EFFECTS OF DIETARY FATTY ACIDS UPON RUMEN METABOLISM AND DIGESTIBILITY IN SHEEP

A Thesis

Presented to

the Faculty of Graduate Studies and Research

The University of Manitoba

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Ronald Alexander Clarke

October 1966

ACKNOWLEDGEMENTS

Gratitude is expressed to Dr. W. K. Roberts for his guidance and assistance throughout the course of the study and for his suggestions and criticisms in the preparation of the manuscript.

The assistance of Mr. J. Woodhouse with the collection of samples is gratefully acknowledged.

Financial assistance for the project was provided by the Horned Cattle Purchase Fund, Manitoba Department of Agriculture and Conservation.

ABSTRACT

EFFECTS OF DIETARY FATTY ACIDS UPON RUMEN METABOLISM AND DIGESTIBILITY IN SHEEP

BY

RONALD ALEXANDER CLARKE

Two metabolism experiments were conducted with sheep to study effects of increasing dietary levels of unsaturated fatty acid upon utilization of various ration components. Four rumen fistulated sheep were used, and each experiment was conducted according to a 4 X 4 Latin square design involving 4 sheep, 4 rations and 4 periods. Each period was of 18 days duration consisting of 10 days for adjustment and an 8 day collection period. Rumen, fecal, and blood samples were collected at specific intervals. In both experiments the treatments were made up by varying the proportions of methyl stearate, oleic, and linoleic acids in the basal ration.

Apparent digestion coefficients for energy, nitrogen, fibre, dry matter, ether extract and crude fat were not significantly affected when increasing levels of unsaturated fatty acids were fed. Although ether extract and crude fat digestibilities were not directly affected by treatment, distinct qualitative changes in rumen and fecal

saturated fatty acid levels were observed. Ruminal fatty acids showed a higher degree of saturation than comparable dietary fatty acids. These qualitative changes were in accordance with high levels of saturated fecal fatty acids observed in the unsaturated fatty acid treatments. The results, in general, suggest that hydrogenation of C-18 polyethnoid fatty acids was occurring within the rumen.

Synthesis of palmitic and oleic acids appeared to occur within the rumen. Rate of synthesis may have been influenced by ruminal levels of these acids which in turn were affected by dietary levels.

Fat digestibilities decreased 11 - 19 digestion units when fecal soap excretion was taken into account. The fatty acids making up these soaps appeared to differ with treatment. Fecal stearate excretion in the form of soaps from the unsaturated fatty acid treatments was about 110% higher than fecal stearate excretion in the saturated fatty acid treatment.

The relative proportions of serum fatty acids were not affected by dietary fatty acids.

No significant differences were observed among treatments in total ruminal VFA concentrations, or was any definite trend deliniated indicating that degree of ration fatty acid unsaturation affected total VFA concentration in

the rumen. In general, as dietary unsaturated fatty acids were increased, the proportions of propionic acid increased and butyric acid decreased.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
LITERATURE REVIEW	3
Some Factors Affecting Fatty Acid Utilization in Monogastrics	3
Fatty Acid Metabolism in the Rumen	9
Effects of Fats and Oils on Digestibilities of Various Ration Components	14
Some Aspects of Volatile Fatty Acid Metabolism	16
Plasma Lipids in Ruminants as Influenced by Dietary Lipids	18
EXPERIMENTAL PROCEDURE	20
Sampling Procedures	22
Sample Preparation and Chemical Analysis	25
Statistical Analysis	29
RESULTS	30
Changes in Fatty Acid Proportions from Ration to Rumen Contents to Feces	33
Changes in Ruminal Fatty Acid Levels with Time after Feeding	40
Daily Consumption vs. Fecal Excretion of Fatty Acids	40

Page

Fecal Excretion of Fatty Acids - Ether Extract Fraction vs. Crude Fat Fraction 45
Protozoa Fatty Acid Composition 48
Serum Fatty Acid Levels 52
Rumen Volatile Fatty Acids. 52
DISCUSSION. 57
Changes in Fatty Acid Proportions from Ration to Rumen Contents to Feces. 58
Changes in Ruminal Fatty Acid Levels with Time after Feeding. 61
Ether Extract vs. Crude Fat on Fecal Extractions. . 61
Protozoa Fatty Acid Composition 63
Serum Fatty Acid Levels 64
Rumen Volatile Fatty Acids. 65
CONCLUSIONS 67
BIBLIOGRAPHY. 69

LIST OF TABLES

Table		Page
1.	Basal Ration	21
2.	Fatty Acid Composition of the Various Fatty Acid Sources	23
3.	Ration Fatty Acid Composition.	24
4.	Experiment I. Apparent Digestion Coefficients of Various Ration Components	31
5.	Experiment II. Apparent Digestion Coefficients of Various Ration Components	32
6.	Experiment I. Fatty Acid Levels in Rations, Rumen Contents (6 Hours) and Feces	34
7.	Experiment II. Fatty Acid Levels in Rations, Rumen Contents (6 Hours) and Feces	35
8.	Experiment I. Fatty Acid Levels in Rumen Contents at Various Times After Feeding. . . .	41
9.	Experiment II. Fatty Acid Levels in Rumen Contents at Various Times After Feeding. . . .	42
10.	Experiment I. Daily Fatty Acid Consumption and Fecal Fatty Acid Excretion	43
11.	Experiment II. Daily Fatty Acid Consumption and Fecal Fatty Acid Excretion	44
12.	Experiment I. Fecal Fatty Acid Excretion of Ether Extract and Crude Fat Fractions.	49

Table Page

13. Experiment I. Protozoa Fatty Acid Composition 50

14. Experiment II. Protozoa Fatty Acid Composition 51

15. Experiment II. Serum Fatty Acids in Lambs Receiving Various Dietary Fatty Acids 53

16. Experiment I. Rumen Fluid Volatile Fatty Acids of Lambs Receiving Various Dietary Fatty Acids. 54

17. Experiment II. Rumen Fluid Volatile Fatty Acids of Lambs Receiving Various Dietary Fatty Acids 55

LIST OF FIGURES

Figure		Page
1.	Experiment I. Stearic and Oleic Acid Levels in the Ration, Rumen Contents, and Feces. . .	38
2.	Experiment II. Linoleic + Oleic and Stearic Acid Levels in the Ration, Rumen Contents, and Feces	39
3.	Experiment I. Fecal Fatty Acid Excretion vs. Fatty Acid Consumption/day.	46
4.	Experiment II. Fecal Fatty Acid Excretion vs. Fatty Acid Consumption/day.	47

INTRODUCTION

Recent uses of inedible fats and oils in animal feeds have provided a stimulus for the elucidation of fatty acid utilization in ruminants. Considerable experimental information is available with respect to fatty acid utilization in monogastrics; this is not the case for ruminants, however. Extrapolation of monogastric data to ruminants is not possible because of inherent differences between monogastrics and ruminants in fatty acid metabolism. The proportions of fatty acids received in the diet of monogastric animals remains relatively constant until subsequent absorption. Ruminants, on the other hand, are capable of changing the proportions of fatty acids in the diet through hydrogenation of unsaturated fatty acids within the rumen, thereby presenting a more saturated mixture of fatty acids for subsequent absorption. If one assumes that metabolism of fatty acids (post-ruminal) is comparable to that of monogastrics, then rumen hydrogenation could have a detrimental effect on digestibility. Young and Garrett (40) have showed in the chick that palmitate and stearate were poorly digested as opposed to their unsaturated analogues. They also found that digestibilities of these acids were significantly improved by the addition of oleic and linoleic

acids to the ration.

The addition of fats or oils to ruminant rations has been shown to affect the digestibility of cellulose, protein, dry matter and organic matter. In addition, cellulose digestibility has been shown to be affected by different fatty acids in the ration. Davison and Wood (13) observed a significant difference in cellulose digestion when oleic acid was compared with stearic acid in a feeding trial. Digestion of cellulose was poorer in rations containing oleic acid.

There is a definite lack of knowledge concerning effects of different dietary fatty acids upon the utilization of such ration components as cellulose, protein, energy, fat and individual fatty acids in ruminants. The present experiments were conducted to determine effects of feeding various proportions of fatty acids (saturated and unsaturated) upon ration utilization and rumen fatty acid metabolism in lambs. The parameters investigated include the following: ration, ruminal and fecal levels of fatty acids; apparent digestibilities of crude fat, ether extract, energy, fibre, dry matter, and nitrogen; and serum fatty acid and rumen volatile fatty acid levels. The fatty acid composition of protozoa was also determined.

LITERATURE REVIEW

Some Factors Affecting Fatty Acid Utilization in Monogastrics

Young and Garrett (40) reported that various dietary components can influence the utilization of fatty acids in the chick. They showed that absorption of fatty acid mixtures high in saturated fatty acids can be improved by antibiotic treatments, and suggested that the effect may be mediated through the elimination of undesirable intestinal microflora. In another experiment the ration levels of crude protein, carbohydrate source, and the ratio of saturated to unsaturated fatty acids in the diet were shown to influence fatty acid absorption. When the level of crude protein was increased from 24 to 28%, an improvement in the apparent digestion of fatty acids from beef tallow was observed. Also, a significant improvement in beef tallow digestibility occurred when corn was used as the principal source of carbohydrate as compared to glucose. This carbohydrate effect was not observed in a later experiment and therefore could not be considered a consistant variable. Young and Garrett (40) also found that by increasing the amount of ration oleic and/or linoleic acids in relation to palmitic acid, a marked increase in the digestibility of palmitic acid occurred. Oleic acid was more efficient in this regard than linoleic acid.

Stearic acid, when fed alone, was digested to the extent of 14%. By the addition of palmitic acid (12% palmitic and 7% stearic in diet), the digestibility of stearic acid was reduced to 2%. When oleic acid was added to this mixture, an improvement in digestibility occurred; however, it was not as high as when oleic acid was added to palmitic acid alone. When linoleic acid was fed with oleic plus a mixture of the above two saturated fatty acids, the highest digestion of the saturated fatty acids was observed. Similar aspects of fatty acid metabolism were investigated by Renner and Hill (29). They determined the digestibility of palmitic and stearic acids with increasing levels of unsaturated fatty acids in chick diets. Animal tallow, containing 50% unsaturated fatty acids, lard containing 60% unsaturated fatty acids, and soybean oil containing 76% unsaturated fatty acids were used in the rations. There were substantial increases in palmitic and stearic acid digestibilities with increasing levels of ration unsaturated fatty acids.

The mechanism by which oleic acid facilitates absorption of saturated fatty acids is not clear. Langworth and Holmes (23) suggested that micelles made up of bile salts, mono-olein and/or oleic acid are formed in the lumen of the intestinal tract. These micelles have the ability

to enhance the emulsification of saturated fatty acids, and presumably make them more available for absorption. Young and Garrett (40) suggested that improved absorption of saturated fatty acids in the presence of oleic acid may be due to a preferential synthesis of mono-olein in the brush border of the mucosal cells and that this mono-glyceride acts as an acceptor of saturated fatty acids to form di- and triglycerides. This hypothesis was supported by the observations of Clark and Hubscher (10) where under in vitro conditions more 1-C^{14} palmitic acid was combined with mono-olein than with monolaurin or monopalmitin. Senior and Isselbacher (32) observed no difference between either the 9 or 8 isomers of monopalmitin or mono-olein in their ability to act as acceptors for palmityl Co-A to form diglycerides in vitro.

