Calcium Soaps of Olive Fatty Acids in the Diets of Manchega Dairy Ewes: Effects on Digestibility and Production

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ABSTRACT

Two experiments were carried out with dairy ewes to determine the effects of supplementation of calcium soaps of olive fatty acids at 10% of the basal diet on digestibility, roughage intake, milk production and composition, and response to early induced ovulation. The addition of calcium soaps of olive fatty acids to the diets of dairy ewes significantly decreased the digestibility of dietary dry matter but not the digestibility of other components. The digestibility of crude fat was enhanced. Voluntary intake of roughage, with fixed concentrate allowances, was slightly, but not significantly, reduced for ewes fed the supplemented diet. After weaning at 35 d postpartum and during the next 5-wk period of twice daily milking, ewes fed the supplemented diet produced more total solids in milk than did ewes fed the basal diet. Ewes fed the supplemented diet also tended to produce more milk, protein, and milk energy and tended to have greater milk fat percentages. The composition of milk fatty acids was changed by the calcium soaps of olive fatty acids. Fewer short- and medium-chain fatty acids (C6:0, C8:0, C10:0, C12:0, C14:0, and C16:0), less C18:2, and more C18:1 and C18:0 were obtained in the milk of ewes fed the supplemented diet. Responses to ovulation induced at 60 d after lambing, while ewes were still lactating, were significantly higher for ewes fed the diet supplemented with calcium soaps of olive fatty acids than for ewes fed the basal diet. The calcium soaps of olive fatty acids appeared to be a useful source of energy for dairy ewes, and dairy ewes may be a good model for the study of the effects of nutrition during early lactation on reproductive performance of dairy ruminants.

Key words: olive fatty acids, calcium soaps, dairy ewes

Abbreviation key: Ca-OFA = calcium soaps of olive fatty acids.

INTRODUCTION

Fat in the diets of dairy ruminants is intended 1) to increase the energy concentration of the diet, 2) to increase the fat content in milk, and 3) to change the fatty acid profile of milk fat. Real increases in useful energy for the lactating ruminant depend on fat digestibility and on the effects of added fat on feed intake, digestibility, and utilization of the rest of the feed components. Voluntary intake of diets with added fat that do not cause energy satiety in ruminants is usually reduced either temporarily, by an effect of fats on the sensory qualities of the diet, or more permanently, by an effect on rumen microbial metabolism, which would imply decreased fiber digestibility (18, 23).

Fiber digestibility may be reduced when extra fat is fed (15, 16, 21, 23). The extent of the reduction in fiber digestibility increases as more fat is added. Fiber digestibility also depends on the fatty acid composition of the fats. The reduction of fiber digestibility is usually greater when the added fat is rich in unsaturated fatty acids (23). The protection of fats is intended to suppress or decrease their negative effects on fiber digestibility.

Various studies (23, 35) on the supplementation of fat to the diet have reported effects on milk composition. Effects of the type of fat fed on both the milk fat content and the fatty acid composition of milk are clear (23, 35). The goal of this study was to report on the effects of supplemental protected fat of olive fatty acids in the diets of dairy ewes on digestibility, intake, milk production, milk composition, and response to early induced ovulation.
MATERIALS AND METHODS

Calcium Soaps of Olive Fatty Acids

Olive fatty acids are by-products obtained from refining olive oil of high acidity. Olive fatty acids were obtained locally (Carbonell & Cia de Córdoba, Córdoba, Spain). As sold, the olive fatty acids contain up to 25% triglycerides; the remainder consists of free fatty acids. The mean content of fatty acids as a percentage of total fatty acids is 9.0% palmitic acid, 0.8% palmitoleic acid, 3.8% stearic acid, 78% oleic acid, 5.5% linoleic acid, 0.5% linolenic acid, 0.2% arachidic acid (maximum), 0.3% behenic acid (maximum), and 0.5% lignoceric acid (maximum). Composition of the calcium soaps of olive fatty acids (Ca-OFA) was 98.7% DM, 0.53% nitrogen, 21.3% ether extract, and, by difference, 76.9% true calcium salt. Calcium content in samples from 10 batches ranged from 10.5 to 11.6% and total ash ranged from 25 to 30%.

