestine (Schelling & Byers, 1984). Per period and per sheep, washed cell suspensions of rumen bacteria (WCS) were prepared as described elsewhere (Van Nevel et al., 1986), and the ratio cytosine : total nitrogen was determined.

Sampling scheme

On day one of each period, WCS of rumen bacteria were prepared and *faeces* samples were taken. On the fourth day, *rumen* fluid and *ileal* contents were sampled. Therefore, 50ml of rumen fluid were taken at 10h and 16h; pH was measured and 5ml used for counting of protozoa (Van Nevel et al., 1986). The rest was acidified (5% v/v H_2SO_4 10N) and stored at –20 °C until analysis. A plastic bag (17.5×12cm) was attached to the open ileal cannula and contents flowing out were regularly collected between 8h and 20h, until a total volume of 600-700ml was obtained. To avoid fermentation, bags were emptied every 30 minutes and contents, collected per day per sheep, were stored at –20 °C. On day five, the *duodenal* canula was opened at 9.00, 12.30, 16.00 and 19.30h, and 250ml of contents were collected. The 1l sample per day and per sheep was stored at –20 °C. These sampling procedures were repeated as follows:

- Day 6, rumen sampling
- Day 7, sampling of ileum and faeces
- Day 8, sampling of rumen and duodenum
- Day 11, sampling of ileum and faeces
- Day 12, sampling of duodenum.

This scheme was followed as well as possible but, on some occasions, animals refused part of the ration, probably due to irritation at the innerside of the duodenal fistula. In this case, sampling was stopped and only continued at the earliest four days after complete recovery of appetite. It was assumed that at that time, steady state conditions were reached again.

Analysis

The proximate composition of feed was determined following the Weende scheme, using EC methods. Total fat was determined by the Soxhlet method after acid hydrolysis. Neutral detergent fibre was obtained as outlined by Goering & Van Soest, (1970). However, in the control period (P4) much too low and probably erroneous rumen digestibilities of NDF were calculated, possibly due to analytical errors, although sampling problems cannot be excluded. Because proper comparison of treatments against control thus became impossible, it was decided to ignore all NDF results of rumen samples. Washed cell suspensions of rumen bacteria were lyophilised and on an aliquot, total nitrogen (TN) was determined by micro-Kjeldahl method, while cytosine was analyzed by HPLC, mainly as described by Koenig (1980). In rumen fluid, volatile fatty acids (VFA), ammonia-N and lactic acid were determined as described elsewhere (Van Nevel et al. 1986; Broudiscou et al. 1990).

For duodenal and ileal samples, analyses were done on total contents and on the liquid phase after filtering contents through a Terylene cloth (pore size : 200μ). Part of these fractions were used as such (fresh material) or after lyophilisation. On fresh material, analysis of dry matter (DM), organic matter (OM), ammonia-N and PEG (Decuypere et al. 1981) were performed. On a lyophilised aliquot, α-amino N (Oddy, 1974), total fat (Soxhlet after acid hydrolysis), cytosine, long chain fatty acids (FA; Lowry & Tinsley, 1976) on an aliquot of the Soxhlet extract, Ruthenium (Van Nevel & Demeyer, 1989) and NDF were determined. On faecal material the following analyses were done: DM, OM, TN, total fat, FA, NDF and PEG.

Calculations

Flows of digesta components at duodenum and ileum were calculated following the double marker method of Faichney (1975) with mathematical reconstitution of digesta. Total tract digestibility was calculated with PEG as sole marker (Schneider & Flatt, 1975).

For all parameters, the three observations per sheep per period were averaged, thus reducing the final number of repetitions from nine to three. Data were submitted to analysis of variance with two factors, sheep and treatment. Comparison of means was done based on the least significant difference (Snedecor & Cochran, 1971). The

Table 4. Effect of soybean oil hydrolysate (SOH) and crushed toasted soybeans (TSB) on fermentation pattern and protozoal numbers in the rumen of defaunated and refaunated sheep.[1,4]

