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EFFECTS OF DEFAUNATION AND METHIONINE IN THE PRESENCE OF PROTOZOA ON THE FLOW OF MICROBIAL LONG CHAIN FATTY ACIDS FROM THE RUMEN OF SHEEP

Pengaruh Defaunasi dan Methionin pada Ternak yang Tidak Didefaunasi Terhadap Aliran Keluar Asam Lemak Rantai Panjang Mikroba Dari Rumen Domba

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ABSTRACT

This study was designed to investigate the effects of the presence or absence of protozoa in the rumen, and of methionine supplement when protozoa are present in the rumen, on microbial production of LCFA. The study used 12 first-cross Merino x Border Leicester wethers (2 years old) with body weights of between 35 and 44 kg, each was equipped with both rumen and abomasum cannulas. All sheep were offered a mix of 400 g of oaten chaff + 400 g of lucerne chaff per day which was delivered in 24 equal portions at hourly intervals via an overhead automatic-feeding machine. Digesta flow was measured using chromium ethylenediamine tetra-acetic acid (CrEDTA) complex and dietary acid-insoluble ash (AIA) as the liquid- and particle-digesta marker, respectively. All sheep were firstly defaunation using detergent alkanate 3SL3, and 8 of them were refaunated with a fresh rumen liquid obtained from a normal donor animal. Thus, there were 8 animals in the refaunated group at the commencement of study, 4 of which were intended to receive methionine supplement. However, comparison of refaunated sheep with or without methionine supplement is based on only 3 sheep per cell; 2 animals (1 of the methionine-supplemented and 1 of the methionine-unsupplemented group) were suitable to be included in the analysis. Comparison of defaunated and refaunated sheep was based on 4 sheep per cell. The study showed that the daily flow of dry matter through the abomasum was higher ($P<0.05$) in refaunated animals, despite the lower liquid outflow rate in these sheep. The concentration of LCFA in total digesta dry matter was not affected ($P>0.05$) by the presence or absence of protozoa in the rumen. However, the presence of protozoa increased concentration of LCFA in particle-digesta dry matter ($P=0.081$), the ruminal production ($P<0.05$) and daily flow of LCFA (0.01). Methionine supplementation did not alter (P 0.05) the concentration of LCFA in digesta, but significantly reduced ($P<0.05$) the flow of LCFA to abomasum.

Key words: Microbial long chain fatty acids, protozoa, methionine

INTRODUCTION

Attempts to maximise the contribution of microbial protein to the host is one of the most important aspects in ruminant nutrition. Eliminating protozoa from the rumen (defaunation) has been studied for over the years as one approach to achieve this goal. This is based on the facts that protozoa are predator for the rest of ruminal microbes and competitor for nutrients needed by the other microbes to grow. Due to sequestration in the rumen, however, the post-ruminal flows and contributions of protozoal cells and

protein to the host are as not much as those of bacteria. The general findings of defaunation studies are increased microbial protein synthesis and efficiency as well as increased availability of protein to be absorbed from the intestines. These effects will be more evident on animal performance when the feeds consumed are low in protein contents, and animals are in conditions where protein is considered as a limiting nutrient.

Apart from nitrogen, other nutrients such as lipids are also produced in the rumen. Lipid production in the rumen is obvious (Harfoot, 1981), but studies on this aspect have not been as extensively undertaken compared to nitrogen. An increase in ruminal production of lipids is undoubtedly beneficial because through this process the wastage of energy in the form of methane could be reduced and the absorbed long chain fatty acids (LCFA) are utilised more efficiently than volatile fatty acids (VFA). While it is known that protozoa are higher in lipid content compared with bacteria, and approximately three-quarters of ruminal lipids is associated with the protozoa (Katz and Keeney, 1969; Keeney, 1970), there has been no study carried out on the effect of defaunation of the ruminal production of lipids/LCFA.

This study aims at investigating the effects of the presence or absence protozoa in the rumen, and of methionine supplement when protozoa are present, on the ruminal production of long-chain fatty acids.

