Effect of Type of Oil and Site of Administration on the Fate of Fatty Acids in Sheep

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Abstract

Safflower oil(S) and methyl myristate(M) were administered in the rumen(R) and abomasum (A) of sheep for 28 days. The digestibility of nitrogen and fiber were not affected by treatment. A larger proportion of fatty acids were digested with SA than with SR while the reverse was found with MR and MA. These differences were reflected in the fecal fatty acid composition. Regardless of treatment, the free fatty acid constituted approximately 75% of the fecal fat. MR and to a lesser extent SR increased the molar % of rumen propionate with a corresponding decrease in acetate. Fatty acid composition of rumen bacteria reflected the composition of the administrated oils. SR resulted in increased stearic and decreased linoleic acids in liver lipids while the adipose tissue remained constant. SA increased linoleic in both tissues. Markedly larger increases in myristic acid were found in adipose and liver lipids with MR compared to MA. Large increases in myristic acid in tissues were associated with decreased linoleic acid.

Introduction

A LARGE PROPORTION of the fatty acids in pasture grass, the natural feed of ruminant animals, consists of linoleic and linolenic acids (1). Yet, the depot fat of ruminant animals contains only 1–3% of diene and triene fatty acids (1). The adipose tissue of other herbivera such as the horse and rabbit contains up to 35% of these unsaturated fatty acids (1). This species difference was partially explained by Reiser (2), who showed that rumen microflora can hydrogenate unsaturated fatty acids.

Until fats became a market surplus and the price was reduced sufficiently to be considered a dietary energy source, relatively little attention was given to fatty acid utilization by ruminant animals. Since 1950 fats from various animal and plant sources have been and are currently being added to commercial ruminant diets. Yet little is known about the utilization of long chain fatty acids and their effect on the utilization of other dietary nutrients.

This study was designed to determine the role of the rumen and different fatty acids on feed digestion and fatty acid deposition in tissues of sheep.

Procedure

Each of 12 wether lambs of approximately 70 lb were fed 800 g daily of the pelleted ration shown in Table I for 28 days. Prior to the start of the trial, 6 lambs were fitted with rumen cannulas and the remainder were fitted with cannulas in the abomasum. During the experimental period both methyl myristate and safflower oil (20 g 3 times daily) were each injected into the abomasum and rumen. Initially and at the end of the study liver, and adipose tissue from the loin region, were biopsied and the tissues analyzed for fatty acids by the procedure described by Marco et al (3).

On the 14th day of study, rumen fluid was withdrawn 6 hr after the daily meal and analyzed for volatile fatty acids by the gas chromatographic procedure described by Erwin et al. (4). In addition, rumen bacteria from sheep in the various treatments were isolated by differential centrifugation. All fluid was centrifuged at 270 g for 15 min. The supernatant was again centrifuged at 17,300 g for 15 min; the supernatant was discarded and the bacteria were resuspended with 0.9% NaCl. This procedure was repeated 3 times. The isolated rumen bacteria were saponified under nitrogen at 50C for 20 hr in 25% NaOH. The free fatty acids (FFA) were converted to methyl esters and analyzed by gas chromatography (3).

During the final 8 days of the study, feces were collected daily, frozen, and an aliquot was analyzed for nitrogen, dry matter, and fiber by conventional AOAC procedures to determine digestion coefficients. Five g of feces, dried in a vacuum oven at 60C, were acidified and extracted with ether (liquid-liquid) in a special glass apparatus. The ether extract was evaporated to dryness by nitrogen and dissolved in 5 ml of pentane. The long chain FFA were analyzed by hydrogen flame gas chromatography using the column mixture proposed by Metcalfe (5). The remainder of the fecal ether extract was evaporated to dryness under nitrogen, saponified in 25% NaOH for 20 hr at 50C, and extracted with pentane. The pentane extract was dried with anhydrous Na_2SO_4 and evaporated with nitrogen. The FA were dissolved in 5 ml pentane and analyzed by gas chromatography with the procedure above. A sample of the feed was saponified, extracted with pentane, and analyzed by the same procedure as the feces. The treatment effects on the digestibility of FA were calculated.

TABLE I

Composition of Basal Ration [a]

Ingredient	%
Chopped alfalfa hay	70
Beet pulp	20
Molasses	10

[a] Fed as ½ in. pellet.