Period	Refaunated control (P4)	Defaunated control (P1)	Refaunated SOH (P3)	Defaunated SOH (P2)	Refaunated TSB (P5)	RSD[5]
VFA[2] (mmol/l)	95.1[a]	109.3[b]	90.3[a]	87.3[a]	88.5[a]	6.6
C2[3] (%)	65.9[bc]	66.1[bc]	65.0[b]	62.9[a]	66.8[c]	1.0
C3 (%)	14.1[a]	19.1[b]	20.7[b]	25.7[c]	14.0[a]	1.0
C4 (%)	17.0[c]	11.7[b]	11.4[b]	8.1[a]	15.7[c]	0.9
Lactate (mmol/l)	0.16[a]	0.26[b]	0.19[a]	0.29[b]	0.19[a]	0.03
NH_3-N (mmol/l)	22.00[c]	15.75[a]	19.09[b]	14.00[a]	25.63[d]	1.71
pH	6.3[a]	6.4[ab]	6.4[ab]	6.5[b]	6.4[ab]	0.1
Protozoa (x10^6/ml)	2.64[b]	0.00	1.53[a]	0.00	2.72[b]	0.39

[1] Rumen samples taken at 16.00h. [2] VFA = total volatile fatty acids. [3] C2, C3, C4 = molar percentages of resp. acetate, propionate and butyrate. [4] Values per row, bearing a different superscript are signif.different (P<0.1). [5] Residual standard deviation.

experimental design confounded periods and treatment, but as it is almost impossible to maintain faunated and defaunated sheep at the same time and place, there is no other way to carry out defaunation experiments. Because mature sheep were used, the effect of time should be negligible, as argued earlier (Siddons et al., 1985).

Results

Rumen fermentation pattern

The effect of the different treatments on fermentation pattern is shown in Table 4. As a same trend was observed for samples taken at 10.00h and 16.00h, only results of the latter are shown. The effect of defaunation in the absence of fat is found by comparison of P4 with P1, while effects of SOH are obtained by comparison of P3-P4 (normal faunated rumen) and P1-P2 (defaunated rumen). Comparing P4 with P5 gives the effect of the crushed toasted soybeans (faunated rumen).

Effect of defaunation. Elimination of protozoa had no effect on acetic acid percentage, while propionic and butyric acid proportions increased resp. decreased. Concentration of total VFA and lactate were somewhat higher in the defaunated rumen, while the opposite was observed for ammonia-N concentrations.

Effect of lipids. In the defaunated rumen, total VFA concentration was decreased after SOH addition whereas ammonia-N concentration tended to be lower, possibly related to a lower org. matter (OM) degradation (Table 5), and/or an increased rumen volume (Broudiscou et al., 1990). Individual VFA proportions were altered : propionate increased accompanied by lower acetate and butyrate percentages. Lactate concentration was low and not changed by SOH. The effect of SOH on fermentation parameters in the refaunated rumen was comparable with the defaunated period, al-

Table 5. Effect of SOH and TSB on digestion of organic matter (OM).[1]

Period	Refaunated control (P4)	Defaunated control (P1)	Refaunated SOH (P3)	Defaunated SOH (P2)	Refaunated TSB (P5)	RSD[2]
Intake (g/d)	959	959	1029	1029	982	
Flow duodenum (g/d)	548.1^{c}	639.9^{a}	701.5^{a}	780.5^{b}	503.0^{c}	45.1
Digested in rumen						
% of intake	42.8^{c}	33.3^{a}	31.8^{ab}	24.2^{b}	48.8^{c}	4.6
% of tot.tract dig.	64.1^{cd}	53.3^{ac}	47.6^{ab}	35.6^{b}	72.2^{d}	7.1
Faeces (g/d)	334^{a}	357^{a}	328^{a}	329^{a}	327^{a}	27
Tot.tract digestib. (%)	65.2^{ab}	62.8^{a}	68.1^{b}	68.0^{b}	66.7^{ab}	2.7
Post-ruminal digestib.						
g/d	214.4^{ab}	283.2^{abc}	373.5^{cd}	451.8^{d}	176.7^{a}	48.7
% of available[3]	39.1^{ab}	44.1^{abc}	53.2^{cd}	57.8^{d}	34.4^{a}	5.1
% of tot.tract dig.	34.3^{ab}	46.7^{bc}	53.2^{cd}	64.4^{d}	27.2^{a}	6.7

[1] Values per row, bearing different superscripts are sign. different ($P<0.05$). [2] RSD = residual standard deviation. [3] % of available in duodenum.

though total VFA concentration was not significantly decreased and molar percentages of acetic acid remained constant. Protozoal counts were lower during P3. Table 4 shows that, compared with SOH, TSB caused only minor changes in fermentation pattern. No difference in protozoal numbers was observed.