MATERIALS AND METHODS

Experimental Animals and Feeding

Twelve first-cross Merino x Border Leicester wethers (2 years old) with body weights of between 35 and 44 kg were used in this study. All animals were surgically prepared with ruminal and abomasal cannulas at the commencement of the study. The ruminal cannula bung was equipped with 2 stainless-steel probes whose inner ends were directed away from one another. To the tip of the probe from which ruminal fluid was collected, a small metal cage covered with gauze was attached and directed to the ventral sac of the rumen. The second probe used for the administration of chromium ethylenediamine tetra-acetic acid (CrEDTA) complex solution directed to the dorsal position in order to prevent a direct sampling of the infused solutions.

All sheep were offered a mix of 400 g of oaten chaff + 400 g of lucerne chaff per day (air dry). The oaten chaff was previously spray treated with urea (2 %, w/w) and a mineral mix (2.8 %, w/w) containing dicalcium phosphate (45.5 %), sodium sulphate (36.4 %), sodium chloride (9.1 %) and trace minerals (Pfizer; 9.1 %). The ration was delivered in 24 equal portions at hourly intervals via an overhead automatic-feeding machine. The oaten and lucerne chaffs were evenly mixed during placement on the belt of the machine. Drinking water was available *ad libitum* throughout the experiment. Animals were allowed to become accustomed to the feed provided daily, then twice daily over several weeks, and then hourly for at least 1 week each before the commencement of the study.

Defaunation and Refaunation Procedures

All sheep were initially defaunated using the detergent alkanate 3SL3 (ICI Australia Ltd.; active ingredient sodium lauryl diethoxy sulphate). Approximately 120 ml of the solution (10% w/v) was administered into the rumen through the ruminal cannula for 4 consecutive days. Feed was not offered during the treatment, but clean water was freely available. Sheep were allowed at least 2 weeks to recover from the treatment prior to the commencement of study. Eight sheep were refaunated by drenching with ruminal fluid (60 ml) from additional refaunated sheep held on similar feed and this was performed for 3 consecutive days. Ruminal fluid in refaunated animals

was transferred from one animal to another in order to unify the rumen environments. Defaunated and refaunated groups were housed in separate rooms in individual metabolic crates. Ruminal fluid of defaunated animals was checked to ensure the absence of protozoa at the commencement and completion of the experiment. The rooms were controlled at a constant temperature of 22 ^{o}C and were well ventilated. Animals were adapted to the new environment for at least 7 d before commencement of the study.

Intraruminal Infusion of CrEDTA Solution

To measure the flow of digesta to the abomasum, the double marker method (Faichney, 1975) was employed using the CrEDTA complex and dietary acid-insoluble ash (AIA) as the liquid- and particle-digesta marker, respectively. A solution containing CrEDTA complex (120 μgCr/ml), prepared according to Binnerts *et al.* (1968), was continuously infused into the rumen. The continuous infusion was made at a constant, measured rate (approximately 0.4 ml/min) through a silastic tubing (1.5 mm internal diameter) by a peristaltic pump. Infusion rate was calculated from the change in mass of the reservoir containing the CrEDTA infusate over periods of 3 - 8 h.

Sampling Procedures

For the enumeration of protozoa, ruminal fluid was withdrawn through the ventrally directed rumen probe into a 20 ml syringe. The first 10 ml was rejected and then 15 ml was flushed back into the rumen 2-3 times before the fluid sample was finally taken and placed into a vial containing 16 ml of a solution of formaldehyde in physiological saline and stored at room temperature.

Samples of abomasal digesta were collected (at 10a.m., 1p.m., 4p.m. 7p.m., 10p.m., 1a.m., 7a.m. and 4a.m) during days 4 and 5 during a 5 day infusion period of the intra-ruminal infusion of the CrEDTA solution. Prior to the collection, the abomasal digesta trapped in the cannula stem were scraped out and discarded. The abomasal digesta sample was collected by placing a bottle underneath the opened abomasal cannula. Abomasal digesta were then divided into 2 portions. The first portion was centrifuged (9,500 *g* x 15 m) to obtain "fluid-rich" and "particle-rich" fractions, while a second untreated portion of about equal weight was kept "as sampled". All of these fractions were stored at -18 ^{o}C.

Counting of Rumen Protozoa

The sample of ruminal fluid was thoroughly shaken and pipetted onto a counting chamber (Hawksley, Sussex, England) of 0.2 mm depth and covered with a double thickness coverslide. Protozoa were counted, aided with a light microscope (x100 magnification), on 48 cells of the counting chamber.