Results and Discussion

Regardless of route of administration, neither safflower oil nor methyl myristate altered the digestibility of nitrogen or fiber in the sheep (Table II). The differences between dry matter digestibility due

TABLE II

Site of Infusion and Type of Fat on Digestibility Coefficient

	% Free acids in fecal fat	Digestibility coefficients			
		Crude fiber	Nitrogen	Dry matter	Fatty acids
Control					90.3
Safflower Oil:					
Rumen Infused	72.8	45.2	62.1	60.3	81.1
Abomasal infused	78.5	42.1	62.5	63.5	96.2
Methyl myristate:					
Rumen infused	73.6	43.5	63.8	63.8	94.0
Abomasal infused	78.1	43.8	61.3	58.8	68.9

TABLE III

Effect of Treatment on the Proportions of Fecal Fatty Acids

	No. C in chain: No. unsaturated bonds											
	12:0	12:1 [a]	14:0	14:1 [a]	16:0	16:1	16:2 [a]	16:3 [a]	18.0	18:1	18:2	18:3
	Weight %											
Diet [b]	.....	.....	8.2	.....	23.1	.....	.....	.....	.....	27.7	26.6	14.4
Control	3.7	.....	25.4	.....	24.1	6.6	6.2	6.6	14.1	7.3	.....	.....
Safflower oil:												
Rumen	1.4	.....	2.1	2.7	15.7	3.0	2.5	1.9	47.1	21.9	0.5	0.5
Abomasum	0.9	.....	3.4	1.5	17.5	1.7	0.2	0.3	14.3	51.0	0.1	1.2
Methyl myristate												
Rumen	1.6	0.2	47.3	5.0	19.8	2.5	2.9	2.6	8.2	3.9	0.7	0.3
Abomasum	2.5	.....	87.4	.....	7.9	.....	0.1	0.1	1.4	0.4	0.1	.....

[a] Estimated by retention time.
[b] 0.281% total fatty acids.

to the type of oil and route of administration shown in Table II could be accounted for by the differences in FA digestion. When safflower oil was infused into the abomasum rather than the rumen, the digestibility of FA was increased from 81% to 96%, while with methyl myristate the reverse was found (94% by rumen, 69% by abomasal administration). The composition of fecal FA obtained by saponification, methylation, and gas chromatographic separation reflects the FA digestibility differences (Table III). The quantity of fecal myristate was greater when sheep were treated with methyl myristate in the abomasum compared to the rumen (47% vs. 87%). Possibly, the esterase activity in the intestine alone is not sufficient and the added lipase activity of the rumen microflora is necessary for maximum absorption of myristic acid via rumen wall. Fecal stearate was markedly higher when sheep were treated with safflower oil in rumen compared to abomasum (14% vs. 47%) (Table III). The lower digestibility of FA from safflower oil (75% linoleic– 1% stearic) when administered in the rumen compared to abomasum, could be explained by the increased fecal stearic acid content. The intestinal absorption of stearic acid in monogastric animals is low compared to the unsaturated FA (6). Reiser (2) demonstrated *in vitro* that rumen microflora can hydrogenate unsaturated FA. Probably, the rumen microflora hydrogenated some of the unsaturated acids contained in safflower oil to stearic, thus rendering a greater proportion of the FA undigestible in the gut. Unfortunately, no data are available concerning the intestinal absorption of different FA by ruminant animals. The percent of FFA in fecal fat from the sheep in this study was approximately 75% regardless of the oil or site of administration (Table II). These FFA may occur as salts in the feces. For the most part, fat digestibility data in ruminant animals has been based on ether extraction of non-acidified feces. Consequently, the values may be misleading because they may not reflect true fecal fat.

Tables IV and V show the effect of type of oil and site of administration on the FA composition of lipids in liver and adipose tissue of sheep. Recently, Ogilvie and MyClymont (7) reported that the FA composition of adipose tissue from one sheep, infused with linseed oil into the duodenum, contained abnormally large proportions of linolenic acid. Similarly, when safflower oil was infused into the abomasum, the proportions of linoleic acid increased 2-fold in the liver, and to a lesser extent in adipose tissue. While the abomasal administration of methyl myristate increased the proportions of myristic acid in liver lipids 4-fold, the myristate proportion of adipose tissue only increased 25%. Like monogastric animals (3) the FA composition of liver and adipose tissue of the sheep reflected the FA administered via the abomasum.