Rumen and post-ruminal digestion

Calculation of nutrients digested in the small intestine revealed some anomalies: the amount of OM disappearing during P4 and P5 was much smaller then the sum of NAN (non-ammonia N), lipid and NDF digested. However, reconstitution factors (Faichney, 1975) for ileal samples were rather high (–0.275 with residual standard deviation of 0.187 versus 0.096 ± 0.192 for duodenal samples). Therefore, we decided to neglect ileal samples and analyses, and to calculate post-ruminal digestion, which is the difference between duodenal flux and faeces. Ortigues et al. (1990) indeed suggested to be cautious when the reconstitution factor is 0.20-0.50.

Digestion of organic matter. In the absence of SOH (comparing P4 with P1), it was clear that duodenal flow of OM was increased by defaunation, thus decreasing rumen digestibility expressed as percentage of intake or as fraction of OM totally digested (Table 5). Administration of SOH reduced rumen OM digestibility in both the defaunated (P1-P2) and refaunated period (P4-P3). Consequently, post-ruminal OM digestion was increased by defaunation (not statistically significant) and by SOH feeding in both defaunated and refaunated animals. The ruminal digestibility of OM with the TSB ration was somewhat higher (P4-P5), with a trend to decreased post-ruminal digestion. Total tract digestibility was not changed.

Table 6. Effect of SOH and TSB on digestion of N-components and efficiency (E) of microbial growth in the rumen.

Period	Refaunated control (P4)	Defaunated control (P1)	Refaunated SOH (P3)	Defaunated SOH (P2)	Refaunated TSB (P5)	RSD[2]
Intake TN[1](g/d)	28.8	28.8	28.8	28.8	31.4	
Flow duodenum						
TN (g/d)[1]	26.5^{bc}	34.6^{a}	29.9^{b}	37.5^{a}	25.3^{c}	1.9
NAN (g/d)[1]	24.8^{bc}	32.8^{a}	27.9^{b}	35.8^{a}	23.1^{c}	1.8
AAN (g/d)[1]	14.1^{a}	21.2^{bc}	17.9^{ab}	23.6^{c}	13.6^{a}	2.0
MN[1]	15.9^{b}	23.7^{a}	16.0^{b}	21.9^{a}	16.1^{b}	1.8
% MN[3]	64.1^{ab}	72.5^{a}	57.5^{b}	61.3^{ab}	70.5^{ab}	8.4
E[4]	39.8^{b}	76.3^{a}	50.3^{b}	93.8^{a}	34.2^{b}	11.7
Faeces TN (g/d)	7.5^{bc}	8.6^{a}	7.4^{bc}	7.8^{b}	6.9^{c}	0.5
Tot.tract digest.						
TN (%)	73.9^{b}	70.0^{a}	74.7^{b}	72.8^{b}	78.1^{c}	1.5

[1] TN, NAN, AAN, MN: resp. total nitrogen, non-ammonia nitrogen, α-amino nitrogen and microbial nitrogen. [2] RSD : residual standard deviation. [3] % microbial nitrogen in NAN. [4] Grams of N incorporated in microbial matter per kg of org. matter fermented ($gNi/kgOM_f$).

Digestion of N-components. Apparent digestibility of N-components in the rumen and intestinal tract including the hindgut is not very meaningful and therefore only the flux of TN, NAN (TN-ammonia N) and AAN (α-amino N) at the proximal duodenum is presented. Defaunation (P4-P1) caused a considerable increase in the amount of N-components leaving the rumen (Table 6). Soybean oil hydrolysate addition had the same effect, but statistical significance was not reached. The TSB ration had no influence. Total tract digestibility of TN was somewhat lower after defaunation, but increased with TSB.

Table 6 also shows that defaunation almost doubled microbial growth efficiency (E). Soybean oil hydrolysate addition also increased E, even above the already high value of P1 but the difference did not reach statistical significance. During the refaunated period, the increase was also considerable but not stat. significant. Crushed toasted soybeans had no effect. The amount of microbial N (MN) leaving the rumen was only increased by defaunation, not by lipid supplementation. This means that the increase in NAN flux after SOH feeding (P2 versus P1 and P3 versus P4) was mainly due to more feed protein leaving the rumen undegraded.