Renner and Hill (30) have shown that chicks fed palmitic acid in a mixture with triolein showed a stepwise improvement in palmitic acid digestion when the palmitic acid to triolein ratio was decreased from 3 to 1. In this case, it would appear that pancreatic lipase hydrolysed the triolein to oleic acid and mono-olein, and the mono-olein moiety could enhance the formation of more readily absorbed micelles as well as acting as an acceptor for palmitic acid in the formation of triglycerides.

The carbon chain length and degree of unsaturation of fatty acids can affect their digestibility. Carrol (7) showed that short chained fatty acids (up to C 10) are completely digested in the rat and that further increases in chain length resulted in a progressive decrease in digestibility. Accordingly, the digestibilities were relatively poor for C 18 and higher saturated fatty acids. The monounsaturated analogues were better digested but showed the same digestibility trend with increasing chain length. Presence of a double bond near the middle of the chain was found to be equivalent, in terms of digestibility, to shortening the chain length by six carbon atoms.

Fat digestibilities have been correlated with the melting points of some natural fats. Crockett and Duel (12) showed that margarine, Crisco, prime steam lard, and bland lard have high digestibility values in the rat and compare favorably with natural fats and oils which have melting points below 50°C. However, when lard was more hydrogenated (melting over a range of 55 - 61°C), there was a marked drop in digestibility when compared to fats of lower melting points. This could be expected since a higher melting point indicates a higher level of fatty acid saturation.

Melting point of fats, when used for the estimation of digestibility of a natural fat, has proven to be

inadequate. The melting point does not accurately reflect the species of fatty acids, however, since natural fats melt over a temperature range which is dependent upon the distribution of fatty acids among the glycerides. Some effects of saturated fatty acid distribution among glyceride molecules on fat digestibility were investigated by Mattson (24). He found that when the saturated fatty acids were distributed evenly among the glyceride molecules, digestibilities were significantly higher than when saturated fatty acids were distributed in a manner such that a portion of the triglycerides were made up entirely of saturated fatty acids. Mattson (24) concluded that fat digestibility was inversely proportional to content of simple triglycerides made up of saturated fatty acids having a chain length of 18 carbon atoms or more. This observation by Mattson could be partly explained by the fact that no unsaturated fatty acids were present within the triglyceride moiety, which would be capable of facilitating fatty acid absorption (23).

Several investigators have noted significant amounts of ether insoluble fatty acids (possibly caused by the complexing of the fatty acid anion with calcium, potassium etc. to form soaps) in the feces of monogastrics and ruminants. Duel et al. (14) determined rapeseed and cottonseed oil digestibilities in humans. They estimated the

fecal excretion of neutral fats, fatty acids and soaps. Cottonseed oil was digested to the extent of 96.5% vs. 99.0% for rapeseed oil. This difference in digestibility was due to significantly higher fecal soap excretion in the cottonseed oil treatment. These workers suggested that calcium ions may have had a greater depressing effect on the fatty acids of cottonseed oil than the fatty acids of rapeseed oil. Cheng et al. (8) designed an experiment to determine effects of including calcium and magnesium in diets relatively rich in natural fats, synthetic triglycerides, or fatty acids, upon fecal soap excretion. They found that the presence of calcium and magnesium in the diet did not change the digestibility of fat with melting points lower than 50°C. This observation helps to explain the high fat digestibilities reported by Crockett and Duel (12) in diets containing high levels of calcium and magnesium, sincetheir fats had melting points under 50°C. The removal of calcium and magnesium from the diet markedly increased digestibility of fats with melting points above 50°C (Cheng et al., 8). In the diet containing calcium and magnesium, trilaurin was digested to the extent of 70.5% whereas in the absence of these divalent rations, the value was 97.3%. Corresponding coefficients of digestibility for trimyristin under these divergent dietary conditions were 37.3 and 76.6%, respectively,

while with tristearin the values were 10.6 and 18.9%. Smaller additions of calcium and magnesium to the trilaurin diet resulted in less depression in digestibility. These data confirmed earlier observations by Boyd et al. (3) in which the depressing effect of ration calcium on fat digestion was related to fatty acid composition of the diet.

The uptake of fatty acids by intestinal mucosal cells appears to be influenced by soap formation. If penetration of fatty acids into mucosal cells occurs on the basis of lipid solubility, as suggested by Johnston and Borgstrom (22), then the presence of fatty acids in the non-ionized state (salts exist as ions in an acid media) should facilitate uptake. Hofman and Borgstrom (21) determined the pK of fatty acids in micellar solutions to be 6.5. In subsequent experiments in which the uptake of fatty acids in micellar solutions by intestinal slices was measured, they found that fatty acids were present predominantly in the non-ionized form.

Fatty Acid Metabolism in the Rumen

Conditions exist within the rumen whereby unsaturated fatty acids can be hydrogenated to their more saturated analogues. This phenomenon was first shown by Reiser (27) who incubated linseed oil in vitro with sheep rumen contents

and found a marked reduction in linolenic acid, accompanied by a corresponding increase in the amount of linoleic acid. He attributed this to hydrogenation of linolenic acid by bacteria found within the rumen. In vivo evidence for hydrogenation was obtained from studies with goats that were fed diets containing either 10% cottonseed or 10% linseed oil for several weeks (Reiser and Reddy, 28). At slaughter, which was 6 hours after the last feed, samples of rumen contents were subjected to iodine number determinations and analyzed for total concentration of long chained fatty acids. Results of this experiment showed that unsaturated fatty acids of the dietary oils had undergone considerable hydrogenation.

Erwin et al. (15) found that when safflower oil was infused into the abomasum of sheep, significantly higher fatty acid digestibilities were obtained than when safflower oil was administered via the rumen. This difference in digestibility was reflected by high fecal stearate excretions (47% of total fatty acids) of sheep administered safflower oil via the rumen as opposed to the abomasal infused sheep (14%). These fecal stearate values could partially account for the differences in digestibility of the safflower oil. This oil contains approximately 75% linoleic acid and a greater proportion of this fatty acid was being hydrogenated when the oil was administered via the rumen as opposed to the

abomasum.

Wood et al. (38) studied metabolism of linoleic acid in the sheep's rumen by isolating the rumeno-reticular area through ligation and then injecting C^{14} labelled linoleic acid into the rumen. They found that only 3 - 6% of the original labelled linoleic acid remained in the rumen after 48 hours; 45% having been completely saturated and 33 - 50% having been hydrogenated to oleic or elaidic acids.

If one assumes that oleic acid is hydrogenated to the extent of only 40% (Shorland et al., 34) and that stearic acid is absorbed only to a limited extent relative to its unsaturated analogues, then low crude fat digestibilities for oils or fatty acid mixtures high in linoleic acid might be expected. This could help explain the high fecal stearate values observed by Erwin et al. (15) when linoleic acid was administered via the rumen. Also, data presented by Roberts and McKirdy (31) could be partially explained by hydrogenation of linoleic acid within the rumen. These workers observed that the fat in a ration containing sunflower seed oil (61% linoleic acid) was significantly less digested than a ration containing rapeseed oil (28% linoleic acid, 30% oleic acid).

Tove (36) suggested that the process of hydrogenation and production of trans acids by rumen microflora

may be closely related. Shorland et al. (34) showed that trans acids were formed by rumen contents. They incubated oleic, linoleic and linolenic acids singly for 48 hours with sheep rumen contents and found trans acids. These acids were formed to the extent of 17, 48 and 67%, respectively. Positional isomers, particularly from linoleic acid, gave rise to a conjugated form (where the double bonds are separated by one linkage) which was resistant to further hydrogenation. Reiser and Reddy (28) reported that in the hydrogenation of linolenic acid (9-10, 12-13, 15-16) the central double bond is preferentially attacked, leading to the formation of dienoic acids with the double bonds separated by more than one methylenic group (i.e. non-conjugated). If hydrogenation by rumen microorganisms parallels that of catalytic hydrogenation, one would expect a wide variety of mono- and dienoic acids to be produced in addition to oleic and linoleic acids.

The role of protozoa in rumen fatty acid utilization was investigated by Gutierrez et al. (19) who incubated E. prostoma with C^{14} labelled fatty acids. This species of protozoa was able to incorporate fatty acids into its cellular components, and hydrogenation of oleic acid also appeared to occur during assimilation. Williams et al. (37) suggested that hydrogenation occurring within the rumen may

be influenced by the particular species of protozoa present.

Rumen microorganisms are capable of synthesizing saturated and unsaturated fatty acids. Erwin and Black (17) reported that protozoa can desaturate stearic acid and suggested the following sequence: stearate ⟶ oleate ⟶ linoleate ⟶ linolenate. Erwin and Black (17) also showed that protozoa have the ability to elongate C_{16} to C_{18} fatty acids. This elongation was found to be quantitatively higher than degradation of C_{18} to C_{16} fatty acids. The major conversion product of both palmitate and stearate was linolenic acid in protozoa. Further results from this experiment showed that the major saturated fatty acids of protozoa were myristic and palmitic acids, whereas linoleic and linolenic acids represented the major unsaturated fatty acids. Furthermore, the ratio of mono-unsaturated fatty acids to their saturated analogues decreased with increasing age of the protozoa, and the amounts of dienoic and trienoic acids remained constant.

Garton and Oxford (20) raised the question of whether ruminants require essential fatty acids and, if so, were they present in bacterial lipids. They found that bacterial lipids from the rumen fluid of hay-fed sheep did not have linoleic or linolenic acid in either of the glyceride or phospholipid fractions, which constituted 48.3 and

39.2% respectively, of total lipids in these bacteria; the remainder being volatile fatty acids. These workers concluded that if the ruminant requires essential fatty acids they must come from the diet and escape hydrogenation in the rumen.

Effects of Fats and Oils on Digestibilities of Various Ration Components

Fats and oils, when added to the diet of ruminants, have been shown to affect apparent digestibilities of cellulose, protein, dry matter, organic matter and calcium. Brooks *et al.* (4) observed the effect of added fat on digestion of cellulose and protein by ovine rumen microorganisms. They found that corn oil significantly decreased cellulose digestion in an artificial rumen. When 170 mg of corn oil was added to 1 gm of dry matter containing 50% cellulose, a 94% decrease in cellulose digestibility occurred. The fat in this experiment did not emulsify and formed a layer in the artificial rumen. Results of a feeding trial (4) showed that cellulose and crude protein digestibility were reduced 52 and 17% respectively, when 32 gm of corn oil were fed daily with a basal ration consisting mainly of cottonseed hulls and some casein (9%). When the daily corn oil allowance was increased to 64 gm, the sheep scoured and the rumen contents were white and turbid. The digestion co-

efficients for cellulose and crude protein in this case were reduced by 70 and 36% respectively. Further results of this experiment showed that concentration of volatile fatty acids in the rumen fluid were also depressed when either corn oil or lard were fed. The effect on cellulose digestibility was partially overcome by addition of alfalfa ash. The authors suggested that the alfalfa ash effect may be due to its buffering capacity or its ability to assist in emulsifying the added fat, thereby preventing coating of the cellulose fibres.

