Bypassing the Rumen in Dairy Ewes: The Reticular Groove Reflex vs. Calcium Soap of Olive Fatty Acids

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ABSTRACT

A 3 × 3 Latin Square experiment was designed to compare 2 ways of bypassing the effects of the rumen with olive oil fatty acids in 'Manchega' dairy ewes. Treatments were a control diet, CaOFA (control diet plus 45 g of olive fatty acids as calcium soap), and OO (control plus 45 g/d of olive fatty acids as olive oil emulsified in skim milk) and bottle-fed to animals trained to maintain the reticular groove reflex). No differences were found in milk, protein, and lactose yields, but fat yield and milk fat content were greater in treatments with added fat (CaOFA and OO). Content of short- and medium-chain fatty acids in milk fat was greater for control treatment than for the other 2 groups, the yield of these fatty acids being similar for all 3 diets, except that of C12:0, which was greater for the control treatment. Content and yield of C18:0 and isomers of C18:1 others than oleic acid were greater in milk from the CaOFA diet than from the other 2 diets. Oleic acid content and yield were greater in milk after OO treatment (23.9% and 16.8 g/d, respectively), intermediate after CaOFA treatment (19.2% and 13.8 g/d, respectively), and lower after control diet (10.7% and 6.52 g/d, respectively). Linoleic acid yield and content were greater in ewes fed the OO diet than in those on the other 2 diets, both of which showed similar data. All these changes indicated that the "protected" olive fatty acids (as calcium soap) were severely affected by the rumen environment and that the use of the reticular groove reflex seems to be a more effective way of bypassing the rumen in adult lactating dairy ewes.

(Key words: reticular groove, calcium soap, olive oil, dairy ewe)

Abbreviation key: CaOFA = calcium soap of olive fatty acids, OO = diet with added olive oil, bottle-fed.

INTRODUCTION

In 1982, Ørskov highlighted the possibility of using the reticular groove reflex to bypass the rumen with protein or any other dietary component in liquid form, bottle-fed to adult, trained animals. Reid et al. (1991) reviewed research on the mechanisms of the reticulo-omasal orifice and those that trigger the closure of the reticular groove. Utilization of the reticular groove reflex in nutrition experiments has been tried effectively, in ruminant lambs (Lawlor et al., 1971), and in ruminant kids (Lindberg, 1991). Lawlor et al. (1971), who attempted to improve the growth of their animals using this procedure, described a behavioral pattern of lambs when sucking from a bottle that is consistently associated with closure of the reticular groove.

Olive oil is the main vegetable fat produced in Spain. The olive oil industry generates fat by-products with the same fatty acid composition as the olive oil. Olive fatty acids and olive oleins (from physical and chemical refining of high-acidity virgin olive oil and solvent-extracted olive oil, respectively) consist of (mainly) unesterified fatty acids. Making them into calcium soap (**CaOFA**) to supplement dairy rations offered the possibility of increasing the oleic acid content of milk fat. Besides, feeding fats to dairy animals in hot climates adds energy to the diet thus avoiding the increase of dietary NFC to dangerous levels, with its corresponding fermentation heat output.

Pérez Alba et al. (1997) found that, in addition to a strong increase of oleic acid content in milk fat of dairy ewes, feeding CaOFA increased the content of other long-chain fatty acids (stearic and octadecenoic isomers others than oleic), and decreased the content of linoleic acid. No reason was found for the lower level of the latter in milk fat of ewes fed CaOFA instead of the control diet, because the olive fatty acids have around 5.5% linoleic acid, whereas milk fat of the control diet had less than 2%. It was not clear why the levels of stearic acid and isomers of octadecenoic acid other than oleic acid increased with CaOFA diet, as the content of these acids in the soap was much lower than it was in milk fat of the ewes fed the control diet. They hypothe-

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Table 1. Milk yield and composition of 3 groups of ewes before the beginning of the experiment (means $\pm$ SD of 6 d).