When the same amount of safflower oil was administered into the rumen, stearic and oleic acid proportions of liver lipids increased markedly while linoleic acid decreased 50%. In the same sheep the composition of adipose tissue remained rather constant with only a slight increase in linoleic acid (4.3%–5.2%). These data certainly support the observations of Reiser (2) that the rumen microflora hydrogenate unsaturated FA. The marked elevation in liver stearate would suggest that stearic acid (probably originating from hydrogenation of the unsaturated acids in safflower oil by rumen microflora) was readily absorbed by the sheep. Relative to the other FA, apparently, saturated acids decreased the proportion

TABLE IV

Type and Site of Infusion on the Fatty Acid Composition of Liver in Sheep

	Chain length: Saturation of liver fatty acids									
	14:0	14:1	16:0	16:1	16:2	18:0	18:1	18:2	18:3	20:4
	Weight %									
Safflower oil:										
Rumen infused										
Initial	0.8	1.4	17.4	2.2	1.3	23.5	21.9	11.9	1.4	14.0
SE			± 0.1	± 0.9		± 6.2	± 3.2	± 0.9		± 7.9
Final	1.0	0.9	15.2	4.2	2.2	34.7	29.2	6.0	1.1	5.4
SE			± 1.0	± 0.1		± 0.1	± 0.8	± 0.6		± 1.8
Abomasal infused										
Initial	0.9	1.2	15.2	3.6	2.4	27.0	22.7	11.2	1.0	10.4
SE			± 2.2	± 0.1		± 0.1	± 0.1	± 0.1		± 1.7
Final	1.0	1.4	17.5	3.0	1.3	21.1	23.0	23.3	1.2	8.4
SE			± 2.8	± 0.1		± 3.9	± 3.4	± 0.8		± 2.5
Methyl myristate:										
Rumen infused										
Initial	1.0	1.7	14.8	3.4	2.6	29.6	23.9	11.4	1.9	13.0
SE	± 0.3		± 0.6			± 0.6	± 1.3	± 1.6		± 0.1
Final	8.6	1.3	22.4	3.6	1.7	24.9	22.1	5.47	1.4	8.3
SE	± 2.0		± 2.3			± 0.8	± 1.4	± 1.5		± 1.2
Abomasal infused										
Initial	0.9	3.0	17.8	3.3	4.2	25.3	24.2	12.3	1.2	8.1
SE	± 0.1		± 2.3			± 2.3	± 1.6	± 0.2		± 3.1
Final	3.2	3.0	16.9	4.7	1.2	17.6	31.5	13.4	1.3	6.6
SE	± 0.9		± 1.8			± 3.5	± 5.5	±1.0		± 1.8

TABLE V

Type of Fat and Site of Infusion on the Fatty Acid Composition of Adipose Tissue in Sheep

	Chain length: Saturation of adipose fatty acids								
	14:0	14:1	16:0	16:1	16:2	18:0	18:1	18:2	18:3
	Weight %								
Safflower oil:									
Rumen infused									
Initial	5.1	0.9	19.8	4.0	2.7	16.6	44.7	4.3	1.5
SE			± 0.5			± 1.6	± 0.2	± 0.1	
Final	5.0	1.6	20.0	4.6	1.8	16.7	43.4	5.2	1.4
SE			± 0.3			± 5.0	± 3.2	± 0.4	
Abomasal infused									
Initial	4.2	1.2	20.6	3.6	2.3	19.6	41.7	5.4	0.9
SE			± 1.6			± 1.4	± 1.1	± 1.4	
Final	3.3	1.7	18.2	3.4	1.9	23.4	37.7	9.2	0.9
SE			± 0.9			± 1.2	± 5.4	± 2.6	
Methyl myristate:									
Rumen infused									
Initial	4.2	1.4	20.8	3.4	2.2	19.7	41.2	5.8	1.3
SE	± 0.1		± 0.2			± 3.9	± 3.8	± 0.1	
Final	15.5	2.5	20.6	4.4	1.9	21.5	29.9	2.98	0.6
SE	± 2.5		± 0.5			± 2.6	± 4.0	± 0.5	
Abomasal infused									
Initial	4.2	0.8	17.8	2.8	2.5	18.7	45.5	4.6	1.7
SE	± 0.5					± 1.6	± 1.0	± 0.4	
Final	4.9	1.6	18.7	3.5	4.2	23.3	38.3	4.6	0.9
SE	± 0.3		± 1.1			± 1.7	± 0.9	± 0.5	

of linoleic acid in liver of sheep. While marked changes occurred in the proportions of liver fatty acids, the composition of adipose tissue remained surprisingly constant.