Experimental Design

The experimental design was intended 1) to assess the effects of Ca-OFA on the digestibility of a basal diet and 2) to determine the effects of the supplementation of the diet of dairy ewes with Ca-OFA on milk production, milk and milk fat composition, voluntary roughage intake, and response to early induced ovulation.

To assess the effects of Ca-OFA on the digestibility of the basal diet, a digestibility experiment (Experiment 1) was arranged using 12 adult lactating ewes fed a roughage and concentrate diet at near three times the maintenance level either alone (n = 6) or supplemented with 10% Ca-OFA (n = 6). Following a 6-wk adaptation period, feces were collected for 8 d. To determine the effects of the supplementation of Ca-OFA to the basal diet on the various parameters, a lactation experiment (Experiment 2) was designed using two treatments: a basal diet or a basal diet supplemented with Ca-OFA. Forty ewes were paired according to age, parity, BW, body condition, number of lambs suckling, and milk production at 7 and 10 d after lambing. Age at mating (1, 2, or 3 yr old), parity (primiparous or second or third parity ewes), and number of lambs (single or twin) were primarily used to form pairs; other traits were used to distinguish pairs further. Each ewe in a pair was randomly assigned to one of two experimental treatments. This experiment started at 12 d after lambing. Ewes were bred by AI on d 60 post-lambing after standard procedures for estrus synchronization and induced ovulation.

Ewes and Housing

Manchega ewes were used. In Experiment 1, 12 3-yr-old ewes in the 3rd mo of the third lactation (mean BW, 64 ± 2.5 kg; mean milk production, 1.44 ± 0.3 kg/d) were housed in digestibility pens (1.25 m²) and received the basal diet (n = 6) or the basal diet supplemented with Ca-OFA (n = 6). In Experiment 2, 100 ewes (1, 2, or 3 yr old) were bred by AI in September after standard estrus synchronization and induced ovulation. The ewes were induced to lamb at 146 d of pregnancy (dexametason deliver i.m.; Resdex®; Schering-Plough, Sègre, France), and 40 ewes were chosen for experimentation (those lambing normally at 149 and 150 d of pregnancy with good milk production at 7 and 10 d after lambing). These ewes were housed in individual pens (1.4 m²) with slatted floors containing two different feed troughs, one in front and another in back. Water was available for ad libitum intake, and the facilities were equipped so that ewes could be machine-milked in the pen.

Diets and Feeding

The same two diets were used in both experiments and contained steam-pelleted concentrates and roughage. The basal concentrate was a mixture of 650 g/kg of ground corn, 230 g/kg of (30% protein) sunflower oil meal, 30 g/kg of fish meal, and 30 g/kg of vitamins, minerals, and sepiolite (air-dry basis) (10,000 IU/kg of vitamin A, 2500 IU/kg of vitamin D₃, 8 mg/kg of vitamin E, 6 g/kg of calcium, 4 g/kg of common salt, 0.5 mg/kg of cobalt, 5 mg/kg of copper, 40 mg/kg of iron, 114 mg/kg of manganese, 0.22 mg/kg of selenium, 1.6 mg/kg of iodine, 150 mg/kg of copper, 300 mg/kg of magnesium, and up to 30 g/kg of sepiolite). The Ca-OFA concentrate was prepared by mixing 200 kg of Ca-OFA with 1000 kg of basal concentrate prior to pelleting. Only one roughage was used in both experiments and treatments. The roughage was composed of 950 g/kg of shredded oat vetch hay and 50 g/kg of olive molasses, which was mixed in a mixer wagon.