Davison and Wood (13) compared a neutral fat with fatty acids upon ration digestion by lambs when fed a low ash, high corn cob ration. Both corn oil and a fatty acid mixture (myristic 2%, palmitic 26%, stearic 16%, oleic 48%, linoleic 8%) decreased digestibility of dry matter, organic matter, cellulose, ash and protein. Both the corn oil and fatty acid mixture significantly increased ether extract digestibility. Corn oil decreased nitrogen retention whereas the fatty acid mixture did not. Stearic or oleic acids alone tended to decrease protein digestibility. A difference was observed in cellulose digestion between stearic and oleic acid treatments, the latter having the most adverse effect. Depression of organic matter and cellulose digestibility by the addition of 5% corn oil was reversed by

adding calcium and phosphorus at levels similar to those supplied by alfalfa ash. Davison and Woods (13) also studied the influence of calcium carbonate and corn oil upon ration digestibility in lambs. They found that calcium carbonate largely overcame the depressing effect of corn oil on digestibility. Also, an increase in apparent digestibility of the corn oil was noted, and the addition of calcium carbonate tended to increase nitrogen retention. Thus, it appears that ruminal calcium requirements, to maintain an optimum digestibility of ration components, are increased in the presence of corn oil.

Some Aspects of Volatile Fatty Acid Metabolism

Volatile fatty acids (VFA) are synthesized by microorganisms within the rumen. These acids (of which acetic, propionic and butyric constitute over 80% of the total) play an important role in energy metabolism of the ruminant. Carrol and Hungate (6) estimated that about 70% of the energy absorbed by the ruminant was in the form of VFA's.

Various dietary factors have been shown to influence VFA production in the rumen. Chou and Walker (9) studied VFA production with respect to levels of grain and hay in the diet. They showed that sheep fed diets high in

wheat produced higher quantities of VFA's than sheep fed alfalfa hay. Production of acetic acid was lower and propionic and butyric acids higher when wheat rations were fed in comparison to alfalfa hay. Also, they observed considerably more variation in VFA production when the diet consisted of only wheat as compared to alfalfa hay. These workers pointed out that the composition of rumen liquor from a sheep is not stable but varies from day to day, and that variations between individual animals receiving the same diet may be greater than the variation resulting from different dietary regimens.

Effects of fineness of grind of alfalfa hay upon VFA production in the rumen was studied by Wright et al. (39). Hay, finely ground and then pelleted, caused a higher concentration of total VFA's in the rumen liquor of sheep (215.1) than coarsely ground hay (173.3) or long hay (164.7 mm/l). The concentration of acetic acid was lowest and propionic and butyric acids highest in sheep fed the pelleted ration. Sheep fed long hay had the highest concentration of acetic acid and the lowest concentration of propionic and butyric acids.

Various long chained fatty acids have been shown to influence VFA production in the rumen. Shaw and Ensor (33) reported that feeding 300 ml of cod liver oil to cows

resulted in a sharp decrease in the molar per cent acetic acid, an increase in propionic and valeric acids, and a decrease in butyric acid, in rumen liquor. When 300 ml of oleic acid were fed, a smaller decrease in the proportion of acetic acid and a smaller increase in propionic acid were observed and the proportion of valeric acid was decreased. Feeding 300 ml of linoleic acid resulted in a sharp reduction in ruminal concentration of acetic acid and an increase in concentration of propionic and butyric acids. Valeric acid production was slightly increased. All three oil treatments resulted in a sharp increase in total VFA concentration in the rumen liquor.

Erwin et al. (15) reported an increase in the molar per cent of propionic with a corresponding decrease in acetic acid in rumen fluid of sheep intraruminally infused with methyl myristate or safflower oil. These results corroborate Shaw's data (33) in which linoleic acid caused a reduction in the rumen concentration of acetate and an increase in propionate.

Plasma Lipids in Ruminants as Influenced by Dietary Lipids

Maynard et al. (25) studied the influence of varying degrees of unsaturation in dietary fat upon plasma lipids and milk fat in dairy cows. They noted that the

extent of change in plasma iodine values obtained from jugular blood was smaller than that of dietary food fat or milk. The exact course of change in blood lipids was not established due to the limited number of determinations but the results did indicate a relationship between the degree of unsaturation in dietary fat and blood lipids. Similar changes in the degree of unsaturation of blood lipids were observed by McCay and Maynard (26) where the feeding of cod liver oil caused a 20% rise in the iodine number of blood lipids in goats.

The experimental evidence from monogastric studies is useful in the analyses and interpretation of post ruminal fatty acid metabolism. Very little information is available, however, with respect to the effect of dietary fatty acids on rumen metabolism which in turn influences the availability of microbial and dietary components.

EXPERIMENTAL PROCEDURE

Six western range lambs, weighing approximately 36 kg each, were selected and fitted with rumen cannulae. These cannulae were made from 500 ml plastic, narrow mouth bottles. The crown of the bottle served as the inner flange and the neck of the bottle projected through the fistula and served as the cannula. An outer flange was made from the bottom of the bottle and was held in place by a hose clamp attached around the cannula, which in turn prevented the cannula from being disloged. Four lambs with the best fitting cannula were used in this study, which consisted of two separate experiments. Each experiment was conducted according to a 4 X 4 Latin square design involving 4 sheep, 4 rations and 4 periods. Each period was of 18 days duration, consisting of 10 days for adjustment (during the last two days harness and fecal collection bags were attached to the lambs) and a 7-day collection period in which total feces excreted were collected. Blood and rumen content samples were collected on the 18th day.

A semi-purified, low ether extract basal ration was used in both experiments (Table 1). This ration contained approximately 0.5% ether extract. Mixtures of various commercial sources of fatty acids were added to the

TABLE 1

BASAL RATION

INGREDIENT	PERCENT
Beet pulp	45.5
Corn starch	14.3
Molasses	9.5
Wheat straw	9.5
Solka floc	9.5
Soy protein	3.8
Dehy. alfalfa	1.0
Mineral[1] - vitamin mixture	1.9
Fatty acid mixture	5.0

[1]Tricalcium phosphate and cobalt-iodized salt (1:1 mixture).

basal ration at a 5% level, by weight, to formulate experimental rations containing increasing levels of unsaturated fatty acids. The commercial sources of fatty acids were methyl stearate, and oleic and linoleic acids. The compositions of these fatty acid sources were determined and are shown in Table 2. The analytical methods are described under "Sample Preparation and Chemical Analysis". The average fatty acid composition of the various experimental rations are shown in Table 3. These rations were mixed in a Hobart mixer every 5 days, bagged, sealed and stored at 1°C until fed. The lambs were fed 397.5 g of ration at each feeding (9 a.m. and 5 p.m.) and about 45 minutes were generally required for total consumption. Water was offered ad libitum. The lambs were kept in individual floor pens and wood shavings were used for bedding.

Sampling Procedures

Feed samples (23 gm) were taken from each 5-day ration mixture at the time of mixing and stored at 1°C until used for chemical analysis.

Fecal collections were made twice daily at a time coincident to feeding for 7 consecutive days. The feces bags were lined with plastic to aid in feces removal and to provide some measure of moisture control. Fecal weights

TABLE 2

FATTY ACID COMPOSITION OF THE VARIOUS FATTY ACID SOURCES[1] (% METHYL ESTERS)

Fatty Acid	Methyl Stearate	Oleic	Linoleic
C_{16}	58.2	4.3	4.0
C_{18}	41.8	----	1.7
$C_{18:1}$	----	78.7	25.0
$C_{18:2}$	----	7.5	65.0
Others	----	9.5	4.3

[1]The 3 fatty acid sources (technical grade) were purchased from Fisher Scientific Co. Ltd.

TABLE 3

RATION FATTY ACID COMPOSITION
(% METHYL ESTERS)

Experiment I

Fatty Acid	Rations 1	2	3	4
C_{16}	50.3	37.7	28.6	8.5
C_{18}	39.0	27.1	16.8	0.5
$C_{18:1}$	1.9	25.2	47.7	77.6
Others	8.8	10.0	6.9	13.6

Experiment II

Fatty Acid	Rations 1	2	3	4
C_{16}	51.7	45.7	37.4	23.0
C_{18}	41.0	33.4	24.4	3.5
$C_{18:1}$	2.2	9.7	19.5	45.6
$C_{18:2}$	0.9	5.9	9.3	14.6
Others	4.2	5.3	9.4	13.3

were recorded and the total feces collected were stored at -18°C in plastic bags.

Rumen samples were taken on the 18th day at 0, 3, and 6 hours after the 5 p.m. feeding. Also, blood samples were drawn from the jugular vein immediately prior to the 5 p.m. feeding. Rumen collections were made by aspirating the rumen contents through a 1/4 inch diameter glass tube which was inserted through the cannula into the central region of the ventral sac. Samples of rumen contents were immediately frozen at -18°C in petri dishes. Rumen fluid was obtained from the 3-hour samples by straining through one layer of muslin cloth and stored at -18°C. Protozoa samples were obtained by straining the 3-hour rumen contents through 2 layers of muslin cloth and centrifuging the fluid at 2300 r.p.m. for 10 minutes. The supernatant was removed and the precipitate washed twice with distilled water, frozen and lyophilized.

Sample Preparation and Chemical Analysis

Total feces collected for each treatment over each period were slightly thawed and mixed in a Hobart mixer. Approximately 200 gm of each sample were taken for dry matter analysis. Dry matter analysis of both feed and fecal samples was carried out in a convection oven at 55°C and

dried until a constant weight was obtained. Subsequent to dry matter determinations, the feed and fecal samples were put through a Wiley mill (screen size 1 mm) to enhance homogeneity. Both feed and fecal samples were analyzed for ether extract according to the A.O.A.C. (1) and crude fat by a method described by Bohman and Lesperance (5). This crude fat method involved hydrolyzing the sample with 0.5N HCl for 12 hours and then ether extracting according to A.O.A.C. (1). Samples of extracts from both methods were stored in capped vials at -18°C until they were analyzed for fatty acids. Analysis of both ration and feces for crude fibre and crude protein were conducted according to A.O.A.C. (1). Gross energy determinations were made using a Parr adiabatic oxygen bomb calorimeter.

Rumen contents and protozoa were dried in a lyophilzer for 36 hours at a pressure less than 75 microns of mercury. The samples were then extracted for 4 hours with ethyl ether, and the extracts stored at -18°C until analyzed. Serum was separated from the blood samples and 10 ml quantities were lyophilized and stored at -18°C until analyzed for fatty acids.

Sample preparation for fatty acid analysis was carried out by the method of Feldman *et al.* (18) which involved dissolving the ether extract in a few drops of

benzene and 1 ml of methylating reagent (94 ml methanol to 6 ml sulfuric acid). The resultant mixture (minus the solvent) was left overnight at 55°C. One ml of water was added to arrest further reaction, and the methyl esters were taken up in pentane. The serum samples were not ether extracted prior to methylation. However, the dried serum was directly subjected to methylation as previously described.

Methyl esters were prepared from extracts of feed and fecal samples both before and after acid hydrolysis in Experiment I. In Experiment II, however, only those samples which had been acid hydrolyzed were methylated for subsequent analysis. Methyl esters were prepared from protozoa and rumen content samples. They were not subjected to acid hydrolysis.