The proportions of myristic acid increased 8-fold in the liver and 4-fold in adipose tissue in sheep administered methyl myristate in the rumen. A decrease in proportion of unsaturated acids in both liver and adipose tissue was similar to the previous reported effect of safflower oil. Tables IV and V show that the deposition of myristate in tissues was greater when methyl myristate was administered in the rumen rather than the abomasum. This may be explained by the higher absorption and/or digestibility of methyl myristate when injected into the rumen compared to the abomasum (Table II). Measurable radioactivity was found in milk of lactating goats 4 hr following oral administration of labelled myristic acid (8). These results together with the sheep data would suggest that a portion of myristic acid was absorbed via rumen wall. Unfortunately, no data are available concerning the absorption sites of various long chain FA in the ruminant animal.

In the ruminant animal the principal source of energy originates from the short chain acid produced from fermentation by rumen microflora. The amount and proportions of these acids have been related to the efficiency of energy utilization. Armstrong et al. (9) showed that the heat increment of acetate was markedly reduced in the presence of propionate in the sheep. Further, increased growth and feed utilization has been related to higher propionate production in the rumen of steers (10). Both methyl myristate and to a lesser extent safflower oil increased the molar percent of propionic acid with a corresponding decrease in acetate in the rumen fluid of the sheep in this study (Table VI). These data would suggest that the oils may increase the caloric efficiency of the other ingredients in the ration, besides their addition of calories per se. This alteration in these fermentation end products may be the result of a change in rumen microflora population and/or a change in bacterial lipids. Marco et al. (3) showed that FA composition of tissues and mitochondria reflect the FA composition of the diet. Like mitochondria, the composition of bacterial lipid reflected the FA composition of the ration. In sheep treated with methyl myristate the proportion of myristate of the total bacterial lipid as linoleic acid increased from 12% to 16% in sheep treated with safflower oil (75% linoleic acid).

Essential FA deficiency syndromes have not been observed in animals with functional rumens. The large proportion of linoleic acid in the rumen bacteria (Table VII) from the control sheep fed a diet that contained only 0.2% TFA suggests that rumen bacteria may synthesize linoleic acid.

If the rumen microflora possess the capacity to hydrogenate unsaturated FA (as indicated by the results of this study when the diet contained approximately 7% of these FA), it is difficult to account for the presence of linoleic acid in the body when many diets such as pasture grasses contain as little as 0.2% TFA. Possibly, it is the protozoa rather than the bacteria that hydrogenate the unsaturated fats. Protozoa and bacteria may compete for hydrogenation and deposition of unsaturated FA. Without a competition phenomenon it would be difficult to explain why the composition of depot fat in ruminant animals remain rather constant even when diets are fed that vary widely in quantity of unsaturated FA (Table VII).

Stearic acid in monogastric animals is poorly absorbed (6). If dietary unsaturated FA become hydrogenated in the rumen, the caloric value would be reduced if we assume that ruminants are similar to monogastric animals in comparative absorption of FA. However, no data are available concerning absorption of long chain FA in ruminant animals. If the protozoa are primarily responsible for FA

TABLE VI

Rumen Fatty Acids from Sheep Administered Safflower Oil and Methyl Myristate

	Molar % of rumen fatty acids [a]				
	Acetate	Propionate	Butyrate	Isovalerate	Valerate
Control	74.1 ± 2.6	15.8 ± 2.3	7.9 ± 0.8	0.9	1.5
Safflower oil	69.2 ± 2.4	19.0 ± 2.2	8.8 ± 0.9	1.6	1.2
Methyl myristate	65.5 ± 2.2	23.0 ± 1.4	9.5 ± 1.0	0.2	1.7

[a] Mean and SE of the mean.

TABLE VII

Fatty Acid Composition of Rumen Bacteria

	Carbon chain length: Unsaturated bonds						
	12:0	14:0	16:0	18:0	18:1	18:2	18:3
Control	5.0	14.1	29.5	14.1	20.1	12.1	3.1
Safflower oil	2.6	6.8	20.0	20.1	30.0	16.3	3.9
Methyl myristate	5.0	44.8	24.7	4.7	10.2	6.4	0.9

hydrogenation, the caloric value of unsaturated fat might be increased by selectively eliminating the protozoa from the rumen.