Laboratory Analysis

Samples of ground feed (1 mm sieve), abomasal digesta and particles were weighed into cleaned, tared filter crucibles (porosity 4). To determine their DM (dry-matter) contents, samples were dried at a constant temperature of 80 ^{o}C for 48 h. The AIA content of DM was determined according to the method of Choct and Annison (1992). Samples of DM were burnt in an ashing oven at a temperature of 480 ^{o}C for at least 8 h. The temperature was taken up gradually (*i.e.* to 150 ^{o}C in the first hour, to 300 ^{o}C in the second hour and then to 480 ^{o}C). The sample was then transferred into an evaporating dish filled with 4 M HCl, until the sample was wetted from underneath. Three-quarters of the crucible was filled with the acid. The evaporating dish was placed on a hot plate and boiled gently for 15 min. After cooling, the crucible was taken out and the acid containing soluble ash was removed by suction. The sample was rinsed with the acid and distilled H_2O. The sample was dried in an oven at 108 ^{o}C for 2 h and transferred into the ashing oven to repeat the procedure of ashing

and removing the acid-soluble ash. The sample was dried at 108 ^{o}C for 6 h and weighed.

Abomasal fluid and mixture of 2 g of abomasal particle-rich samples and 8 g of milli Q (extremely pure distilled) water, were centrifuged (9,500 g x 30 min) to obtain supernatants. The concentration of chromium in the supernatants was determined with an atomic absorption spectrophotometer (Perkin-Elmer, Connecticut, USA; Model 360). Stock standard solution (1000 μgCr/ml) was prepared from K_2CrO_4 (Binnerts *et al.*, 1968).

Analysis of Long Chain Fatty Acids (LCFA)

Extraction

The method of Viviani *et al.* (1966) was used to isolate the total LCFA from feed and digesta (Figure 1), which were then quantified by titration (Dole and Meinertz, 1960). Because these methods had not been previously used in this laboratory, it was required to set up the apparatus and evaluate the procedures. The method was chosen because: (i) it measures total (both free and bound) LCFA; (ii) it uses low temperatures and an antioxidant to avoid modifying the LCFA profile; (iii) it excludes non-LCFA lipid components which are of no benefit to the animal; and (iv) it provides an extract ready for methylation and analysis by gas chromatograph.

No other method which could provide all these benefits was found after extensive literature review. The principle of the method is as follows:

1. LCFA are saponified with methanolic-KOH;
2. LCFA soaps are then trapped in an aqueous phase while unsaponifiable materials are removed in the non-polar phase;
3. pH is then reduced to liberate free LCFA;
4. LCFA are absorbed back into a new non-polar solvent leaving other aqueous impurities behind;
5. The quantity of LCFA is determined by titration.

Samples were weighed (0.75 g for abomasal digesta and particles, 0.25 g for feed) into wide-neck McCartney bottles. Methanolic-KOH containing hydroquinone (10 ml of 10 M KOH, 25 ml of methanol, 0.05 g of hydroquinone, 15 ml milli Q water) was added: 3.75 ml for digesta samples and 4.75 ml for feed samples. This mixture was incubated in an oven with a constant temperature of 30 ^{o}C for 3 days. To each bottle, 10 ml of milli Q water was added, followed by the same volume of freshly distilled petroleum spirit (B.P. 40-60 ^{o}C). The mixture was shaken and allowed to stand and form two layers with a sharp separation. The petroleum spirit (the upper layer) containing unsaponifiable materials was removed by a mild suction. This removal of unsaponifiable substances was repeated twice with the same volume of petroleum spirit (10 ml). The water phase was acidified by addition of 1.3 ml of 9 M H_2SO_4 and 10 ml of petroleum spirit was added to force LCFA from the water phase into fresh non-polar solvent. The mixture was shaken thoroughly, and the petroleum phase containing extracted LCFA was removed under suction into another clean McCartney bottle. This procedure of extraction was repeated 5 times with the same volume of petroleum spirit (10 ml). The volume of petroleum in the collecting McCartney bottle was reduced through evaporation with nitrogen gas after each extraction to allow the addition of all of the solvent rinses in one bottle. The petroleum spirit extract was washed twice with milli Q water and evaporated to dryness with nitrogen gas. Methanol (8 ml) was added to the sample, and this was stored under nitrogen at - 18 ^{o}C prior to titration.