Fatty acid analysis of the various samples was done by gas-liquid chromatography, using a Burrel Kromotog KD gas chromatograph with a thermal conductivity detecting unit. Helium was used as the carrier gas. The column was 3 m long with a 3 mm inside diameter and was packed with 80% diataport W and 20% diethylene glycol succinate ($^{W}/w$). The fatty acid separations were done at a column temperature of 200°C and a helium flow rate of 50 ml/minute. A disc integrator was used to measure chromatogram peak areas and

some of the fatty acids were identified according to their retention time in comparison to that of known fatty acids.

Rumen fluid samples were prepared for VFA analysis according to the method of Erwin et al. (16). This involved thawing the samples and then adding 1 ml of metaphosphoric acid (25%) to 5 ml of rumen fluid. After standing at room temperature for 30 minutes, the contents were centrifuged at 3000 r.p.m. for 10 minutes, and the supernatant removed for analysis.

Volatile fatty acid determinations of the rumen fluid were carried out using the flame ionization detecting unit of the Burrel gas chromatograph. Hydrogen flow into the burner jet was set at 28 ml/minute. Nitrogen was used as the carrier gas at a flow rate of 45 ml/minute, and the air flow was 350 ml/minute. The collector plate potential was 250 V., and the column was operated at a temperature of 150°C. The column packing was neopentylglycol succinate (2% H_3PO_4) on 60-80 mesh firebrick.

Quantitative procedures involved the injection of known amounts of standard acids (acetic, propionic, butyric and valeric) and determining recorder response in relation to the quantity of acid injected. Standard fatty acid mixtures were analyzed routinely to guard against instrument variation. A rumen fluid sample size of

0.8 ml gave a satisfactory response at an attenuation of 2.

Statistical Analysis

Data from these experiments were subjected to analysis of variance (Cochran *et al.*, 11), and in some cases treatment means were compared using Duncan's multiple range test (Steele and Torrie, 35).

RESULTS

No difficulties were experienced with the sheep during Experiment I. In Experiment II, period 1, however, one sheep lost the cork stopper in the cannula, thus permitting partial loss of its rumen contents. A similar event took place during period 2, where one sheep dislodged its cannula. Collections during these periods were discontinued, and in both cases the animals had sufficient time to readjust before beginning the next period. At conclusion of Experiment II, these two lambs were fed the diets that were being fed at the time the difficulties arose and the particular period repeated.

No significant treatment differences were observed for energy, nitrogen, fibre, dry matter, ether extract, or crude fat digestibilities in the two experiments. Digestion coefficients of all items in Experiment I (Table 4) were considerably higher than for the same items in Experiment II (Table 5).

The ether extract digestibilities shown in Tables 4 and 5 are 11 - 19 digestion units higher than the corresponding crude fat digestibilities. This indicates that a portion of the fecal fat was in the form of soaps and shows that the ether extract digestibility values were

TABLE 4

APPARENT DIGESTION COEFFICIENTS OF VARIOUS RATION COMPONENTS

Experiment I

ITEM	Rations 1	2	3	4
ENERGY	78.6±0.8	77.8±1.4	77.1±1.6	74.4±1.7
NITROGEN	66.9±2.9	63.8±1.7	63.3±1.0	62.8±1.2
FIBRE	67.3±1.0	66.2±1.4	63.9±4.1	65.3±1.4
DRY MATTER	79.2±0.7	78.6±1.2	78.0±2.0	77.5±1.0
ETHER EXTRACT	86.5±1.6	86.2±1.0	86.1±0.6	84.6±0.7
CRUDE FAT	69.4±5.1	71.0±4.4	72.1±2.5	68.8±3.7

TABLE 5

APPARENT DIGESTION COEFFICIENTS OF VARIOUS RATION COMPONENTS

	Experiment II			
	Rations			
ITEM	1	2	3	4
ENERGY	72.1±2.2	72.8±2.5	71.6±1.7	71.1±1.8
NITROGEN	60.5±2.9	59.4±4.5	58.2±1.9	58.2±1.9
FIBRE	56.1±3.4	57.7±3.4	54.0±3.4	51.6±5.1
DRY MATTER	72.6±2.0	73.9±2.2	73.2±1.5	72.4±2.0
ETHER EXTRACT	82.9±1.5	83.0±1.3	83.7±1.3	83.5±0.4
CRUDE FAT	63.8±5.0	69.1±3.0	72.4±2.1	70.6±3.6

underestimating the quantity of fat that was excreted in the feces.

Changes in Fatty Acid Proportions from Ration to Rumen Contents to Feces

The relative changes in proportions of fatty acids from ration to rumen contents to feces are shown in Tables 6 and 7. In Experiment I, palmitate supplied 50.4, 37.8, 28.7 and 8.3% of total fatty acids in rations 1 to 4 respectively. The rumen contents obtained 6 hours after feeding reflected palmitate levels in rations 1-3 (50.3, 38.7 and 29.8% respectively), but in ration 4 the ruminal level was considerably greater than that of the ration (24.3 vs. 8.3%). Fecal levels of palmitate for sheep fed rations 1-3 (46.5, 32.4 and 25.0%) in general reflected dietary levels of palmitic acid. Sheep fed ration 4, however, had higher palmitate levels in the feces than that present in the diet (14.6 vs. 8.3%). In all cases, fecal palmitate levels were lower than ruminal levels.

Stearic acid supplied 39.0, 27.2, 16.9 and 0.4% of the total fatty acids in rations 1-4 of Experiment I. Unlike palmitate, however, the stearic acid levels in the 6-hour rumen contents were considerably higher than dietary levels of stearic acid (42.2, 41.3, 37.8 and 34.3% for

TABLE 6

FATTY ACID LEVELS[1] IN RATIONS, RUMEN CONTENTS (6 HOURS) AND FECES

Experiment I

	ITEM											
	Rations				Rumen Contents				Feces			
FATTY ACID	1	2	3	4	1	2	3	4	1	2	3	4
PALMITIC	50.4	37.8	28.7	8.3	50.3	38.7	29.8	24.3	46.5	32.4	25.0	14.6
STEARIC	39.0	27.2	16.9	0.4	42.2	41.3	37.8	34.3	42.7	51.7	54.7	67.0
OLEIC	1.9	25.4	47.6	77.6	3.7	15.8	26.0	44.3	5.1	8.5	13.5	13.5

[1]Levels are calculated as a percentage.

TABLE 7

FATTY ACID LEVELS[1] IN RATIONS, RUMEN CONTENTS (6 HOURS) AND FECES

Experiment II

	ITEM											
	Rations				Rumen Contents				Feces			
FATTY ACID	1	2	3	4	1	2	3	4	1	2	3	4
PALMITIC	51.7	45.7	37.4	23.0	40.2	34.3	24.7	12.6	42.9	35.6	26.7	12.0
STEARIC	41.0	33.4	24.4	3.5	49.7	40.2	32.5	17.6	46.5	53.5	55.4	59.7
OLEIC	2.2	9.7	19.5	45.6	7.9	17.3	27.0	50.9	3.5	5.5	12.6	23.0
LINOLEIC	1.0	5.9	9.3	14.6	0.7	1.9	11.4	11.8	0.4	0.3	0.5	0.4

[1]Levels are calculated as a percentage.

treatments 1-4 respectively). The fecal levels of stearic acid were considerably higher than those found in either the ration or rumen contents. This was especially noticeable in the sheep fed ration 4 where stearic acid levels in the diet, rumen contents and feces were 0.4, 34.3 and 67% respectively.

Oleic acid supplied 1.9, 25.4, 47.6 and 77.6% of the total fatty acids in the four rations of Experiment I. Although ruminal levels of oleic acid (3.7, 15.8, 26.0 and 44.3%) showed the same trend as that found in the diets (rations 1 to 4), the differences among treatments were not nearly as great. Fecal oleic acid made up a relatively small proportion of the total fecal fatty acids (5.5, 8.5, 13.5 and 13.5% for treatments 1 to 4 respectively).

Ration 1 - Experiment I was comparable in composition to ration 1 - Experiment II. Rations 2 to 4, however, contained increasing levels of linoleic acid (Table 7).

In Experiment II, palmitate supplied 51.7, 45.7, 37.4 and 23% of total fatty acids in rations 1 to 4, respectively. The rumen contents obtained 6 hours after feeding reflected palmitate levels in the rations. Also, fecal levels of palmitate for sheep fed rations 1-4, in general, reflected dietary levels of palmitic acid.

Stearic acid supplied 41.0, 33.4, 24.5 and 3.5% of the total fatty acids in rations 1-4 of Experiment II. Again, as in Experiment I, the stearic acid levels in the 6-hour rumen contents were considerably higher than dietary levels of stearic acid (49.7, 40.2, 32.5 and 17.6% for treatments 1-4 respectively). As in Experiment I, the fecal levels of stearic acid were considerably higher than those found in either the ration or rumen contents. This was especially noticeable in those sheep fed ration 4, where stearic acid levels in the diet, rumen contents and feces were 3.5, 17.6 and 59.7% respectively.

Oleic acid comprised 2.2, 9.7, 19.5 and 45.6% of the total fatty acids in the 4 rations. Ruminal levels of oleic acid (7.9, 17.3, 27.0 and 50.9%) were all higher than corresponding dietary levels, which is unlike the results for Experiment I, where rumen oleic acid levels were less than dietary levels in 3 treatments. Linoleic acid levels decreased in the rumen with respect to levels found in the diet. Negligible amounts of linoleic acid were found in the feces of sheep fed the 4 rations.

The results shown in Tables 6 and 7 are depicted graphically in Figures 1 and 2 respectively. A point of interest is the increasing proportion of stearic acid appearing in the feces when dietary levels of unsaturated