Feeding Management

In Experiment 1, ewes were fed three equal meals at 0800, 1500, and 2200 h. Ewes fed the basal diet received 1.2 kg of concentrate and 1.2 kg of roughage daily; ewes fed the diet supplemented with Ca-OFA received 1.44 kg of Ca-OFA concentrate and 1.2 kg of
roughage daily. Orts were collected before each meal and kept for each ewe. At the end of the collection period, ewes with more than 0.4 kg of total orts were eliminated from the experiment. In Experiment 2, roughage was fed for ad libitum intake separately from the concentrate. Ewes fed the basal diet received 1 kg of concentrate daily, and ewes fed the diet supplemented with Ca-OFA received 1.2 kg of the Ca-OFA concentrate daily in three equal meals at the times indicated previously. Roughage was always available in a different trough. Fresh roughage was added three or four times daily from an individual container, and daily orts were collected at 0800 h into another individual container. Fresh roughage containers were weighed weekly, refilled, sampled, and weighed again. Orts containers were weighed, sampled, and emptied.

**Procedures**

**Milk production.** In Experiment 1, ewes were hand-milked twice daily. In Experiment 2, the double milking technique using oxytocin (2 to 3 IU of oxytocin administered i.v.; Loburmon; Fort Dodge Veterinaria, Vall de Bianya, Girona, Spain) was followed twice a week until weaning. After weaning, the ewes were machine-milked and hand-stripped twice daily; milk from machine-milking and hand-milking were pooled. Oxytocin was used every day before each milking in both experiments. Individual milk production was obtained by weighing milk produced over a 4-h period twice weekly before weaning, and weighing milk produced during a morning and evening milking once weekly after weaning. Before and after weaning, milk production was expressed on a 24-h basis. After milks were weighed, they were thoroughly mixed and sampled. Individual weekly samples were composited and analyzed.

**Body condition and BW change.** Body condition was measured at lambing by five different trained persons. The mean was obtained for each ewe. Body weight was measured weekly at the same time (1100 h).

**Response to early induced ovulation.** The response to early induced ovulation was measured by counting the corpora lutea on the ovaries through laparoscopy at 7 d after AI. Ovulation was induced after placement of progestogen pessaries (Chrono-gest®; Intervet, Salamanca, Spain) on d 46 after lambing. The pessaries were removed on d 58 when an i.m. injection of pregnant mare serum gonadotropin (400 IU; Foligon®; Intervet) was administered. Artificial insemination was carried out on d 60 after lambing at 48 and 60 h after the removal of the pessaries. Corpora lutea were directly observed by five qualified persons using a television monitor.

**Chemical analysis.** Samples of concentrates (basal and Ca-OFA) and roughage were assayed for moisture (48 h at 100°C), ash (48 h at 550°C), crude protein [Kjeldahl procedure; (2)], calcium content (2, 19), total fat using the chloroform-methanol method (3), crude fiber (2), NDF and ADF (30), and gross energy (Parr adiabatic bomb calorimeter, model 1271; Parr Instruments Co., Moline, IL). The same analyses were performed on feces samples from ewes in Experiment 1. Milk samples were assayed for total solids (48 h at 80°C), total protein [Kjeldahl procedure; (2); nitrogen × 6.38], fat [Gerber procedure; (7)], and ash (using residues of total solids analysis as previously described). The composition of milk fatty acids was assessed by GLC (2). Samples of milk fat were obtained by centrifugation of 20 ml of milk at 33,000 × g for 10 min at 34°C and removal and retention of the upper layer. This layer was extracted using the Folch method [as described by Christie (9)] and was used for GLC analysis of fatty acids. Cold methylation was performed. To 30 drops of fat, 1 ml of hexane and 0.5 ml of 10% potassium hydroxide in methanol were added. After stirring and waiting, the clean phase was injected in a gas chromatograph with a 50-m high polar capillary column (0.20 mm i.d. and 0.20 μ thickness; BPX 70; Sugelabor, Madrid, Spain). Helium was used as the carrier gas. The oven temperature was kept constant at 185°C. Temperatures of the injector and the flame ionization detector were set at 270 and 280°C, respectively. Identification of peaks was performed using fatty acid standards and, when in doubt, GLC coupled with mass spectrometry. Conditions for GLC were as described for milk fatty acid analysis. The apparatus for mass spectrometry analysis was a mass spectrometer VG AutoSpec (Fisons Instruments, Manchester, UK), a high resolution mass spectrometer with trisector geometry electromagnetic-electric sectors. Conditions for mass spectrometry analysis of peaks of methylated fatty acids were ionization mode, electron impact; source temperature, 250°C; data acquisition, scanning from 33 to 500 at 1.5 s per scan; and mass spectra library, National Institute of Standards and Technology (used to compare mass spectra data obtained to reveal each compound analyzed). The GLC-mass spectrometry interface was held at 250°C during the analysis. Results were expressed as grams of fatty acid/100 g of total fatty acids.