Digestion of the lipid fraction. Except for the TSB period (P5),the amount of total lipid leaving the rumen equalled intake, suggesting microbial lipid synthesis was insignificant (Table 7). Reasons for the lower value during P5 can be twofold : the flux could be underestimated or lipid extraction of the samples could be incomplete. Total tract digestibility of lipid increased considerably by SOH or TSB feeding, while defaunation had no effect. The data for post-ruminal digestion again showed that digestibility, expressed as fraction of lipid available in proximal duodenum, increased in periods when SOH or soybeans were fed. Intestinal digestibility of SOH-fatty acids was higher than the lipid fraction in TSB. As could be expected, lipids are

Table 7. Effect of SOH and TSB on digestion of lipid fraction.

Period	Refaunated control (P4)	Defaunated control (P1)	Refaunated SOH (P3)	Defaunated SOH (P2)	Refaunated TSB (P5)	RSD[3]
Intake total lipid (g/d)	30	30	100	100	106	
Flow duodenum						
Tot.lipid (g/d)	30.4[a]	29.6[a]	100.9[b]	102.7[a]	88.6[b]	7.4
FA (mmol/d)[1]	48.6[a]	54.8[a]	256.1[bc]	279.0[c]	237.8[b]	18.9
Faeces						
Tot.lipid (g/d)	12.6[a]	12.3[a]	17.4[ab]	19.6[b]	27.4[c]	3.1
FA (mmol/d)[1]	13.5[a]	14.7[a]	31.4[a]	33.0[a]	60.9[b]	10.7
Total tract dig. of total lipid (%)	57.9[a]	59.2[a]	82.6[b]	80.4[b]	74.2[c]	3.0
Post-ruminal digestib.						
Tot. lipid (g/d)	17.7[a]	17.4[a]	83.5[c]	83.1[c]	61.2[b]	7.9
% of available[2]	58.3[a]	58.7[a]	82.8[c]	80.7[c]	68.9[b]	3.4
% of tot.tract dig. FA	102.2[b]	97.9[ab]	101.2[b]	103.0[b]	78.0[a]	9.1
% of available	72.2[a]	72.9[a]	87.8[b]	87.7[b]	74.4[a]	4.6

[1] FA: long chain fatty acids, determined on Soxhlet extract. [2] digested as % of available in duodenum.
[3] RSD: residual standard deviation.

only digested post-ruminally, except during P5, again indicating that lipid flow at the duodenum during this period was perhaps underestimated.

Discussion

Rumen fermentation pattern

Defaunation caused changes in fermentation pattern (P4-P1) in agreement with other work (Jouany et al., 1988; Broudiscou et al., 1990). The considerable decrease in concentration of ammonia-N is not due to changes in degradation of feed protein, as the higher NAN flow to the duodenum during P1 is fully accounted for by the higher MN flow (P4-P1 : Table 6). Increased use of ammonia-N for bacterial protein synthesis after defaunation lowered its concentration in the rumen (Kayouli et al., 1986; Ushida et al., 1990; Hsu et al., 1991). During the defaunated period (P1-P2) SOH addition influenced molar proportions of individual VFA in agreement with literature data (Van Nevel & Demeyer, 1988). This is a consequence of a selective toxic effect of SOH on individual bacterial species or fungi, as no protozoa were present (Prins et al., 1972; Henderson, 1973; Maczulak et al., 1981). Broudiscou et al. (1990) did not find an effect on butyric acid (C4) proportions under similar experimental conditions, but C4 percentages were already low in periods without SOH, indicating that the final effect is depending on ration composition. The lower concentration of total VFA after addition of SOH (P2) is probably due to an increased rumen liquid volume (Broudiscou et al., 1990), or/and to decreased fermentation of

OM in the rumen (Table 5). Soybean oil hydrolysate addition during the refaunated period (P4-P3) caused similar effects, but the decrease in total VFA concentration was not statistically significant. The lower ammonia-N concentration was probably the result of lower feed protein degradation, as indicated by the lower OM digestibility (Table 5), but an increase in rumen liquid volume cannot be excluded.