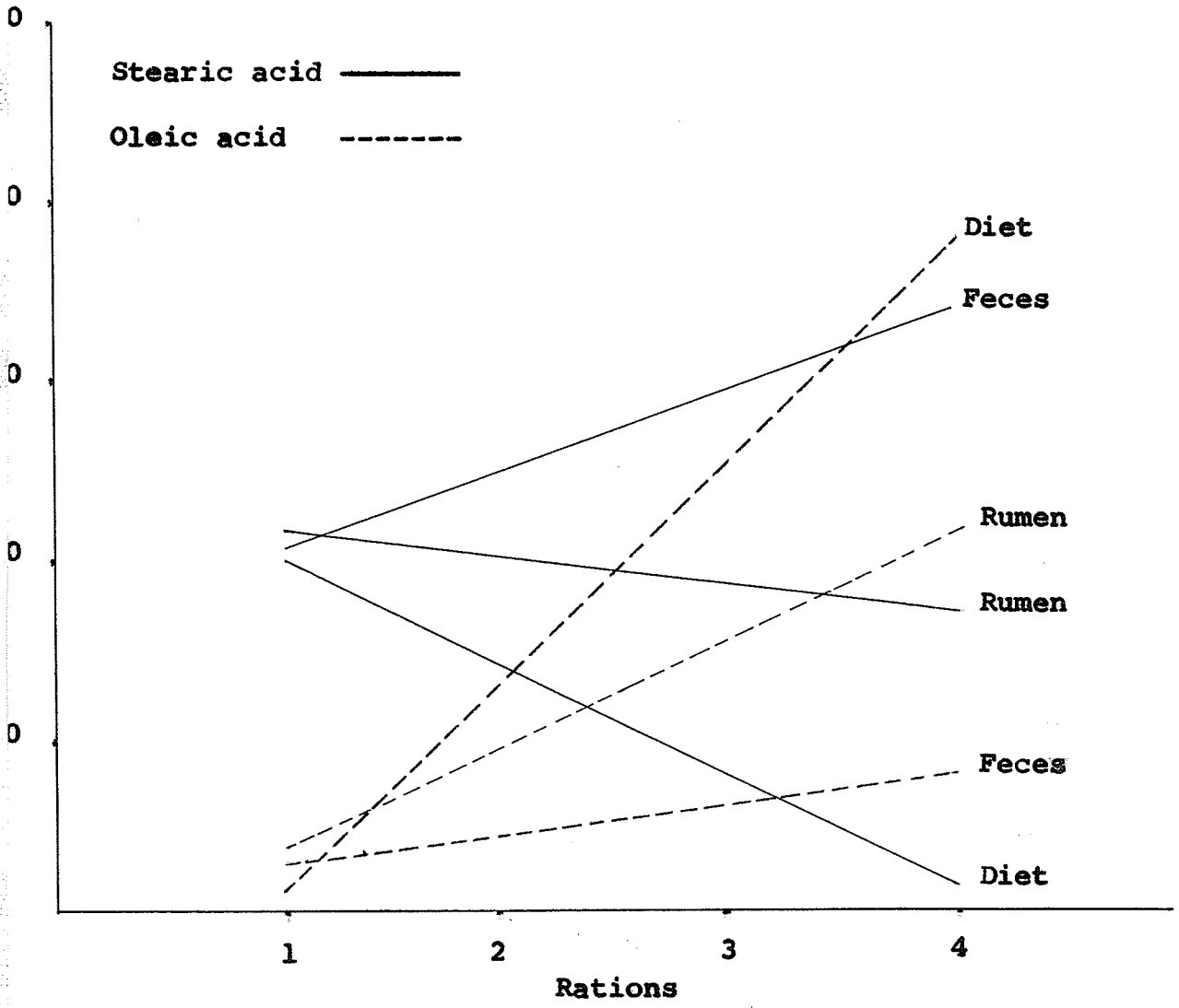

Figure 1. Experiment I. Stearic and oleic acid levels in the ration, rumen contents, and feces.

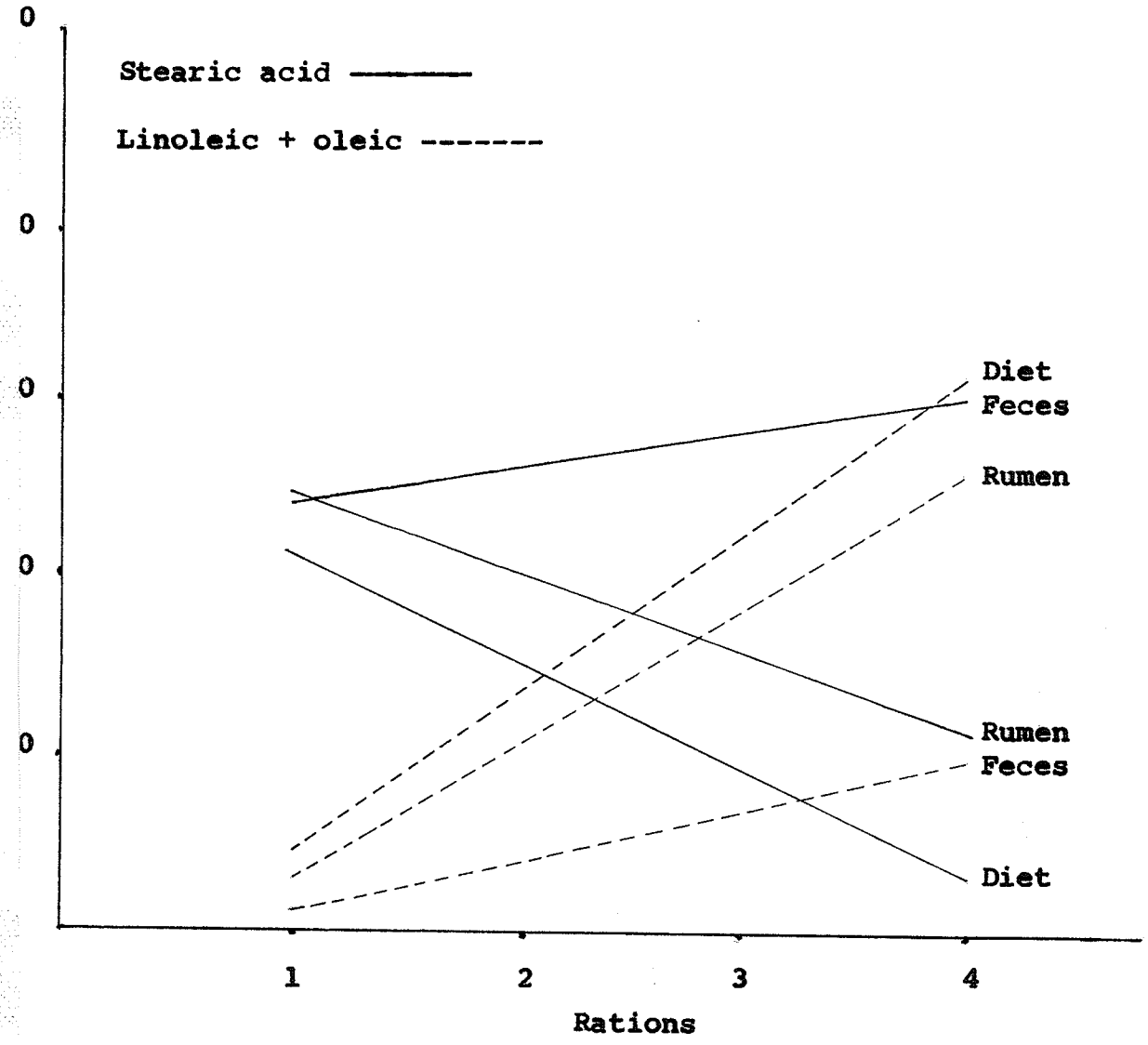

Figure 2. Experiment II. Linoleic + oleic and stearic acid levels in the ration, rumen contents, and feces.

fatty acids increased.

Changes in Ruminal Fatty Acid Levels with Time after Feeding

In Experiment I, the ruminal levels of palmitic, stearic and oleic acids in the 0, 3 and 6-hour samples were almost identical (Table 8). The 0-hour sample, which was taken immediately prior to the 5 p.m. feeding, was in fact a sample taken 9 hours after the last feeding (9 a.m.). Therefore, in order to detect a change with time in fatty acid levels within the rumen, a comparison between the 0 and 3-hour samples would be most appropriate since a greater time lapse would probably accentuate any change in fatty acid levels. Rumen palmitic and stearic acid levels of Experiment II (Table 9) were comparable to those of Experiment I. However, linoleic acid levels in Experiment II ration 4 decreased by 27% from 0 to 3 hours after feeding and a corresponding increase in oleic acid (41.5 to 49.3%) was observed. Similarly, linoleic acid decreased from 3 to 6 hours after feeding and a corresponding increase in oleic acid (49.3 to 57.9%) was observed.

Daily Consumption vs. Fecal Excretion of Fatty Acids

Data showing daily consumption and excretion of fatty acids (Tables 10 and 11) are deliniated in Figures 3

TABLE 8

FATTY ACID LEVELS[1] IN RUMEN CONTENTS
AT VARIOUS TIMES AFTER FEEDING

	Experiment I											
	ITEM[2]											
	0 Hours				3 Hours				6 Hours			
FATTY ACID	1	2	3	4	1	2	3	4	1	2	3	4
PALMITIC	46.0	34.0	23.4	15.4	46.1	36.6	28.2	12.5	50.3	38.7	29.8	14.2
STEARIC	45.1	43.6	42.0	32.5	44.4	40.4	39.3	32.1	42.2	41.3	37.8	34.3
OLEIC	4.3	16.8	22.8	49.0	4.3	16.5	27.6	49.0	3.7	15.8	26.0	44.3

[1]Levels are calculated as a percentage of total methyl esters.

[2]1 to 4 represents the experimental rations.

TABLE 9

FATTY ACID LEVELS[1] IN RUMEN CONTENTS
AT VARIOUS TIMES AFTER FEEDING

Experiment II

	ITEM[2]											
	0 Hours				3 Hours				6 Hours			
FATTY ACID	1	2	3	4	1	2	3	4	1	2	3	4
PALMITIC	45.5	37.6	26.4	15.1	46.6	39.0	27.1	14.5	40.2	34.3	24.7	12.6
STEARIC	45.9	45.5	36.2	18.7	44.6	43.2	34.8	18.6	49.7	40.2	32.5	17.6
OLEIC	3.9	10.6	22.6	41.5	3.7	11.1	27.7	49.3	7.9	17.3	27.0	57.9
LINOLEIC	0.6	2.8	11.2	22.3	0.5	30.0	8.6	16.2	0.7	1.9	11.4	11.8

[1]Levels are calculated as a percentage of total methyl esters.

[2]1 to 4 represents the experimental rations.

TABLE 10

DAILY FATTY ACID CONSUMPTION (C) AND
FECAL FATTY ACID EXCRETION (E) GM

Experiment I

FATTY ACID	Rations[1] 1		2		3		4	
	C	E	C	E	C	E	C	E
PALMITIC	21.7	6.1	16.0	4.0	12.4	3.0	3.6	2.1
STEARIC	16.8	5.6	11.5	6.4	7.3	6.6	0.2	9.4
OLEIC	0.8	0.7	10.7	1.0	20.7	1.6	33.7	1.9

[1]These values were determined using the crude fat method of analysis.

TABLE 11

DAILY FATTY ACID CONSUMPTION (C) AND
FECAL FATTY ACID EXCRETION (E) GM

Experiment II

FATTY ACID	Rations[1] 1		2		3		4	
	C	E	C	E	C	E	C	E
PALMITIC	27.4	8.2	23.9	6.0	19.2	3.8	11.5	1.8
STEARIC	21.7	8.9	17.5	9.0	12.5	7.9	1.8	8.8
OLEIC	1.2	0.7	5.1	0.9	10.2	1.8	22.9	3.4
LINOLEIC	0.5	0.1	3.1	0.01	4.8	0.01	7.3	0.1

[1]These values were determined using the crude fat method of analysis.

and 4. Palmitic acid was supplied in amounts of 21.7, 16.0, 12.4 and 3.6 gm/day in rations 1 to 4 of Experiment I and 6.1, 4.0, 3.0 and 2.1 gm were recovered in the feces (Table 10). Stearic acid increased in the feces when decreasing amounts were fed (the ration supplying 16.8, 11.5, 7.3 and 0.2 gm vs. fecal values of 5.6, 6.4, 6.6 and 9.4 gm). Excretion of oleic acid in the feces of sheep fed the 4 rations was low in comparison to the amounts fed.

Palmitate consumption and excretion in Experiment II (Table 11) followed the same general pattern as observed in Experiment I. Fecal stearic acid did not reflect dietary intake. However, it did differ from Experiment I in that the amount excreted daily did not increase as the ration levels of unsaturated fatty acids increased. A greater quantity of fecal oleate was excreted by sheep fed ration 4 - Experiment II than fed ration 4 - Experiment I (3.4 vs. 1.9 gm/day). Daily fecal excretion of linoleic acid ranged from 0.1 to 0.01 gm in the 4 sheep.