**Statistical analysis.** Analysis of variance with one factor (supplementation with Ca-OFA) at two levels (positive or negative) were performed for all data from Experiment 1. Weekly data (e.g., milk production, feed intake) were averaged before and after weaning in Experiment 2. Because of different conditions before and after weaning (i.e., adaptation
to Ca-OFA of ewes fed the supplement before weaning, use of ewes sucking singles or twins before weaning versus twice daily machine-milking of ewes after weaning), data from before and after weaning were analyzed separately.

The model used in Experiment 2 for data obtained before weaning was \( Y_{ijkl} = \mu + T_i + A_j + L_k + b_1 (\text{IMP})_l + b_2 (\text{IMFC})_l + b_3 (\text{IMPC})_l + e_{ijkl} \), where \( Y_{ijkl} \) = dependent variable; \( \mu \) = overall mean of the population; \( T \) = the effect of the treatment; \( A \) = effect of the age of the ewes; \( L \) = litter size; IMP, IMFC, and IMPC = data for milk production, milk fat content, and milk protein content, respectively, obtained for each ewe before Experiment 2 began; and \( e \) = residual error. The model used for data after weaning was \( Y_{ijkl} = \mu + T_i + A_j + L_k + e_{ijkl} \), and terms are as previously defined. No covariates were used because data for milk production and composition are strongly influenced by sucking frequency of the lambs, and the effects of twins are greater than that of singles; these effects disappear when lambs were removed from the ewes. Data regarding the composition of the milk fatty acids were analyzed using the model \( Y_{ij} = \mu + T_i + b_1 (\text{IFAC})_j + e_{ij} \), where \( Y \), \( \mu \), and \( T \) were as previously defined and IFAC = fatty acid composition from milk samples taken from each ewe before Experiment 2 began (7 and 10 d after lambing). We used the GLM procedure of SAS (24) to perform calculations. The null hypothesis was rejected at \( P < 0.05 \).

RESULTS

Experiment 1

Results of the digestibility experiment are presented in Table 1. Data from two ewes fed the diet supplemented with Ca-OFA and from one ewe fed the basal diet were discarded because their total orts were greater than 400 g. No significant differences between digestibility values of the basal diet and of the diet supplemented with Ca-OFA were found, except for DM (\( P = 0.034 \); higher for ewes fed the basal diet) and crude fat (\( P = 0.012 \); higher for ewes fed the diet supplemented with Ca-OFA). Dry matter content of the diet supplemented with Ca-OFA had a higher concentration of ash, which partially accounted for the difference in DM digestibility. However, digestibilities of the rest of the components in the diet supplemented with Ca-OFA tended to be slightly lower than those in the basal diet (2.3% less for NDF, 1.5% less for OM, and 0.7% less for ADF and gross energy). The higher digestibility of crude fat for ewes fed the diet supplemented with Ca-OFA (13.5%) can be explained by the higher lipid content of that diet. The mean apparent digestibility of crude fat in the Ca-OFA supplement was 0.77 as calculated by difference. Digestibility of gross energy was similar for ewes fed both diets, which implies higher energy intake for ewes fed the diet supplemented with Ca-OFA because gross energy content of their diet was higher (Table 2) and because those ewes were offered 240 g/d more concentrate. The calculated difference in digestible energy intake was about 3.85 MJ/d.

Experiment 2

Five ewes fed the basal diet and one ewe fed the diet supplemented with Ca-OFA acquired clinical mastitis during the experiment and were removed. Data from these ewes and from ewes in their respective pairs were discarded.