Feeding TSB had only a minor influence on rumen fermentation pattern. Ammonia-N concentration was higher (P5-P4), possibly related to a slightly higher N intake, while feed protein degradability seemed to be higher (Table 6: difference NAN-MN). Protozoal numbers were decreased by SOH addition, as found earlier (Czerkawski, 1973; Ikwuegbu & Sutton, 1982; Sutton et al., 1983; Broudiscou et al., 1990). Comparison of the decrease in total VFA and protozoa indicates that the latter effect was not due to an increased rumen liquid volume after SOH administration, but the result of toxic effects of the fatty acids.

This experiment has shown that an equal amount of lipid, fed as TSB (approx. 35% of ration DM) had only minor effects on rumen fermentation pattern, which is especially important for acetic-and propionic acid proportions, related to lower milkfat content after fat supplementation (refs: see Palmquist, 1984). Feeding of whole roasted soybeans (max.24% of ration DM) or 1kg or 2kg of full-fat rapeseed had also no effect on VFA pattern in the rumen of lactating cows (Murphy et al., 1987; Knapp et al., 1991). It has been suggested that whole beans form a natural protection against release and subsequent hydrolysis of glycerolesters, whereby the former process is more determining than the latter, because normally, lipolysis proceeds very rapidly (Doreau et al., 1989). Consequently, differences between SOH and TSB observed in this experiment should rather be due to protection against immediate release of fatty acids in the case of TSB than to the difference : glycerolesters in TSB against free fatty acids in SOH.

Finally, it is clear that the effect of fatty acids on rumen fermentation pattern was not solely due to the decreasing or sometimes disappearing protozoa, as with defaunated animals, similar effects were found, be it to a lesser extent for most of the parameters.

Ruminal digestibility

Effect of defaunation. The data for digestibility of different feed components in different sites of the gastrointestinal tract, obtained during the control period (P4) agree with other work (e.g. Sutton et al., 1975; Ikwuegbu & Sutton, 1982; Sutton et al., 1983; Pallister & Smithard, 1987; Jenkins & Fotouhi, 1990; Ushida et al., 1990). Defaunation lowered rumen digestibility of OM, probably due to lower NDF digestion, and increased the flow of MN to the duodenum, which confirms earlier work (Kayouli et al., 1986; Ushida et al., 1990). Increased MN flows from rumen to duodenum were due to elimination of protozoa as predators of bacteria, with subsequent lower lytic and degradation activity of bacterial protein (Demeyer & Van Nevel, 1979; Kayouli et al., 1986).

Effect of soybean oil hydrolysate. Rumen digestibility of OM was clearly decreased

by SOH feeding both in the defaunated and refaunated state. This observation may be related to a lowered NDF digestion, as reported by other authors for the normal faunated rumen (Palmquist, 1984; Doreau et al., 1989; 1991b). The final effect of supplemental fat on ruminal digestibility of OM and NDF is related to the amount and nature of the fat (glycerolesters versus fatty acids;animal versus vegetable fat; protected fat) and the composition of the basal ration, as in literature no effect as well as inhibition was reported (Ikwuegbu & Sutton, 1982; Sutton et al., 1983; Boggs et al., 1987; Broudiscou, 1988; Jenkins & Fotouhi, 1990; Bock et al., 1991; Doreau et al., 1991a,b; Krysl et al., 1991; Ohajuruka et al., 1991). Broudiscou (1988) investigating the effect of linseed oil observed that the oil considerably decreased rumen digestibility of the hemicellulose fraction in defaunated as well as refaunated sheep, while cellulose was not influenced.

In defaunated sheep, direct effects on crude fibre degrading bacteria, and perhaps fungi are the reason for the lower digestibilities, while in the normal rumen, the effects are the result of actions on protozoa, as well as on bacteria (Henderson 1973; Maczulak et al., 1981).

The flow of N components from the rumen tended to increase by SOH. In earlier experiments, this increase was explained by the lower protozoal counts in the rumen (Ikwuegbu & Sutton, 1982; Sutton et al., 1983). However, we observed the same tendency in protozoa-free sheep (P1-P2), while it can be calculated that the increased NAN flow was due to increased feed N and not microbial N as observed by defaunation. Table 6 indeed shows that SOH had no influence on MN flow (P1-P2 and P4-P3), as also reported by Jenkins & Fotouhi (1990). This is in contradiction with Sutton et al. (1983), who found that, after addition of linseed oil and coconut oil, the increased total N flow to the duodenum was entirely due to increases in microbial N flow. However, in their experiments, the oils had a much more drastic effect on protozoal numbers compared with our data. On the other hand, in a subsequent experiment, linseed oil also increased total N flow, but more than could be accounted for by increases in microbial protein synthesis (Ikwuegbu & Sutton, 1982).