	Group 1	Group 2	Group 3
Milk yield (g/d)	948 $\pm$ 233	938 $\pm$ 72.2	1029 $\pm$ 140
Fat (%)	7.43 $\pm$ 0.230	7.31 $\pm$ 0.438	7.57 $\pm$ 0.082
Protein (%)	6.19 $\pm$ 0.580	6.32 $\pm$ 0.098	6.24 $\pm$ 0.373
Lactose (%)	5.57 $\pm$ 0.158	5.46 $\pm$ 0.057	5.37 $\pm$ 0.120
Total solids (%)	20.32 $\pm$ 0.806	20.26 $\pm$ 0.523	20.24 $\pm$ 0.311

sized that partial biohydrogenation of the fatty acids in the calcium soap could be the cause of these findings.

Our aim in this paper was to compare 2 ways of bypassing the effects of the rumen with olive oil on ewe's milk production and composition: the reticular groove reflex and calcium soap of olive oil fatty acid.

MATERIALS AND METHODS

Animals and Housing

Nine 18-mo-old 'Manchega' dairy ewes in the second month of their first lactation, weighing 54.2 ± 2.6 kg, from a group of artificially reared animals were used. Since birth, these animals were bottle-fed on their dams' colostrum and milk for 1 wk, and thereafter with milk replacer. They were eating enough solid feed that they could be weaned at 45 d of age. From that time on, they were fed daily 250 mL of reconstituted milk replacer (12% by weight) throughout their lives, as well as a normal solid diet. They were housed individually in 1.40-m² raised floor cages with trough and water supply, in a temperature-controlled room ($21 \pm 1^\circ\text{C}$) artificially lit for 16 h daily. All of them had lambed and reared a single lamb. These were weaned when 45 d old and thereafter the ewes were milked twice a day.

Experimental Design

According to milk yield (measured over the 6 d before the beginning of the experiment, Table 1), 3 groups of 3 ewes each were made. These groups were randomized as a 3×3 Latin Square, treatments being control, control plus 45 g/d of olive fatty acids given as calcium soap pelleted into the concentrate part of the diet (**CaOFA**), and control plus 45 g of olive fatty acids given as olive oil emulsified in 1 L of reconstituted (8% DM) skim milk (**OO**). Periods lasted 3 wk and sampling was carried out on the last 5 d of each period.

Diets

The control diet had a solid and a liquid part. The solid part was made of 700 g of pelleted concentrates, 700 g of alfalfa hay, and 200 g of beet pulp, per day,

Table 2. Ingredient composition of both concentrates (basic and calcium soap, CaOFA) used in the experiment as percentage of air-dry matter.

Ingredient	Mixes of concentrate	
	Basic concentrate	CaOFA concentrate
Ground barley	75.0	69.1
Soybean meal	12.0	11.0
Sunflower meal	10.0	9.21
Dicalcium phosphate	1.00	0.92
Common salt	0.35	0.32
Vitamin/mineral mix ¹	0.5	0.47
Sepiolite	1.50	1.38
Calcium soap of olive fatty acids	. . .	7.92

¹Minimum concentration in the mix: Vitamin A, 10,000 IU/kg; vitamin D₃, 2500 IU/kg; vitamin E, 8 mg/kg; calcium, 6 g/kg; common salt, 4 g/kg; cobalt, 0.5 mg/kg; copper, 5 mg/kg; iron, 40 mg/kg; manganese, 114 mg/kg; selenium, 0.22 mg/kg; iodine, 1.6 mg/kg; lime, 115 mg/kg; sulfur, 150 mg/kg; magnesium, 300 mg/kg; and sepiolite, up to 1 kg.

per ewe. The liquid part of the control diet was made of (per ewe, per day) 80 g of dry skim milk, 5 g of glycerol, 0.5 g of soybean lecithin, and warm water up to 1 L. The concentrate for the CaOFA diet had 8.6 kg of CaOFA added to 100 kg of basal concentrate, before pelleting. Therefore, the solid part of the CaOFA diet was made of 760 g of basal concentrate plus CaOFA, 700 g of alfalfa hay, and 200 g of beet pulp, per day, per ewe. The liquid part of this diet was the same as the control diet. The solid part of the OO diet was the same as the control diet. The liquid part of the OO diet comprised 50 g of olive oil emulsified in 1 L of reconstituted skim milk (80 g of dry powder) with 0.5 g of soybean lecithin. Both diets with added fat (CaOFA and OO) were isonitrogenous and isocaloric, whereas the control diet (also isonitrogenous) had less energy because it had no added fat. The composition of diets and dietary ingredients are shown in Tables 2 and 3.