Titration

Three ml of the sample in methanol were transferred into a conical centrifuge tube for titration with CO_2-free NaOH (0.005 M). Bromothymol blue (0.02% in ethanol) was used as indicator (2 drops). Nitrogen gas was delivered to the bottom of the tube during titration to expel CO_2. Palmitic acid solution

(0.3 g crystallised palmitic acid in 100 ml of methanol) was used as the standard. Linearity of the titration over a range of 0 - 60 μmoles palmitic acid was shown to have an r^2 of 0.9975.

Mathematical Procedures

The flow of digesta to abomasum was calculated according to the double marker method of Faichney (1975). All data collected were analysed with analysis of variance using Minitab spreadsheets. A one way analysis of variance was used initially to test for effects of methionine (in the refaunated group). Where no effect was found, data for all refaunated sheep were pooled and a one way analysis of variance was made to compare defaunated and refaunated sheep.

RESULTS AND DISCUSSION

Results

There were initially 8 animals in the refaunated group at the commencement of study, 4 of which were intended to receive methionine supplement. Unfortunately, 2 animals (1 of the methionine-supplemented and 1 of the methionine-unsupplemented group) began refusing feed and were removed from the experiment prior to digesta-flow studies. Thus, comparison of refaunated sheep with or without methionine supplement is based on only 3 sheep per cell. Comparison of defaunated and refaunated sheep is based on 4 sheep per cell.