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Quantitative Analysis of 1-Olefins by Programmed Temperature Gas Chromatography

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Abstract

Gas chromatography has been extremely valuable for the analysis of materials used in the manufacture of surfactants. Temperature programming, which makes possible the efficient separation of wide boiling range mixtures, has extended the usefulness of the technique.

In the work described, mixtures of 1-olefins from C_8–C_{18} in chain length were completely separated on a silicone rubber column in 17 min. The peaks were sharp, symmetrical and almost evenly spaced. A flame ionization detector was used.

Using synthetic mixtures, it was found that over the C_8–C_{18} range, peak area percent agrees very closely with weight percent. On a large number of measurements, the average deviation between area percent and weight percent was 0.5% absolute, with a range of ± 2%. No bias in instrument response was observed with respect to chain length. Average carbon numbers calculated from chromatographic data were accurate to within ± 0.2 units.

Chromatographic results were compared with mass spectrometer data and were found to be significantly more precise and accurate. Furthermore, olefins could be detected in concentrations low enough that they were missed by the mass spectrometer (0.1%).

Introduction

BRANCHED-CHAIN OLEFINS, particularly tetrapropylene, have long been used as intermediates in the manufacture of detergents. With improved technology, straight-chain 1-olefins are expected to become available soon in large volume and at commercial prices. As such, they are regarded as basic raw materials and valuable intermediates for the detergents of the future. The need arises, therefore, for a rapid, reliable method for analyzing commercial mixtures of 1-olefins in the C_8–C_{18} range. Gas chromatography has proved useful for analyzing detergent intermediates and related materials in the past (1–4), and we have found this technique, with the addition of programmed column temperature, to be useful also for 1-olefins.

Experimental

An F&M Model 609 gas chromatograph was used in this work. The instrument was equipped with a flame ionization detector and a Disc integrator. Silicone gum rubber (SE-30) was found to be a very efficient stationary phase for separating 1-olefins in the C_8–C_{18} range. The column used was 2 ft long, ¼ in. O.D. stainless steel packed with 20% SE-30 on 60–80 mesh Chromosorb P.

Separation was best when the column temperature was programmed upward from 75C at a linear rate of 9C per minute. Flow rate of the helium carrier gas was 30–35 cc per minute. Injection port and detector temperatures were 270C and 325C, respectively. Sample size was varied between 0.1 and 1.0 μl. To check quantitative response, a series of nine synthetic mixtures was prepared from pure, even-numbered 1-olefins in the C_8–C_{18} range. The mixtures contained from two to six components ranging in concentration from 5–95%.

Results and Discussion

A typical programmed temperature chromatogram of an experimental olefin mixture is shown in Figure 1. Figure 2 shows the chromatogram of the same sample run under isothermal conditions. The increased efficiency of programmed temperature operation in separating mixtures spanning a wide boiling-point range is strikingly demonstrated. Not only is resolution improved by temperature programming, but higher boiling components (i.e., C_{16}, C_{17}, and C_{18} olefins), so diffused in isothermal operation as to be

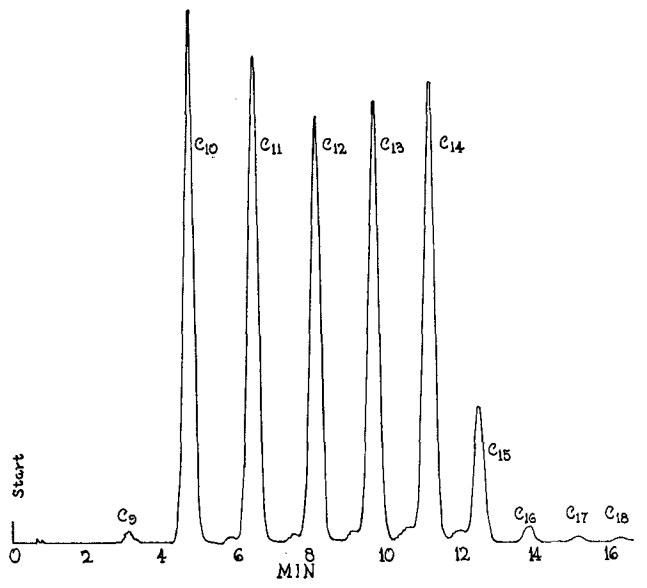

FIG. 1. Programmed temperature gas chromatogram of experimental olefin.