Procedures

Feeding. Each ewe received its daily diet (solid and liquid portions) in 2 equal parts at 0800 and 2000 h. The emulsion of the olive oil for the corresponding ewes was freshly prepared before each meal, as follows: the needed weight of olive oil was heated to 70°C , then 1% (wt/wt) of soybean lecithin was added, followed by slow addition of warm reconstituted skim milk (65°C), and the mixture was blended by mechanical stirring. Feeding bottles were filled and immediately given to the ewes.

Milking. The ewes were hand-milked and stripped out before feeding times. They were given 2 to 3 IU of oxytocin i.v. (Oxiton, Laboratorios Ovejero, León, Spain) before milking to prevent milk retention in the

Table 3. Composition of all dietary components used in the experiment as percentage of air-dry matter.

	Basic concentrate	CaOFA concentrate	Alfalfa hay	Beet pulp	Calcium soap ¹	Olive oil	Dry skim milk
DM	90.1	89.5	91.4	90.7	89.1	99.9	98.0
CP	16.5	15.0	15.9	10.0	...	...	36.5
NDF	18.2	16.2	45.4	43	...	...	...
Ether extract	2.0	7.64	2.3	0.9	75.0	99.9	0.1
Ca	0.35	1.26	1.66	0.98	12.1	...	1.50
P	0.62	0.58	0.27	0.11	...	...	1.41
True fat ²	66.1	90.4	48.2	44.1	99.9	99.9	99
Fatty acids ³							
C14:0	0.001	0.001	0.138	0.038	...	...	...
C16:0	0.277	0.785	0.311	0.091	6.71	8.9	...
C16:1	0.001	0.042	0.011	0.001	0.47	0.7	...
C18:0	0.011	0.245	0.035	0.012	2.93	4.0	...
C18:1	0.203	4.715	0.115	0.165	58.0	77	...
C18:2	0.782	1.032	0.288	0.051	4.00	5.3	...
C18:3	0.076	0.103	0.350	0.010	0.38	0.5	...
C≥20	0.001	0.050	0.011	0.016	0.7	0.8	...

¹Included in calcium soap (CaOFA) concentrate.

²Triacylglycerols or free fatty acids as percentage of ether extract.

³As percentage of the dietary component in as-fed basis.

udder. To calculate milk yields, only milkings during the last 5 d (10 milkings per ewe) in each period were used. Two composite milk samples per ewe were taken in the last 4 d of each period. One was used for infrared analysis; the other was kept frozen at -24°C until the fatty acid analysis was performed.

Chemical Analysis

Samples of feed were analyzed for DM, CP, NDF, and ether extract (AOAC, 1990). Saponifiable and nonsaponifiable fractions in ether extract were measured by hydrolysis-saponification (Christie, 1982), and fatty acids were measured in the saponifiable fraction by gas chromatography. Data on Ca and P content were drawn from published data on feedstuffs composition (FEDNA, 2002). Milk contents of protein, fat, lactose, and total solids were analyzed using infrared spectroscopy (AOAC, 1990; Milko Scan 104 a/b, Foss Electric, Hillerød, Denmark). After thawing, samples of milk for fatty acid analysis were centrifuged at $35,000 \times g$ (17,000 rpm) and 34°C for 15 min. Then the tubes were placed into crushed ice to cool them before extracting the upper lipidic layer. Milk fat was extracted from this layer by the Folch method (Christie, 1982). Milk fat and olive fatty acids in the olive oil were methylated using the cold methylation procedure described by Pérez Alba et al. (1997). The saponifiable fractions of ether extracts and CaOFA were methylated according to the acid procedure (acetyl chloride) described by Christie (1982). Fatty acid analyses were performed using a GC HP 6890 with a 48-m capillary column (0.25 mm i.d. and a $0.25\text{-}\mu\text{m}$ film) from Hewlett Packard (Palo

Alto, CA). For milk fatty acids, oven temperature conditions were as follows: 50°C held for 4 min, then increased $5^{\circ}\text{C}/\text{min}$ up to 230°C , and held for 8 min.