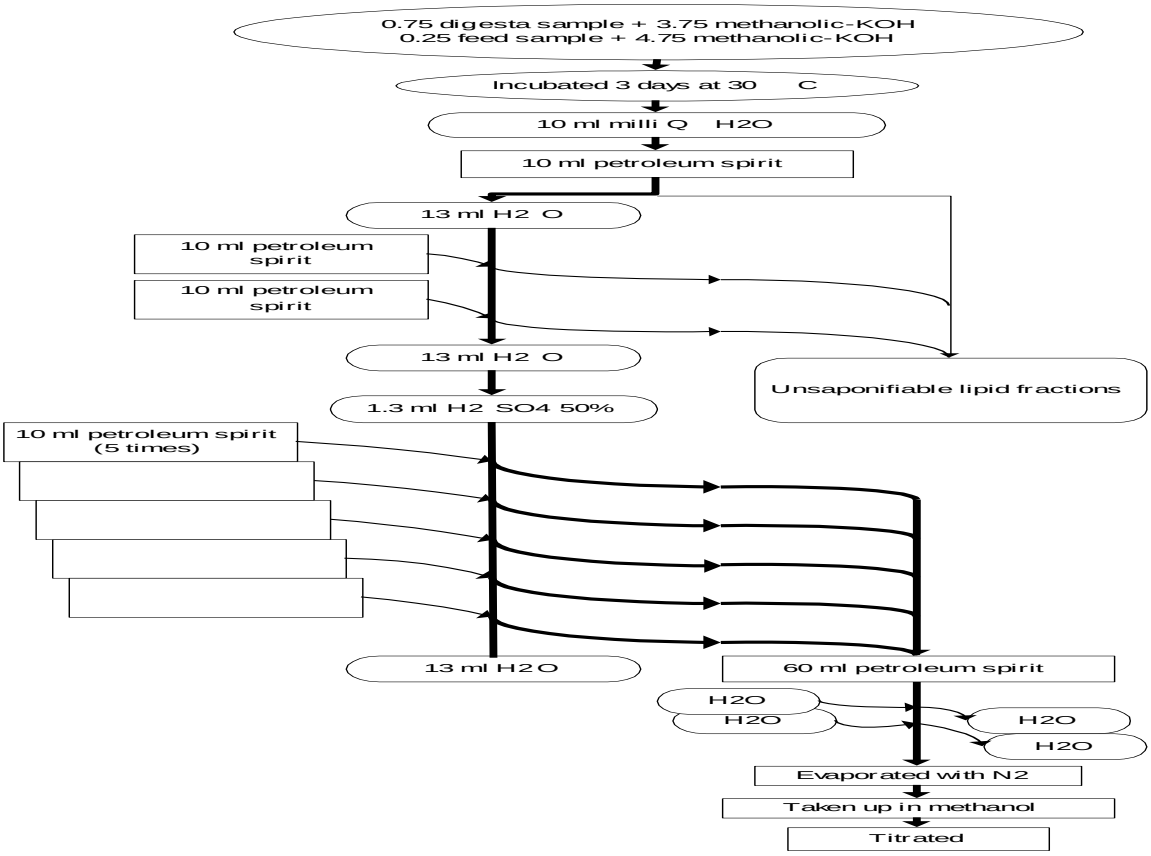

Figure 1. Procedure for Extracting Total LCFA From Diet and Abomasal Digesta (Viviani *et al.*, 1966). Subsamples of Final Extract Were Titrated In Triplicate.

As shown in Table 1, the concentrations of total LCFA in the DM of abomasal digesta as sampled and the particle-phase of abomasal digesta were not affected (P>0.05) by methionine supplementation. The concentration of total LCFA in the DM of abomasal digesta as sampled also did not differ (P>0.05) between defaunated and refaunated animals. The presence of protozoa in the rumen, however, tended to increase the concentration of total LCFA in particle-phase of abomasal digesta (P=0.081). With the exception of two animals (nos. 6 and 10), the concentration of total LCFA in digesta as sampled was higher than in the particle-phase of abomasal digesta, regardless of methionine supplementation and faunation status of the rumen.

The abomasal flow of DM (g/d) was significantly greater in refaunated than in defaunated animals (P<0.05). Supplementation of methionine reduced the total flow of LCFA (P<0.05) and the outflow of microbial LCFA produced in the rumen. On the other hand, the presence of protozoa in the rumen increased the daily flow of LCFA and their production in the rumen (P<0.01 and <0.05, respectively).

Discussion

The Abomasal Flow Of Total LCFA

Microbial lipids synthesised in the rumen contribute to the post-ruminal concentration and flow of LCFA (Harfoot, 1981). The contribution of the animal's own lipids rather than microbial lipids to the net gain in LCFA flow across the rumen is negligible (Noble, 1981). Previous studies of rumen lipid production have frequently been of limited use because the determination of lipid has included non-LCFA fractions and excluded much of the bound microbial LCFA. Microbial lipids contain some LCFA which are in an unesterified form (ULCFA), either free and fully protonated or ionic and so bound to the cationic sites of protein or of carbohydrate molecules (O'Leary, 1962; Viviani *et al.*, 1966). Other microbial LCFA are present in an esterified form (ELCFA). Owing to the acidic conditions in the abomasum (pH 2.00 to 2.5), however, there is no ionic form of ULCFA present in abomasal digesta (Bauchart, 1993). Even if ULCFA-proteins/carbohydrate complexes were present, all LCFA would have been extracted in the LCFA extraction procedure employed in this study. Viviani *et al*. (1966) found that the recovery of the total ruminal LCFA extracted by this procedure was the same as the sum of the ELCFA plus free and bound ULCFA in microbes when the esterified and unesterified forms were extracted individually.

Table 1. Concentration of Total LCFA in Abomasal Digesta And Their Flow To Abomasum In Defaunated and Refaunated Sheep Consuming 400 g of Oaten Chaff + 400 g Of Lucerne Chaff

Parameter	Defaunated				Refaunated						Statistical Effects	
					– M			+ M			M	P
Sheep no.	1	2	3	4	6	7	8	9	10	11		
Protozoal counts (x 10^{-5}/ml)	-	-	-	-	na	7.3	7.9	6.7	8.1	na		
Dry matter (DM) intake (g/d)	733.3	733.3	733.3	733.3	741.9	741.9	741.9	741.9	741.9	741.9		
Total LCFA intake (g/d)	4.2	4.2	4.2	4.2	5.3	5.3	5.3	5.3	5.3	5.3		
Total LCFA concentra tion in abomasal digesta												
digesta as sampled (g/ kgDM)	40.2	34.1	32.4	28.0	32.2	30.6	35.5	36.0	24.1	33.4	ns	ns
particle digesta (g/kg DM)	24.5	27.2	24.8	23.2	32.3	25.7	29.0	29.5	26.1	32.8	ns	= 0.081
DM flowing to abomasum (g/d)	332.6	340.4	352.3	358.5	438.4	399.1	444.5	359.2	444.2	370.1	ns	< 0.05
Total LCFA flowing to abomasum												
total flow (g/d)	11.8	10.9	10.8	9.6	14.4	13.2	16.2	12.1	10.5	12.2	< 0.05	< 0.01
rumen production (g/d)	7.6	6.6	6.6	5.4	8.8	7.9	10.9	6.9	5.2	6.9	= 0.052	< 0.05
(% of total)	35.8	38.9	39.0	43.9	38.9	40.2	32.8	43.7	50.4	43.3	= 0.059	ns