Fecal Excretion of Fatty Acids - Ether Extract Fraction vs. Crude Fat Fraction

In Experiment I, the difference between the quantity of fatty acids in the fecal ether extract fraction and fecal crude fat fraction was assumed to be due to their occurring

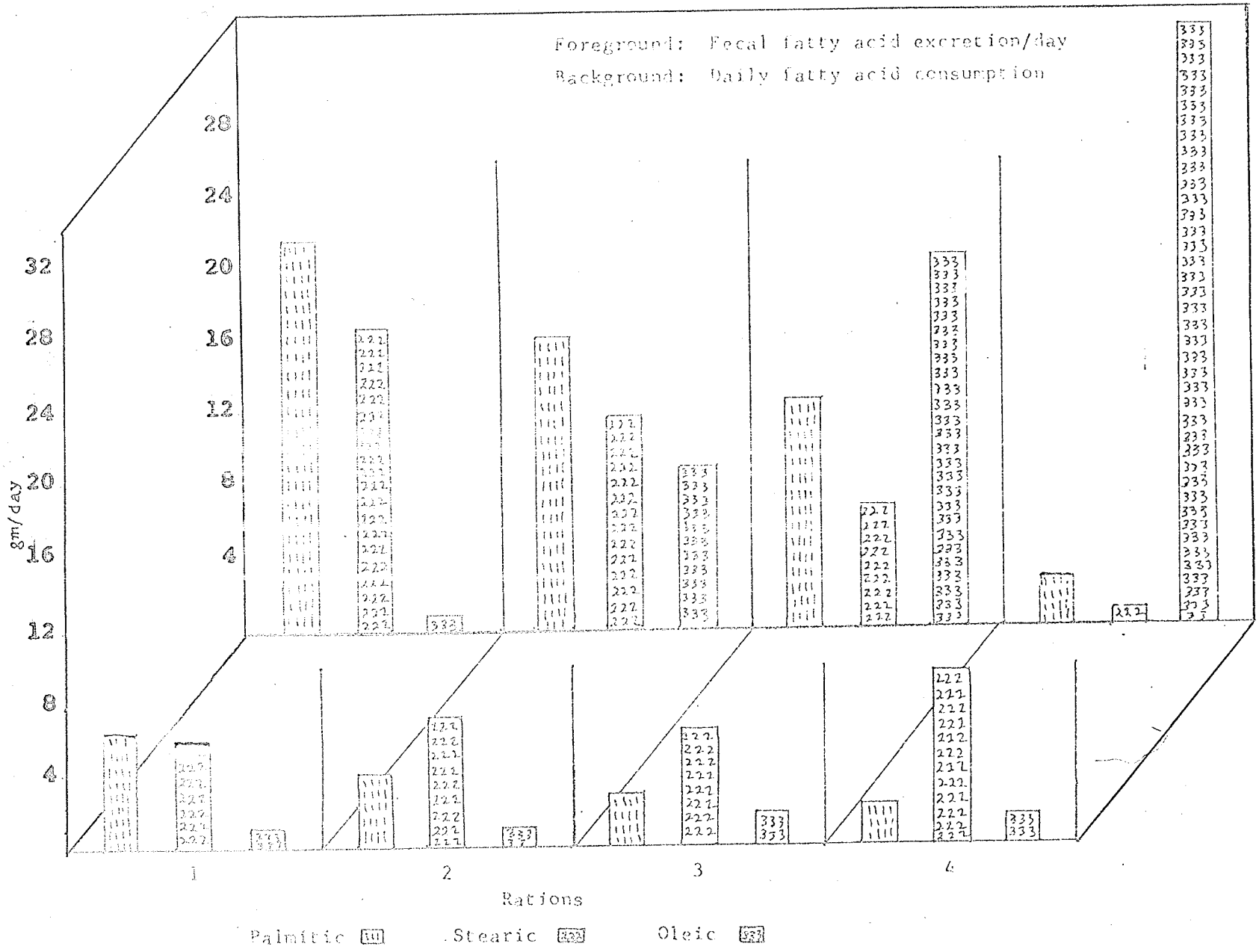

Figure 3. Experiment I. Fecal fatty acid excretion vs. fatty acid consumption/day.

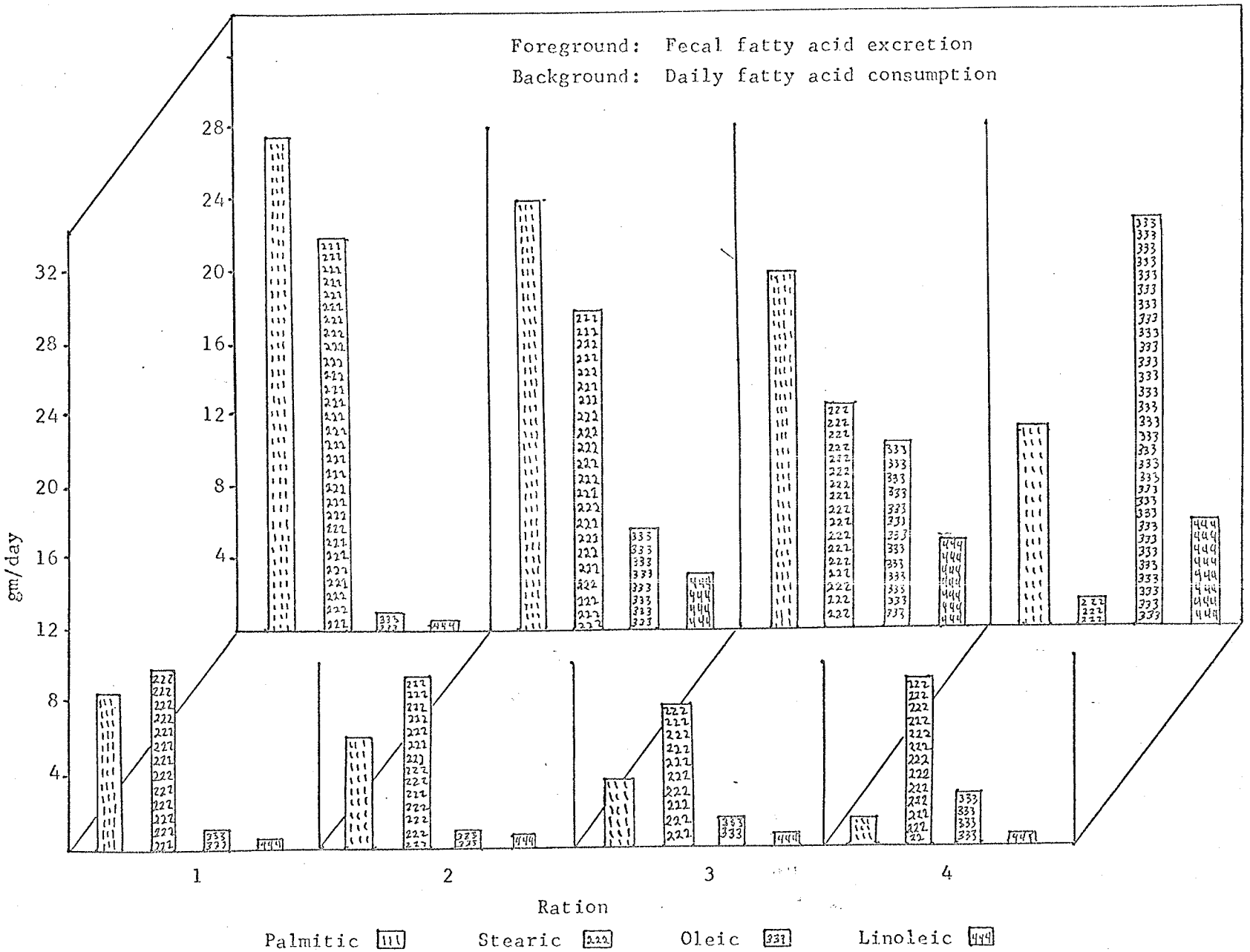

Figure 4. Experiment II. Fecal fatty acid excretion vs. fatty acid consumption/day.

partly as soaps in the fecal crude fat fraction. Data presented in Table 12 provides an estimation of fecal soap formation for each of the fatty acids. The crude fat to ether extract ratios for palmitic acid decreased slightly as the level of ration unsaturation increased (rations 1 to 4). Crude fat to ether extract ratios for stearic acid, on the other hand, showed an increase through rations 1 to 4. In fact there was a 110% increase in fecal stearic acid soaps in treatment 4 compared to treatment 1. The crude fat to ether extract ratios for oleic acid showed a tendency to decrease in rations 1 to 4. Amounts of total fecal soaps excreted were a little higher for treatments 1 and 4 than for treatments 2 and 3.

Protozoa Fatty Acid Composition

The fatty acid composition of protozoa obtained from lambs under the four dietary regimens (Tables 13 and 14) reflected to some degree the fatty acids in rumen contents. However, some differences were observed. Stearic acid levels of protozoa were generally higher than stearic acid levels of the rumen contents with the exception of treatment 1, in both experiments. Differences between the percentages of stearate in protozoa and rumen contents became greater when the level of ration unsaturated fatty

TABLE 12

FECAL FATTY ACID EXCRETION OF ETHER EXTRACT (E.E.) AND CRUDE FAT (C.F.) FRACTIONS (GM)

Experiment I

	Rations							
	1		2		3		4	
ITEM	CF	EE	CF	EE	CF	EE	CF	EE
PALMITIC	6.1	2.9	4.0	2.3	3.0	1.8	2.1	1.2
STEARIC	5.6	2.9	6.4	3.2	6.6	3.2	9.4	3.8
OLEIC	0.7	0.1	1.0	0.4	1.6	0.9	1.9	1.5
PALMITIC $^{CF}/_{EE}$	2.1		1.7		1.6		1.7	
STEARIC $^{CF}/_{EE}$	1.9		2.0		2.0		2.5	
OLEIC $^{CF}/_{EE}$	7.0[1]		2.3		1.8		1.3	
TOTAL FECAL SOAPS	6.5		5.5		5.3		6.9	

[1]Due to negligible quantities of this fatty acid in the feces, it cannot be considered a valid measurement.

TABLE 13

PROTOZOA FATTY ACID COMPOSITION[1]
(% METHYL ESTERS)

Experiment I

	Rations			
FATTY ACID	1	2	3	4
PALMITIC	51.3±2.1	42.0±0.7	34.5±2.5	18.5±7.5
STEARIC	37.2±0.7	42.1±0.4	44.7±1.1	40.7±8.8
OLEIC	5.3±1.0	12.0±2.1	17.2±2.1	35.4±5.5

[1]Via ethyl ether extraction.

TABLE 14

PROTOZOA FATTY ACID COMPOSITION[1]
(% METHYL ESTERS)

	Experiment II			
	Rations			
FATTY ACID	1	2	3	4
PALMITIC	47.2±1.8	34.9±3.6	28.5±1.7	16.0±2.2
STEARIC	36.9±3.6	43.0±3.5	42.1±4.7	33.5±8.9
OLEIC	7.9±0.8	18.0±4.0	23.7±4.7	46.2±4.6
LINOLEIC	2.5	1.2	1.2	2.4

[1]Via ethyl ether extraction.

acids increased. The amount of oleic acid in rumen contents was higher than in protozoa. This was particularly evident in Experiment I. In addition, oleic acid levels in protozoa of Experiment I showed a tendency to be lower than levels in protozoa in Experiment II.