Data Analysis

All data were analyzed using the GLM procedure of the SAS statistical software (1988) from the SAS Institute (Cary, NC) taking into account the effects of period, group, and ewes into groups, treatment, and the interaction period $\times$ treatment. Paired comparisons between means of treatments were studied with the *t*-test.

RESULTS

All ewes showed sucking behavior characteristic of healthy preruminant lambs before and during the experiment. One ewe took 48 h (4 bottle feedings) to fully accept the olive oil emulsified into the liquid skim milk, but the others accepted it readily from the beginning. The solid part of the diet was eaten in its entirety between meals, most of it in the first 30 min after it was offered.

The analysis of the data showed some effects of the feeding periods on the results. As expected, milk, fat, protein, and lactose yield were affected by feeding periods, as were protein contents. No effect of feeding periods on milk fat content or milk fatty acid composition was observed. There were also effects of the group on milk, protein, and lactose yields. Interaction between periods and treatments was not observed. Only the effects of treatments on the results will be discussed here.

Table 4. Results of milk, fat, protein, and lactose yield (g/d), and milk composition (as percentage of milk) from Manchega dairy ewes when fed 1 of 3 dietary treatments: control (C), control plus calcium soap of olive fatty acids (CaOFA), and control plus olive oil emulsified in skim milk and bottle-fed (OO).

	Dietary treatments			SEM	<i>P</i>	<i>P</i> values of paired comparisons		
	C	CaOFA	OO			OO vs. CaOFA	OO vs. C	CaOFA vs. C
Milk yield (g/d)	728	767	737	33.6	0.63	0.54	0.76	0.36
Fat (%)	8.68	9.35	9.86	0.179	0.002	0.07	0.001	0.02
Protein (%)	6.55	6.32	6.61	0.095	0.12	0.05	0.67	0.11
Lactose (%)	5.146	5.233	5.179	0.025	0.08	0.15	0.37	0.03
Fat (g/d)	62.0	71.2	72.0	2.32	0.02	0.79	0.01	0.02
Protein (g/d)	47.1	48.0	48.4	1.61	0.84	0.87	0.58	0.70
Lactose (g/d)	37.2	40.2	38.2	1.86	0.53	0.71	0.46	0.28
GE ¹	994	1096	1099	36.7	0.11	0.96	0.07	0.07

¹GE = Gross energy production (kcal/d) calculated from milk components yield.

Results of milk yield and composition are in Table 4. No differences in milk, protein, and lactose yields were found, but there were clear differences in fat content and yield between treatments. Lactose content showed a trend to be greater ($P = 0.08$) in milk of the ewes when fed the CaOFA diet than the other 2 diets. Milk protein content tended to be lower when ewes were fed the CaOFA diet ($P = 0.12$). Ewes on treatments with added fat (CaOFA and OO) had greater milk fat content. A comparison between treatments CaOFA and OO showed a trend to induce more milk protein and fat contents for the OO diet ($P = 0.05$ and $P = 0.07$). On the other hand, lactose content showed a trend to be greater in milk after CaOFA treatment ($P = 0.15$). Fat yield was similar in milk from ewes when fed any type of fat and lower in ewes when fed the control diet.

Results of fatty acid composition of milk fat are shown in Table 5. Adding fats to the control diet decreased the short- and medium-chain fatty acid contents of milk fat (C8:0 to C14:0). Palmitic acid (C16:0) content in

milk fat was unaffected by diet, but longer chain fatty acids were distinctly affected. Stearic acid (C18:0) and isomers of C18:1 others than oleic acid (6.69 and 2.20%) were greater in milk of ewes fed the CaOFA diet, and similarly lower ($P = 0.003$ and $P = 0.04$) in milk fat of ewes fed the control (3.72 and 1.33%) or the OO diet (4.49 and 1.56%). On the other hand, oleic acid (C18:1-9c) content was greater (23.9%) in milk fat of ewes fed the OO diet, intermediate (19.2%) in that of ewes fed the CaOFA diet, and lower (10.7%) in ewes fed the control diet ($P = 0.001$). Content of C18:2 was significantly greater in milk fat of ewes fed the OO diet (2.70%) than in milk fat of ewes fed the CaOFA (1.62%) or the C (1.80%) diets ($P = 0.001$).