ns = not significant
na = not available
M = methionine main effect
P = protozoa main effect

This study clearly supports previous reports that the amounts of total LCFA leaving the rumen are generally higher than those consumed by animals in the diet (*e.g.* Sutton *et al.*, 1970; Bickerstaffe *et al.*, 1972; Hogan *et al.*, 1972). Total LCFA flowing out from the rumen consist of dietary LCFA which are adsorbed onto particulate matter and microbial LCFA derived from the incorporation of dietary LCFA and *de novo* synthesis. When the amount of LCFA ingested by animals is subtracted from the total LCFA flowing out from the rumen, the value obtained can be interpreted as the quantity of total LCFA derived from *de novo* synthesis by ruminal microbes. In making this calculation, it is assumed that no LCFA are catabolised in the rumen (Bickerstaffe *et al*., 1972). In this study, approximately 40 % of the total LCFA available for absorption by the animal were of microbial origin.

Effect of Defaunation

Bacterial pool size and amino acid outflow are generally higher in defaunated compared with refaunated animals (Williams and Coleman, 1988). However, it does not follow that a greater LCFA outflow will occur in defaunated ruminants. The full protozoal protein mass is not available for post-ruminal digestion due to sequestration of protozoa in the rumen (Coleman, 1989). Indeed, the flow rate of protozoa is only 20-40 % of that of liquid (Williams and Coleman, 1992), indicating many protozoa are retained and lysed in the rumen. There is, however, a fundamental difference in the ruminal metabolism of protein and of LCFA; this being that protein is degraded within the rumen (mainly by protozoa) but LCFA are not (Bickerstaffe *et al*., 1972). Consequently, an effect of protozoa on LCFA outflow will be seen only if they synthesise LCFA to a greater or less extent than the bacteria they replace. Protozoal predation of bacteria in the rumen *per se* will not reduce LCFA outflow as it does for amino acid flow for which ruminal deamination is possible.

The finding that protozoa in the rumen significantly increased the ruminal LCFA production and the daily flow of total LCFA to the abomasum indicates a greater *de novo* synthesis of LCFA in protozoa than in the bacteria they replace. This is expected considering that protozoa contain more lipid than do bacteria, and up to 75 % of microbial lipid in the rumen is protozoal in origin (Katz and Keeney, 1969; Keeney, 1970).

In addition to the possible contribution of protozoal cell lipid to the higher flow of LCFA in refaunated animals, some protozoal LCFA may also flow out from the rumen unbound to the protozoal cells. If protozoal cells are lysed in the rumen, their lipids as well as those of engulfed bacteria will be released to the medium. The released lipid will be hydrolysed and the products of hydrolysis (ULCFA) will be taken up by the existing microbes or alternatively will be adsorbed onto particulate matter which flows out from the rumen.

Effect of Methionine

The mode of action of methionine or methionine hydroxy analog (MHA) to affect the synthesis of LCFA or lipids by microbes has been previously studied. *In vitro* study with non-ruminal bacteria (O'Leary, 1959) showed that the labelled carbon in the methyl group of methionine was incorporated mostly into an "unknown compound", which was later found by the author to be a cyclopropane fatty acid (O'Leary, 1962). While the presence of this LCFA in ruminal microbes has not been shown to date, Patton *et al.* (1970) demonstrated that supplementing methionine hydroxy analog decreased the free ULCFA in ruminal fluid and apparently promoted the formation of an unidentified polar lipid. The consequence of the reduction of free ULCFA will be reduced biohydrogenation of unsaturated LCFA since hydrogenation is dependent on the presence of LCFA in their free form (Hawke and Silcock, 1969). If this is true, the proportion

of unsaturated LCFA flowing out from the rumen may be higher in animals receiving methionine. Fatty acid profiles of digesta were not, however, determined.