Before weaning, ewes fed the basal diet appeared to produce more milk, but not more milk energy, than did ewes fed the diet supplemented with Ca-OFA. The
opposite result occurred after weaning (Table 3). The difference was due to a lower intake of Ca-OFA concentrate before weaning by ewes fed the diet supplemented with Ca-OFA because 2 to 3 wk were needed for those ewes to adapt to the supplement. Estimated differences in digestible energy and total protein amounted to 6 and 12% less, respectively, for ewes fed the diet supplemented with Ca-OFA. After weaning, when all of the ewes were consuming the whole allocation of concentrates, ewes fed the diet supplemented with Ca-OFA produced 11% more milk ($P = 0.063$), 9% more total fat ($P = 0.114$), 5.6% more fat as a percentage of milk ($P = 0.091$), 4% more total protein ($P = 0.205$), and 5.3% less protein as a percentage of milk ($P = 0.035$).

Roughage intake before and after weaning was slightly less for ewes fed the diet supplemented with Ca-OFA. Total intake after weaning was slightly higher for ewes fed the diet supplemented with Ca-OFA (2%) when a higher proportion of concentrates was consumed, which suggests that more digestible energy (estimated at 5%) was available to these ewes at the same protein intake. Although all of the ewes were gaining BW at the time of AI, rate of gain tended to be higher for ewes fed the diet supplemented with Ca-OFA (24%). These ewes were also gaining more BW for a longer period of time. Therefore, responses to early induced ovulation from ewes fed the diet supplemented with Ca-OFA can be explained in terms of the extra energy consumed by these ewes from weaning to breeding (flushing). We do not know if the same amount of extra energy (5%) supplied by other nonfat supplements (e.g., basal concentrate) would have produced the same response.

Composition of milk fatty acids is presented in Table 4. Almost 90 peaks in the chromatograms were detected, which corresponded to milk fatty acids. In this study, only the fatty acids that amounted to more than 1% of total fatty acids were considered, and most of these fatty acids were previously identified using fatty acid standards. Short-chain fatty acids (C$_{6:0}$ to C$_{12:0}$) decreased by 30 to 50% when Ca-OFA was added to the diets. The proportion of C$_{14:0}$ fell by 21 to 32%, and C$_{16:0}$ fell by 7 to 11%. Concentrations of C$_{18:0}$ increased about 30%, and the proportion of C$_{18:1}$ increased 40 to 80%. Two peaks that individually amounted to approximately 1% of total fatty acids were eluted before the oleic acid. These peaks were identified by GLC coupled with mass spectrometry. The mass spectrometry spectra obtained from the first of these two peaks corresponded to trans-9-octadecenoic acid and to cis-10-octadecenoic acid; the spectra from the second of these two peaks corresponded to trans-6-octadecenoic acid and to cis-11-octadecenoic acid. The value of both peaks was added and is presented as C$_{18:1}$ isomers (Table 4) because the amount of each fatty acid represented in each peak could not be calculated. These isomers of C$_{18:1}$ increased about 200%. C$_{18:2}$ decreased in the milk of ewes fed diets supplemented with Ca-OFA. These changes were measured in milk from 19 d (1 wk after the beginning of the experiment) to 70 d after lambing.

### TABLE 3. Results of Experiment 2. Least squares means of milk production, fat and protein content of milk, intake, BW change, and ovulation data from the beginning of the experiment (12 d after lambing) to weaning (period 1; 24 d) and the next 5 wk after weaning (period 2; 35 d).