Individual fatty acid yields in milk fat, calculated as described by Jensen (1999), are shown in Table 6. No differences were observed in total yield of most short- and medium-chain fatty acids of milk fat, except for C12:0 (greater in milk of ewes fed the control diet, $P = 0.04$). A trend of C10:0 to be lower for CaOFA diet ($P =$

Table 5. Fatty acids content in milk fat (as percentage of total fatty acids) from Manchega dairy ewes when fed 1 of 3 dietary treatments: control (C), control plus calcium soap of olive fatty acids (CaOFA), and control plus olive oil emulsified in skim milk and bottle-fed (OO).

	Dietary treatments			SEM	<i>P</i>	<i>P</i> values of paired comparisons		
	C	CaOFA	OO			OO vs. CaOFA	OO vs. C	CaOFA vs. C
C4:0	3.64	3.70	3.21	0.249	0.35	0.19	0.24	0.16
C6:0	3.23	2.83	2.84	0.190	0.28	0.97	0.17	0.16
C8:0	3.14	2.46	2.71	0.163	0.03	0.29	0.09	0.01
C10:0	9.73	6.78	7.68	0.473	0.003	0.20	0.01	0.001
C12:0	5.41	3.52	4.01	0.242	0.000	0.18	0.002	0.000
C14:0	11.7	8.87	9.14	0.567	0.01	0.35	0.01	0.01
C16:0	21.9	21.9	21.5	1.58	0.95	0.88	0.87	0.99
C16:1	1.23	1.32	1.21	0.086	0.64	0.38	0.86	0.48
C17:0	0.48	0.32	0.33	0.050	0.09	0.88	0.06	0.048
C17:1	0.22	0.17	0.18	0.032	0.45	0.81	0.34	0.24
C18:0	3.70	6.70	4.50	0.485	0.003	0.01	0.29	0.001
C18:1	10.7	19.2	23.9	1.00	0.001	0.01	0.001	0.001
iso 18:1	1.33	2.20	1.56	0.215	0.04	0.055	0.48	0.02
C18:2	1.80	1.62	2.70	0.118	0.001	0.001	0.001	0.31
C18:3	0.30	0.60	0.38	0.535	0.01	0.01	0.48	0.002

Table 6. Amounts of fatty acids in milk (g/d) from Manchega dairy ewes when fed 1 of 3 dietary treatments: control (C), control plus calcium soap of olive fatty acids (CaOFA), and control plus olive oil emulsified in skim milk and bottle-fed (OO).

	Dietary treatments			SEM	<i>P</i>	<i>P</i> values of paired comparisons		
	C	CaOFA	OO			OO vs. CaOFA	OO vs. C	CaOFA vs. C
C4:0	2.26	2.64	2.43	0.213	0.47	0.51	0.57	0.23
C6:0	2.00	2.01	2.13	0.167	0.83	0.63	0.59	0.95
C8:0	1.94	1.74	2.01	0.144	0.41	0.21	0.75	0.33
C10:0	6.00	4.80	5.60	0.416	0.14	0.18	0.51	0.06
C12:0	3.35	2.49	2.88	0.210	0.04	0.21	0.14	0.01
C14:0	7.18	6.41	6.56	0.485	0.51	0.83	0.38	0.28
C16:0	13.7	15.8	15.5	1.25	0.48	0.85	0.35	0.26
C16:1	0.757	0.950	0.851	0.0713	0.20	0.34	0.37	0.08
C17:0	0.292	0.242	0.233	0.0381	0.51	0.87	0.29	0.36
C17:1	0.139	0.121	0.119	0.0218	0.78	0.94	0.53	0.58
C18:0	2.25	4.77	3.17	0.472	0.01	0.034	0.19	0.003
C18:1	6.52	13.8	16.8	1.087	0.001	0.08	0.0001	0.001
iso 18:1	0.834	1.55	1.10	0.156	0.02	0.07	0.26	0.01
C18:2	1.09	1.15	1.90	0.103	0.001	0.001	0.0001	0.70
C18:3	0.200	0.432	0.258	0.0428	0.01	0.01	0.35	0.002