The present study indicates that the daily total flow of LCFA was significantly reduced by methionine supplement, even though the daily flow of DM was not altered by the supplement. The ruminal production of total LCFA was also lower in methionine-supplemented animals compared with the control animals. This is in contrasts with previous observations made by O'Kelly and Spiers (1990) that methionine increased the lipid synthesis by ruminal microbes *in vitro*. Two factors may have contributed to this discrepancy. First, the synthesis of lipids by particle-phase microbes was not accounted for in the study of O'Kelly and Spiers (1990); these microbes may respond differently to methionine supplement compared with liquid-phase bacteria. Second, O'Kelly and Spiers (1990) determined the synthesis of total lipids rather than LCFA (as in the current study).

In contrast to the reports of O'Kelly and Spiers (1990) and in like manner to results of this study, Patton *et al.* (1970) found that supplementing cows with 40 and 80 mg of MHA tended to reduce the concentration of lipid in the rumen, but this was not significant. In that study, it was also found that the methionine hydroxy analog supplement significantly reduced the percentage of stearic acid by inhibiting reduction of oleic and linoleic acids. The digesta-flow study reported here is probably the first to show the suppressive effect of methionine on LCFA synthesis. The reasons why methinone reduced the LCFA outflow are still not known at this stage, and this requires further study.

The Concentrations Of Total LCFA in Digesta

There was a tendency for the concentration of LCFA in particle digesta of refaunated animals to be higher than that of defaunated animals. It has been demonstrated by many authors (*e.g.* Merry and McAllan, 1983; O'Kelly and Spiers, 1988; Hegarty *et al.*, 1994) that the lipid content of particle-phase bacteria is higher than that of fluid-phase bacteria. Therefore, the changes in the numbers of bacteria associated with both phases of digesta will obviously have an effect on the concentration of LCFA in digesta. It is unlikely, however, that the greater LCFA concentration in particle digesta of refaunated than of defaunated animals is due to the increased numbers of particle-phase bacteria. Orpin and Letcher (1984) found that defaunation increased the number of liquid-phase bacteria but not of particle-phase bacteria.

Gram-negative bacteria are known to contain more lipid than do Gram-positive bacteria (Salton, 1960) and it is possible that the higher LCFA concentration in particle digesta of refaunated sheep was due to a higher proportion of Gram-negative relative to Gram-positive bacteria. Ushida *et al*. (1986) observed an increase in the number of Gram-negative bacteria but not of Gram-positive bacteria due to defaunation, while other workers (Hsu *et al*., 1991) found that defaunation increased the numbers of both groupings equally.

The difference between defaunated and refaunated groups in the concentration of total LCFA in particle digesta in the abomasum may also be due to the changes in the concentration of LCFA within the bacterial cells. Hegarty *et al.* (1994) found that there was a tendency for both particle- and liquid-phase bacteria to have higher lipid contents when protozoa were present in the rumen, irrespective of the availabilities of nitrogen and sulphur or the kinetics of rumen liquid. The higher LCFA content of particle-digesta DM in refaunated animals could be partly due to the higher lipid content of particle-phase bacteria and a greater flow of these bacteria in refaunated sheep. In summary, it is postulated that the higher LCFA concentration in the particle phase of

abomasal digesta may be partly due to the contribution of protozoa with higher lipid contents compared with bacteria, and/or a shift in the population from mostly fluid to mostly particle attached species, and/or a shift from Gram- positive to Gram-negative species.

CONCLUSIONS

From results of this experiment, the following conclusion can be drawn: (i) the daily flow of dry matter through the abomasum was higher in refaunated animals, despite the lower liquid outflow rate in these sheep; (ii) The concentration of LCFA in total digesta dry matter was not affected by the presence or absence of protozoa in the rumen, but, the concentration of LCFA in particle-digesta dry matter, the ruminal production and daily flow of LCFA, were significantly higher in refaunated than in defaunated animals; and (iii) methionine supplementation did not alter the concentration of LCFA in digesta, but significantly reduced the flow of LCFA to abomasum.

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