<table>
<thead>
<tr>
<th>Item</th>
<th>Basal diet</th>
<th>Ca-OFA Diet</th>
<th>$P$</th>
<th>Basal diet</th>
<th>Ca-OFA Diet</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>SE</td>
<td>$\bar{x}$</td>
<td>SE</td>
<td>$\bar{x}$</td>
<td>SE</td>
</tr>
<tr>
<td>Milk, kg</td>
<td>46.7</td>
<td>2.29</td>
<td>44.6</td>
<td>2.13</td>
<td>0.476</td>
<td></td>
</tr>
<tr>
<td>Fat, kg</td>
<td>3.42</td>
<td>0.270</td>
<td>3.67</td>
<td>0.259</td>
<td>0.474</td>
<td></td>
</tr>
<tr>
<td>Protein, kg</td>
<td>2.04</td>
<td>0.089</td>
<td>1.94</td>
<td>0.831</td>
<td>0.383</td>
<td></td>
</tr>
<tr>
<td>Total solids, kg</td>
<td>8.10</td>
<td>0.480</td>
<td>8.13</td>
<td>0.450</td>
<td>0.952</td>
<td></td>
</tr>
<tr>
<td>Ash, kg</td>
<td>0.42</td>
<td>0.021</td>
<td>0.39</td>
<td>0.019</td>
<td>0.266</td>
<td></td>
</tr>
<tr>
<td>Fat, %</td>
<td>7.30</td>
<td>0.299</td>
<td>8.20</td>
<td>0.372</td>
<td>0.084</td>
<td></td>
</tr>
<tr>
<td>Protein, %</td>
<td>4.40</td>
<td>0.092</td>
<td>4.36</td>
<td>0.091</td>
<td>0.762</td>
<td></td>
</tr>
<tr>
<td>IC, $^3$ kg/d</td>
<td>0.85</td>
<td>0.060</td>
<td>0.80</td>
<td>0.056</td>
<td>0.458</td>
<td></td>
</tr>
<tr>
<td>IR, $^4$ kg/d</td>
<td>1.30</td>
<td>0.058</td>
<td>1.22</td>
<td>0.054</td>
<td>0.302</td>
<td></td>
</tr>
<tr>
<td>BW Gain, kg</td>
<td>0.089</td>
<td>0.431</td>
<td>0.246</td>
<td>0.401</td>
<td>0.771</td>
<td></td>
</tr>
<tr>
<td>CL, $^5$ per ewe</td>
<td>0.29</td>
<td>0.258</td>
<td>1.58</td>
<td>0.258</td>
<td>0.296</td>
<td></td>
</tr>
</tbody>
</table>

$^1$n = 14.
$^2$Basal diet plus 10% calcium soaps of olive fatty acids (Ca-OFA; n = 14).
$^3$Intake of DM of concentrate.
$^4$Intake of DM of roughage.
$^5$Corpora lutea.
DISCUSSION

Digestibility

Data from Experiment 1 showed the usual trend found by most researchers when measuring the digestibility of diets with supplemental fats, whether protected or unprotected (16, 25). Whether there was a shift in the site of digestion of a proportion of the digested OM because of the partially unprotected fat (22, 25) could not be determined in this experiment. If the site of digestion did shift for total OM, particularly fiber, the net consequence would be a waste of microbial mass synthesized in the hindgut. Conversely, free oils have been shown to increase the efficiency of microbial protein synthesis, probably because of their defaunating effects (25). Cerkawski and Clapperton (12) found an increase of 11% of bacterial matter per gram of linseed oil included in the substrate used for their artificial rumen, Rusitec. Olubobokun et al. (21) found increased nitrogen digestion and retention when 8% calcium soap from tallow was substituted for the same proportion of ground corn in the diets of sheep. If these results are valid, the waste of microbial protein caused by more carbohydrates (NDF) digested in the hindgut could be partially or totally offset or even exceeded by increased protein synthesis in the rumen. No data regarding nitrogen retention were obtained in this experiment. Ushida et al. (27) determined that the supplementation of fats to diets that are rich in starch may cause a lower pH and lower fiber digestibility in the rumen because starch ferments quickly and more of it is made available to bacteria by defaunation. Data from our lab from ewes fed Ca-OFA at 10% of dietary DM did not support the hypothesis of Ushida et al. (27). A higher rumen pH was detected when the partially protected fat Ca-OFA was fed to the ewes (L. M. Pérez Alba, S. De Souza Cavalcanti, and M. Pérez Hernandez, 1996, unpublished data).