0.14) was also observed. Long-chain fatty acid yields and contents (percentage of total fatty acids) were affected by dietary treatments in the same way. Thus, stearic acid and isomers of C18:1 (different from oleic acid, given as a sole figure) yields were greater in milk in ewes fed the CaOFA diet than those fed the other 2 diets ($P = 0.01$ and $P = 0.02$). Oleic and linoleic acid yields were lower when the CaOFA diet was fed instead of the OO diet (P values for paired comparisons: 0.08 and 0.001). The control diet showed the lowest oleic acid yield of all diets.

DISCUSSION

Reflex of the Reticular Groove

No radiological test of passage through the reticular groove was done in this experiment. We assumed that the reticular groove was functioning properly because of the way the animals sucked the liquid part of the diet. In fact, there is good evidence that the sucking behavior of the animals is a reliable indicator of proper closure of the groove. Thus, in the study by Lawlor et al. (1971), all lambs showing the typical suckling behavior had sucked the radiopaque mix entirely into the abomasum, whereas those showing a poor or atypical suckling pattern had the radiopaque mix partially or totally in the rumen. Training 2- or 3-d-old lambs to drink from the bottle instead of from their mothers' teats was a difficult part in the work of Lawlor et al. (1971). However, our animals were never allowed to suckle their dams. They were retired from their mothers as soon as they were born and were bottle-fed fresh colostrum within 1 h of birth. Therefore, the lambs immediately and naturally accepted the bottle teat, and

the typical suckling behavior (with excitement, wagging tails, general nervousness) was maintained into adulthood by reinforcing the reflex daily with 250 mL of reconstituted milk replacer. All the animals showed the typical suckling behavior before, during, and after the experiment.

Effects of Dietary Treatments on Milk Composition and Yield

Feeding fats to dairy ruminants decreases milk protein content. This assessment on dairy cows is well tested and documented in the literature (Storry et al., 1980; Wu and Huber, 1994) and is also found in dairy ewes (Pérez Alba et al., 1997). Although milk protein yield was nearly identical for all the treatments, milk protein content tended to be lower when the CaOFA diet was given. Milk protein and fat yields of ewes fed the CaOFA and OO diets were nearly equal, but the contents of milk protein and fat for ewes fed the CaOFA diet were 4.4 and 5.2% lower, respectively, than those for the OO diet. This suggests that a dilution effect (Wu and Huber, 1994) occurred when the CaOFA diet was given. If that was the case, the ewes on the CaOFA diet should have produced more milk than ewes on the OO diet. In fact, milk yield was 4.1% greater with the CaOFA diet, which was not statistically significant, probably because the variability in milk yield between individual ewes and groups was greater than that of milk fat and protein content.

Lactose content in milk was 1% greater after the CaOFA diet than after the OO diet. Due to the low variability in lactose content in milk (inherent to its osmotic effect), this small difference showed a P value of 0.15. Lactose yield with the CaOFA diet was 5.2%

greater than with the OO treatment, but the *P* value was too high, probably for the same reason as given above. Despite the lack of significant differences in milk and lactose yields in ewes fed the diets with added fat, the cause of the observed dilution effect should be sought in the different lactose content in milk produced after those diets.

Other osmotic agents in milk are proteins and electrolytes, yet lactose remains the strongest. Protein content was lower in milk after the CaOFA diet. No data for milk electrolyte content were obtained in this experiment, but this is probably not the cause of the dilution effect. Milk osmotic pressure must be equal to that of the blood. Thus, if the concentration of one of the osmotic agents in milk increases, then that of the other(s) must decrease (Kaufmann and Hagemester, 1987).