The different treatments had very interesting effects on the efficiency of microbial protein synthesis (E). The largest increase is caused by defaunation (Table 6 : P4-P1), which is known from earlier papers (Kayouli et al., 1986; Ushida et al., 1990). This large increase was the result of a higher MN flow and a decrease of OM fermented in the rumen. Soybean oil hydrolysate addition increased E in presence or absence of protozoa, although not significantly. Here, the increase was entirely due to the lower OM fermented in the rumen as MN flow remained unchanged. Higher E after fat supplementation was also found by several authors (Ikwuegbu & Sutton, 1982; Sutton et al.; 1983; Tamminga et al., 1983; Boggs et al., 1987; Jenkins & Fotouhi, 1990). The E values were extremely high during the defaunated periods, but agree well with Sutton et al. (1983). However, E has a rather relative significance, as the final amount of amino acids entering the duodenum is the most important factor.

Valid use of cytosine as microbial marker assumes that all cytosine of feed origin is completely degraded in the rumen. It remains possible that during the defaunated period (P1), in line with a decreased ruminal OM digestion, cytosine degradation

was also lowered. The latter can result in an overestimation of MN flow at the duodenum. However, increases in duodenal MN flow after defaunation were often found, using microbial markers other than cytosine (Kayouli et al., 1986; Ushida et al., 1990).

Post-ruminal digestibility

In line with decreased digestibility of OM in the rumen after defaunation or SOH addition, digestion of this component in the post-ruminal tract was increased, above the amount of fat in the diet. This shift in digestion from rumen to lower gut after defaunation of the rumen or feeding supplemental fat has often been observed (Ikwuegbu & Sutton, 1982; Sutton et al., 1983; Kayouli et al., 1986; Boggs et al., 1987; Jenkins & Fotouhi, 1990; Ushida et al., 1990). As a result of this compensation, fat supplementation had in most cases no effect on total tract digestibility of OM and fibre (Tamminga et al., 1983; Olubobokun et al., 1985; Boggs et al., 1987; Pallister & Smithard, 1987; Jenkins & Fotouhi, 1990; Doreau et al., 1991a; Emanuelson et al., 1991; Ohajuruka et al., 1991), although decreases have also been reported, specially due to lower degradation of the fibre fraction (Ikwuegbu & Sutton, 1982; Sutton et al., 1983; Doreau et al., 1991b).

Soybean oil hydrolysate or TSB increased considerably post-ruminal digestion and total tract digestibility of total lipid, indicating that supplemented fat had a higher digestibility than the lipid in the basal ration. This agrees with earlier work reporting high intestinal digestion of several fat supplements, with unsaturated fatty acids showing a better digestibility than saturated ones (Andrews & Lewis, 1970a,b). As we did not determine fatty acid composition of the lipid fraction entering the duodenum, we do not know if the proportion unsaturated versus saturated fatty acids also played a role in improved digestibility of total lipid observed here. The higher digestibility of fatty acids (FA determined on Soxhlet extract) versus total lipid (as % of available in duodenum) is due to the fact that total lipid (Soxhlet after acid hydrolysis) contains substances, other than fatty acids and less digestible. As FA content of the ration was not determined, calculation of the proportion of FA in total lipid could not be calculated.

Conclusion

It is clear that the change in rumen fermentation pattern when fats or fatty acids are fed, is not entirely due to a decrease in protozoal numbers, but the resultant of actions on the total microbial population. Only defaunation drastically increased MN flow to the duodenum. Consequently, only drastical effects of fats or fatty acids on protozoal numbers will cause a higher flow of MN to the lower digestive tract. Moderate effects, as observed in this experiment, were accompanied by a higher NAN flow (not statistically significant) to the duodenum, which was fully accounted for by more feed protein escaping rumen degradation, as microbial N flux was not changed. The higher E during the defaunated state can be explained by the absence of protozoa as predatory and lytic factors. This also indicates that in the normal ru-

men, microbial growth is more related to presence of ciliates and predation than to available energy.

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