Serum Fatty Acid Levels

No significant differences were detected in the percentages of palmitic, palmitoleic, stearic, oleic and linoleic acids in serum with respect to the presence of different dietary fatty acids (Table 15). Unsaturated fatty acids made up the largest proportion of serum fatty acids.

Rumen Volatile Fatty Acids

Ruminal levels of VFA's are presented in Tables 16 and 17. No significant differences were observed among treatments in total VFA concentrations, or was any definite trend observed indicating that degree of ration fatty acid unsaturation affected total VFA concentrations in the rumen.

The ruminal levels of acetic acid were comparable in both experiments. In both cases, a gradual increase in acetic acid levels was observed through rations 1 to 3. The trend was interrupted, however, by the low levels of ruminal acetate in sheep fed ration 4 in both experiments.

TABLE 15

SERUM FATTY ACIDS IN LAMBS RECEIVING
VARIOUS DIETARY FATTY ACIDS
(% METHYL ESTERS)

Experiment II

	Rations			
FATTY ACID	1	2	3	4
PALMITIC	21.4	25.1	22.3	18.1
PALMITOLEIC	7.2	5.4	6.8	6.5
STEARIC	16.4	16.4	19.0	17.0
OLEIC	32.4	30.7	30.5	30.8
LINOLEIC	17.9	17.0	19.1	27.5
OTHERS	4.7	5.4	2.3	0.1
TOTAL SATURATED	37.8	41.5	41.3	35.1
TOTAL UNSATURATED	57.5	53.1	56.4	64.8

TABLE 16

RUMEN FLUID VOLATILE FATTY ACIDS OF LAMBS RECEIVING VARIOUS DIETARY FATTY ACIDS

Experiment I

ITEM[1]	Rations 1	2	3	4
ACETIC	59.7±1.8	63.8±0.8	67.5±2.8	59.9±2.0
PROPIONIC	25.1±0.3	21.4±0.7	20.8±2.4	27.3±0.7
BUTYRIC	15.2±1.6	14.8±0.3	11.7±1.4	13.5±0.6
$C\text{-}2/C\text{-}3$	2.4	3.0	3.3	2.2
Total VFA's $^{um}/ml$	170.0	151.2	148.2	189.3

[1]Acetic, propionic and butyric acids are expressed as molar percent.

TABLE 17

RUMEN FLUID VOLATILE FATTY ACIDS OF LAMBS RECEIVING VARIOUS DIETARY FATTY ACIDS

Experiment II

ITEM[1]	Rations 1	2	3	4
ACETIC	60.7±2.2	60.2±1.6	61.8±1.4	58.5±2.1
PROPIONIC	24.3±1.6	25.3±2.5	28.5±1.7	30.2±3.2
BUTYRIC	15.0±2.0[a]	14.5±2.1[a]	9.7±1.3[b]	11.3±1.4[ab]
C-2/C-3	2.5	2.4	2.2	1.9
Total VFA's um/ml	110.9	126.1	142.9	121.3

[1]Acetic, propionic and butyric acids are expressed as a molar percent.

[ab]Treatment means with similar superscripts are not significantly different.

Ruminal levels of propionic acid decreased from rations 1 to 3, and increased in ration 4 (Experiment I). In Experiment II, however, the propionate level was shown to increase steadily with increasing ration unsaturation. Although statistical significance ($P > .05$) was not detected, there is some indication that propionic acid made up a greater proportion of the total VFA's with increasing levels of dietary unsaturated fatty acids.

Ruminal levels of butyric acid in general decreased in both experiments as ration levels of unsaturated fatty acids increased. In Experiment II, a significant ($P < .05$) difference among treatments was observed in butyric acid level.

Acetate to propionate ratios were calculated and although no significant differences were observed among treatments, a definite trend was shown in Experiment II, indicating that as the degree of ration unsaturation increased, the acetate to propionate ratio decreased. An almost opposite trend was observed in Experiment I.

DISCUSSION

These experiments failed to reveal any relationship between apparent digestibilities of various ration components and the level of dietary unsaturated fatty acids. These data are corroborated by the results of Roberts and McKirdy (31) in that dry matter and energy digestibilities were not significantly affected by rapeseed oil, sunflower seed oil, or animal tallow treatments. Davison and Woods (13), on the other hand, found differences in crude fibre digestibilities when rations containing stearic acid were compared to those containing oleic acid.

The higher apparent digestion coefficients obtained in Experiment I than in Experiment II are difficult to explain. This difference between experiments cannot be attributed to the addition of linoleic acid in Experiment II since ration 1 was the same in both experiments. The only apparent variables were temperature, relative humidity, and age of lambs. Experiment I was conducted in late summer and Experiment II was conducted during December and January. The room temperature and relative humidity were slightly lower and age of the lambs about 3 months older during Experiment II. Whether or not these factors were responsible for the lowered apparent digestibilities in

Experiment II is a moot point.

Changes in Fatty Acid Proportions from Ration to Rumen Contents to Feces

Palmitic acid level in rumen contents (24.3%) of sheep fed ration 4 in Experiment I was considerably higher than the dietary level (8.3%), suggesting net synthesis of palmitic acid within the rumen. In Experiment II - ration 4, however, palmitic acid in the diet (23%) was considerably higher than in rumen contents (12.6%) and also higher than the dietary level in Experiment I. These data could suggest that rumen synthesis of this acid was controlled by ruminal levels of palmitic acid. This sizeable decrease in palmitate level from ration to rumen contents might have been the result of elongation of C_{16} to C_{18} fatty acids. Erwin and Black (17) showed that protozoa are capable of elongating C_{16} to C_{18} fatty acids. In Experiment I - ration 4 the dietary palmitate level was relatively low, and in this treatment the fecal palmitate level dropped to one-half that of the ruminal level. Ration 4 of Experiment II provided fairly high levels of palmitate (23%), however, and the fecal level was the same as the rumen level (12%). It is possible that synthesized palmitic acid was present in a form that was more readily

absorbed from the lumen of the small intestine than the palmitic acid supplied in the diet.

Data suggesting that dietary C_{18} unsaturated fatty acids were being hydrogenated to stearic acid are deliniated in Figures 1 and 2. In Experiment I (Figure 1) ruminal levels of stearic acid markedly increased in relation to dietary levels through rations 1 to 4. Also, since decreasing dietary stearate levels were concomitant to increasing dietary oleic acid levels, the results suggest that hydrogenation of oleic to stearic acid was occurring within the rumen. The decrease in slope between dietary oleate and ruminal oleate (Figure 1) was almost inversely related to the change in slope between dietary stearate and ruminal stearate, which also suggests that ruminal hydrogenation to stearic acid was occurring. Comparable results were obtained in Experiment II, although not as pronounced (Figure 2). The unsaturated fatty acid fraction (oleic and linoleic acids) remained at relatively high levels in the rumen suggesting less efficient hydrogenation. In general, the results of these experiments are in agreement with those reported by other workers (15, 27, 28, 34, 38) where evidence has been obtained indicating hydrogenation of unsaturated fatty acids within the rumen.

In both experiments fecal stearate levels increased much faster than rumen stearate levels when the proportions of dietary unsaturated fatty acids were increased. This could suggest that either further hydrogenation of linoleic and/or oleic acids occurred postruminally, thereby increasing the proportion of fecal stearic acid; or selective absorption of the unsaturated fatty acids occurred in the small intestine and thus caused a relative increase in fecal stearic acid. The latter suggestion has little merit, however, since the apparent digestion coefficients for crude fat (Tables 4 and 5) remained relatively constant with increasing unsaturation of dietary fatty acids. Alternatively, there may have been delayed hydrogenation in the rumen. Tove (36) noted that natural fats (present in feed) were more efficiently hydrogenated than pure unsaturated fatty acids. He suggested there was less opportunity for hydrogenation of pure unsaturated fatty acids than of those present in natural feeds because of their faster rate of passage through the rumen.

Sheep receiving ration 1 in both experiments showed higher rumen levels of unsaturated fatty acids than for dietary levels. This would suggest that a net synthesis of these acids occurred within the rumen. Erwin

and Black (17) have shown that rumen microorganisms are capable of synthesizing unsaturated fatty acids, and protozoa were shown to desaturate stearic to oleic acid. In the present study oleic acid made up 3.7% of the fatty acids in the rumen and 5.1% in the feces of sheep fed ration 1 in the first experiment. Assuming that oleic acid is preferentially absorbed in the small intestine, compared to its saturated analogue, one would expect fecal oleic levels to be lower than rumen levels. However, it might be that the increase in fecal oleate was due to endogenous secretion of unsaturated fatty acids posterior to the area of absorption.

Changes in Ruminal Fatty Acid Levels with Time after Feeding

The fatty acid composition of rumen contents taken at 0, 3 and 6 hours after feeding failed to give a clear indication of oleic or linoleic acid hydrogenation. The relative concentrations of stearic and oleic acids changed very little with respect to the three sampling periods.

Ether Extract vs. Crude Fat on Fecal Extractions

Fecal soaps made up 40-60% of fecal fat from all four treatments in Experiment I (Table 12). The decreasing trend in crude fat to ether extract ratios for fecal palmit-

ate corresponded to increasing unsaturation in the diet. This suggests that less palmitic acid was complexed as soaps in sheep fed rations containing decreasing palmitic acid levels. When small amounts of palmitic acid were in the diet (ration 4), there was an increase in the relative proportions of palmitic acid entering the duodenum and a substantial decrease in fecal palmitate levels (Table 6). In Experiment II, the proportions of palmitate in rumen contents and feces of sheep fed ration 4 were identical, and, as previously mentioned, it was suggested that rumen palmitic acid synthesis was occurring. This indicates greater utilization of palmitate mediated through some effect of the microorganisms.

The crude fat to ether extract ratios for fecal stearate increased with increasing levels of unsaturated fatty acids in the diet. In this case, one might postulate that hydrogenation of unsaturated fatty acids by rumen microorganisms may enhance salt formation since the majority of fecal stearate in the highly unsaturated treatments must have arisen from hydrogenation of oleic acid. Fecal soap excretion of stearate was 10% higher in the unsaturated than in the saturated fatty acid diets. Roberts and McKirdy (31) fed animal tallow (47.8% oleic plus linoleic acids), rapeseed oil (58.8% oleic plus linoleic

acids), and sunflower seed oil (85.7% oleic plus linoleic acids) to steers. The calculated crude fat to ether extract ratios in the feces were 2.0, 3.0 and 3.5 respectively, which suggests higher fecal soap excretions with increasing dietary levels of unsaturated fatty acids.

A decreasing trend in crude fat to ether extract ratios for fecal oleate suggests that smaller proportions of fecal oleate were in the form of soaps with increasing quantities of dietary oleic acid. The fecal oleate values from which these rations were calculated were quite low and showed considerable variation, thus their importance may be limited.