Speculating that the above mentioned differences in milk and lactose yields after diets CaOFA and OO (both featuring the same intake and content of ingredients and nutrients) were significant, their likely cause should be discussed.

Fats fed to lactating ruminants may decrease the synthesis of short- and medium-chain length fatty acids in the mammary gland, lowering the amount of glucose used as NADPH in this synthesis. The spared glucose can be used to produce lactose in the udder, and more lactose drains more water into milk, thus increasing milk yield, due to the strong osmotic effect of lactose. Thus, if milk protein and fat productions are not increased, their percentages in milk would decrease. No significant differences in yield of C6:0 to C14:0 milk fatty acids were found between treatments CaS and OO, but adding them together, 1.73 g less of these acids (amounting to 2.40% of total milk fat output) were found daily in milk of CaOFA fed ewes.

From this reasoning, coupled with the trend to greater lactose content in milk of ewes fed the CaOFA diet, it could be suggested that the olive fatty acids fed as calcium soap (but not as olive oil through the reticular groove) caused a dilution effect on the main milk components.

Effect of Treatments on Milk Fat Fatty Acid Composition

Content of total short- and medium-chain length fatty acids as percentage of total fatty acids is greater with the C diet than with the CaOFA or OO diets. This is expected because vegetable fats fed to dairy ruminants usually increase milk fat yield and the proportion of long-chain fatty acids in milk fat. Therefore, dilution of the short- and medium-chain fatty acids in the milk fat would be expected. But the yield of these fatty acids should not change when only a dilution effect is taking

place. This is not observed clearly here, as yield of C12:0 is significantly lower for CaOFA treatment, the yield being intermediate (nonsignificant) with the OO diet. A trend in the same direction is observed for C10:0. That seems to indicate that a reduction in short- and medium-chain fatty acid synthesis has occurred. This supports the hypothesis of a dilution effect caused by the CaOFA diet (Wu and Huber, 1994).

No data of the recently identified fatty acid 10 *trans*,12 *cis*-18:2 (Baumgard et al., 2000), which inhibits the synthesis of short- and medium-chain fatty acids in the udder, are available in this study. Yet, the lower yield of these fatty acids probably indicates some degree of synthesis inhibition. No differences in palmitic acid content in milk fat were found. The palmitic acid in milk fat may have 2 origins: it can be synthesized by the mammary gland and it can be taken up preformed from the blood. Palmitic acid content in olive oil fatty acids is about 9%. The likely lower synthesis in the udder or the dilution in the milk fat with the CaOFA or OO diets could be offset by greater uptake of palmitic acid from dietary origin.

Bypassing the rumen with olive oil fatty acids should have the same effect on the main long-chain fatty acid composition of the milk fat, independently of the method used. These effects could be predicted from the fatty acid compositions of olive oil and milk fat from the ewes fed the control diet. Stearic acid (similarly low in both fats) should not change. Oleic acid should increase strongly in milk fat. Linoleic acid (low in olive oil and very low in milk fat in ewes fed the control diet) should increase its concentration. All of these changes can be observed in the milk fat of ewes fed the OO diet. Differences appeared in ewes fed the CaOFA diet compared with the OO diet. More stearic acid, less oleic and linoleic acids, and more isomers of octadecenoic acid others than oleic (coming mainly from rumen desaturation of linoleic acid, as described by Bauman et al., 1999) indicate that rumen biohydrogenation of the saponified fatty acids has occurred.

CONCLUSIONS

Olive oil fatty acids were poorly protected from rumen biohydrogenation when fed as calcium soap. The lower than expected contents of oleic and linoleic acids and the increased contents of stearic acid and isomers of oleic acids in milk fat of ewes fed the calcium soap support the above statement.

Training ewes to accept bottle-feeding in a manner typical of healthy preruminant suckled lambs seems to be an effective method of bypassing the rumen with vegetable fats emulsified in reconstituted skim milk. The high levels of oleic and linoleic acids in milk fat of

ewes fed with the OO diet and the similar content of stearic and C18:1 isomers others than oleic acid after C and OO diets further support our proposal.

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