Protozoa Fatty Acid Composition

The difference between ruminal and protozoa stearic acid levels became greater with increasing dietary unsaturated fatty acids. This suggests that protozoa were hydrogenating unsaturated fatty acids. Guttierrez *et al.* (19) showed that protozoa were able to hydrogenate oleic to stearic acid during incorporation into cellular components. The possibility also exists that protozoa were selecting stearic acid specifically from the ruminal media. In Experiment II, the difference between stearate levels of protozoa and rumen contents, with increasing levels of

dietary unsaturated fatty acids, was more pronounced. Possibly linoleic acid is more efficiently hydrogenated by protozoa. The linoleic acid levels in protozoa from sheep fed ration 1 in both experiments were higher than values obtained for rumen contents. The possibility exists that protozoa can synthesize linoleic acid and that this difference appeared because of low levels of linoleic acid entering the rumen from the diet.

Serum Fatty Acid Levels

There were no significant differences among treatments in serum fatty acid levels. This can be explained by the fact that unsaturated fatty acids were being hydrogenated within the rumen and the fatty acid mixture entering the duodenum was of relatively constant composition, irrespective of dietary fatty acids. Any differences in serum fatty acid composition reflecting the treatments would probably be modified by liver metabolism and mixing with systemic blood before arrival at the jugular vein where sampling occurred. Thus, one would expect little treatment effect upon the relative proportions of serum fatty acids. Another interesting point was the relatively high proportion of unsaturated fatty acids in the serum, in comparison with what one would expect to be available

for absorption. This could be explained by the fact that unsaturated fatty acids of endogenous origin were entering the circulatory system. Serum data in the present studies are not necessarily in agreement with data reported by Maynard et al. (25) and McCay and Maynard (26). These workers observed an increase in the iodine values in blood plasma lipids of cows and goats, respectively, where high levels of unsaturated fatty acids were fed. Behrman (2) noted a significant difference in blood plasma fatty acids of sheep when unsaturated fatty acids were infused post-ruminally and compared to feeding the same fatty acids. By infusing fatty acids post-ruminally, monogastric conditions were simulated, and one would expect serum fatty acids to reflect, to a certain degree, dietary fatty acids.

Rumen Volatile Fatty Acids

Rumen fluid levels of propionic acid (Experiment II) increased, although not significantly, with increasing levels of dietary unsaturated fatty acids. Shaw and Ensor (33) reported an increase in rumen fluid propionic acid when cod liver oil (high in linoleic acid) was fed to dairy cows, which in general is in agreement with the propionic acid data of Experiment II. This increase in ruminal propionate with increasing levels of ration unsaturated fatty

acids was not observed in Experiment I. The lack of dietary linoleic acid in Experiment I may have been the reason for the lack of increase in ruminal propionate. Shaw and Ensor (33) reported that linoleic acid was more efficient than oleic in this regard.

CONCLUSIONS

Experimental results provided a basis for the following conclusions:

1. No significant treatment effects on energy, nitrogen, fibre, dry matter, ether extract, or crude fat digestibilities were detected.
2. Rumen saturated fatty acid levels in comparison to dietary levels suggest that hydrogenation of C_{18} polyethnoid fatty acids occurs within the rumen.
3. Relative proportions of rumen fatty acids do not appear to change with time after feeding (0, 3 and 6 hours).
4. Palmitic acid synthesis occurs within the rumen of sheep fed diets low in this acid. The results suggest that synthesis may depend upon ruminal levels of this acid.
5. Oleic acid synthesis occurs in treatments low in this acid. Oleic acid synthesis may be dependent upon ruminal levels of this acid as influenced by dietary levels.
6. Fecal levels of stearic acid for sheep fed saturated fatty acid diets are quite similar to those fed unsaturated fatty acid diets.

7. Fat digestibility decreases from 11-19 digestion units when the excretion of fecal soaps are taken into account.

8. When dietary unsaturated fatty acids are relatively high, the fecal stearate soap excretion is increased by 110% compared to highly saturated dietary fatty acids.

9. The relative proportions of serum fatty acids show no response to various dietary fatty acids.

10. No significant differences are observed among treatments in total VFA concentrations, or is any definite trend deliniated indicating that degree of ration fatty acid unsaturation affects total VFA concentration in the rumen.

11. There is some indication that propionic acid makes up a greater proportion of the total VFA's when levels of dietary unsaturated fatty acids are increased.

12. Ruminal levels of butyric acid, in general, decrease as ration levels of unsaturated fatty acids increase.

13. Although no significant differences were observed in acetate to propionate ratios among treatments, a definite trend was observed in Experiment II indicating that as the degree of ration unsaturation increases, the acetate to propionate ratio decreases.

BIBLIOGRAPHY

1. Association of Official Agricultural Chemists. 1960. Official Methods of Analysis. 9th ed. Washington, D.C.

2. Behrman, H. R. 1965. The effect of feeding and duodenal infusion of oleic and linoleic acids on lipid digestion in sheep. Masters Thesis, University of Manitoba.

3. Boyd, O. F., C. L. Crum and J. F. Lyman. 1932. The absorption of calcium soaps and the relationship of dietary fat to calcium utilization in the white rat. J. Biol. Chem. 95:29-36.

4. Brooks, C. C., G. B. Garner, C. W. Gehrke, M. E. Muhrer and W. H. Pfander. 1954. The effect of added fat on the digestion of cellulose and protein by ovine rumen microorganisms. J. Animal Sci. 13:758-764.

5. Bohman, V. R. and A. L. Lesperance. 1962. The effect of dietary fat on digestion and blood composition of cattle. Proc. West. Sec. Am. Soc. Animal Production, 13:lx-l.

6. Carrol, E. J. and R. E. Hungate. 1955. The magnitude of the microbial fermentation in the bovine rumen. Appl. Microbiol. 2:205-214.

7. Carrol, K. K. 1958. Digestibilities of individual fatty acids in the rat. J. Nutrition, 64:399-410.

8. Cheng, L. S., M. G. Morehouse and H. J. Duel Jr. 1949. The effect of the level of dietary calcium and magnesium on the digestibility of fatty acids, simple triglycerides, and some natural and hydrogenated fats. J. Nutrition, 37:237-250.

9. Chow, K. C. and D. M. Walker. 1964. The effect on the rumen composition of feeding sheep diets supplying different starches. J. Agric. Sci. 62:7-13.

10. Clark, B. and G. Hubscher. 1961. Biosynthesis of glycerides in subcellular fractions of intestinal mucosa. Biochem. Biophys. Acta. 46:479-494.

11. Cochran, W. G., K. M. Autrey and C. Y. Cannon. 1941. A double changeover design for dairy cattle feeding experiments. J. Dairy Sci. 24:941-947.

12. Crockett, M. E. and H. J. Duel Jr. 1947. A comparison of the coefficients of digestibility and rates of absorption of several natural and artificial fats as influenced by melting point. J. Nutrition, 33:187-193.

13. Davison, K. L. and W. Woods. 1960. Influence of fatty acids upon digestibility of ration components by lambs and upon cellulose digestion in vitro. J. Animal Sci. 19:54-59.

14. Duel Jr., H. J., R. M. Johnson, C. E. Calbert, J. Gardner and B. Thomas. 1949. Studies on the comparative nutritional value of fats. J. Nutrition, 38:369-379.

15. Erwin, E. S., W. Sterner and G. J. Marco. 1963. Effect of type of oil and site of administration on the fate of fatty acids in sheep. J. Am. Oil Chem. Soc. 40:344-347.

16. Erwin, E. W., G. J. Marco and E. M. Emery. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768-1770.

17. Erwin, J. and K. Black. 1963. Lipid metabolism of of ciliated protozoa. J. Biol. Chem. 238:1618-1624.

18. Feldman, G. L., H. T. Jonsson, T. W. Culp and R. H. Gowan. 1962. Fatty acid composition of embryonic fat organ lipids. Poultry Sci. 41:1851-1855.

19. Gutierrez, J., P. P. Williams, R. E. Davis and E. J. Warwick. 1962. Lipid metabolism of rumen ciliates and bacteria. Appl. Microbiol. 10:548-551.

20. Garton, G. A. and A. E. Oxford. 1955. The nature of bacterial lipids in the rumen of hay fed sheep. J. Sci. and Agric. 6:142-148.

21. Hofman, A. F. and B. Borgstrom. 1963. Hydrolysis of long chained monoglycerides in micellar solutions by pancreatic lipase. Biochem. Biophys. Acta. 70:317.

22. Johnston, J. M. and B. Borgstrom. 1963. Unpublished results - cited in Advances in Lipid Research 1963. Edited by R. Paoletti and D. Kitchensky.

23. Langworth, C. F. and A. D. Holmes. 1915. Digestibility of some animal fats. U. S. Dept. of Agric. Bull. 310:1-22.

24. Mattson, F. H. 1959. The absorbability of stearic acid when fed as a simple or mixed triglyceride. J. Nutrition, 69:338-342.

25. Maynard, L. A., C. M. McCay and L. L. Madsen. 1936. The influence of one food fat of varying degrees of unsaturation upon blood lipid and milk fat. J. Dairy Sci. 19:49.

26. McCay, C. M. and L. A. Maynard. 1935. The effect of ingested cod liver oil, shark liver oil, and salmon oil upon the composition of the blood and milk of lactating cows. J. Biol. Chem. 109:29.

27. Reiser, R. 1951. Hydrogenation of polyunsaturated fatty acids by the ruminant. Fed. Proc. 10:236.

28. Reiser, R. and H. G. R. Reddy. 1956. The hydrogenation of dietary unsaturated fatty acids by the ruminant. J. Am. Oil Chem. Soc. 33:155-156.

29. Renner, R. and F. W. Hill. 1961. Factors affecting the absorbability of saturated fatty acids in the chick. J. Nutrition, 74:254-258.

30. Renner, R. and F. W. Hill. 1961. Utilization of fatty acids by the chicken. J. Nutrition, 74:259-264.

31. Roberts, W. K. and J. A. McKirdy. 1964. Weight gains, carcass fat characteristics and ration digestibility in steers as affected by dietary rapeseed oil, sunflower seed oil and animal tallow. J. Animal Sci. 23:682-687.

32. Senior, J. R. and K. J. Isselbacher. 1962. Direct esterification of monoglycerides with palmityl coenzyme A by intestinal epithelial subcellular fractions. J. Biol. Chem. 237:1454-1460.

33. Shaw, J. C. and W. C. Ensor. 1959. Effect of feeding cod liver oil and unsaturated fatty acids on rumen VFA and milk fat content. J. Dairy Sci. 42:1238-1240.

34. Shorland, F. B., R. O. Weenink, A. T. Johns and I. R. C. McDonald. 1957. The effect of sheep rumen contents on unsaturated fatty acids. Biochem. J. 67:328.

35. Steele, R. D. G. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw Hill, Toronto.

36. Tove, S. B. 1960. Milk lipids symposium. J. Dairy Sci. 9:1354-1360.

37. Williams, P. P., J. Gutierrez and R. E. Davis. 1963. Lipid metabolism of rumen ciliates and bacteria. Appl. Microbiol. 11:260-264.

38. Wood, R. D., M. C. Bell, R. B. Grainger and R. A. Teekell. 1963. Metabolism of labelled linoleic 1-C^{14} acid in the sheep rumen. J. Nutrition, 79:62.

39. Wright, P. L., A. L. Pope and P. H. Phillips. 1963. Effect of physical form of ration upon digestion and volatile fatty acid production *in vivo* and *in vitro*. J. Animal Sci. 22:586-591.

40. Young, R. J. and R. L. Garrett. 1963. Effect of oleic and linoleic acids on the absorption of saturated fatty acids in the chick. J. Nutrition, 81:321-329.