However, not all researchers have found a trend toward decreased digestibility values of most dietary components because of the inclusion of calcium soap in the diet. Bayourthe et al. (4) found the opposite trend when high percentages of calcium soap were included in the diet of sheep (12%; 98 g/d of extra fat). In our study, total DM and Ca-OFA intakes were more than double those observed by Bayourthe et al. (4), and digestibility results were not significantly lower than those obtained from ewes fed the basal diet. These facts support the use of high concentrations of calcium soaps for diets offered to dairy ruminants at the beginning of lactation.

The estimated value of digestible energy was 16.0 MJ/kg (air-dry basis) of the Ca-OFA supplement (98.7% DM), which appeared to be very low. However, the supplement caused negative, although not significant, changes in the digestibility of total OM. Typical values have been found by various researchers for some fats (28, 33), but those studies always kept added fat at or below 5% of total intake. Many other studies (1, 12, 32) have found strong

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**TABLE 4. Effect of supplementation of calcium soaps of olive fatty acids (Ca-OFA) to a basal diet on main fatty acid pattern of fat from the milk of ewes. Data are presented as least squares means.**

<table>
<thead>
<tr>
<th>FA1</th>
<th>Samples from 7 d of initiation</th>
<th>Samples from 58 d of initiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal diet</td>
<td>Ca-OFA Diet</td>
</tr>
<tr>
<td>C6:0</td>
<td>2.23</td>
<td>1.57</td>
</tr>
<tr>
<td>C8:0</td>
<td>2.57</td>
<td>1.68</td>
</tr>
<tr>
<td>C10:0</td>
<td>8.62</td>
<td>5.17</td>
</tr>
<tr>
<td>C12:0</td>
<td>4.83</td>
<td>2.98</td>
</tr>
<tr>
<td>C14:0</td>
<td>10.39</td>
<td>8.13</td>
</tr>
<tr>
<td>C16:0</td>
<td>22.98</td>
<td>21.48</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.94</td>
<td>0.79</td>
</tr>
<tr>
<td>C18:0</td>
<td>9.57</td>
<td>12.47</td>
</tr>
<tr>
<td>C18:1</td>
<td>13.78</td>
<td>19.33</td>
</tr>
<tr>
<td>iso-C18:1</td>
<td>2.03</td>
<td>5.71</td>
</tr>
<tr>
<td>C18:2</td>
<td>1.70</td>
<td>1.20</td>
</tr>
</tbody>
</table>

1Fatty acids; FA amounting to less than 1% of total FA are not shown.
2n = 14 for each diet.
3Oleic acid.
4Mixture of C18:1 isomers that are not oleic acid. These isomers were identified by mass spectrometry coupled with GLC.
variation in the energy value of fats when included in the diets of ruminants.

Under the assumption that the digestible energy provided by the supplement of Ca-OFA is equal to the metabolizable energy (i.e., that no changes occurred in energy lost as CH₄ and urine from supplemental dietary fats fed to ruminants) and that this metabolizable energy was used with an efficiency of 0.62 (ratio of milk energy to metabolizable energy), this extra digestible energy (3.85 MJ) should have allowed a 0.50-kg increase in daily milk production (7% fat). If the energy content of BW gain in the lactating ewe was 26 MJ/kg and efficiency was 0.95 that of milk production, a BW gain of 87 g could be expected. However, for ruminants fed diets supplemented with fats, Cerkawski (11), Van der Honing et al. (28), and Wainman et al. (34) have found a decrease in energy lost as CH₄ and urine. The amount of decrease in loss of gaseous and urinary energy exceeded the increase noted for fecal energy. Thus, values of metabolizable energy were greater for diets with added fat (2 to 4%), even though digestible energy was lower than that for the control, unsupplemented diet.

**Feeding Trial**

Before weaning, ewes fed the basal diet consumed 12% more protein and 6% more energy than did ewes fed the diet supplemented with Ca-OFA. The reduced concentrate intake of these ewes was caused by the lack of an adaptation period to Ca-OFA, which illustrated the faultiness of the design (supplementation of a basal diet). Morand-Fehr et al. (18) determined that acceptability of supplemental concentrates with added fat was decreased but that eventually dairy cows adapted to the concentrates. Adaptation was complete within 3 wk in this experiment. Both before and after weaning, the total amount of roughage consumed was slightly, but not significantly, lower for ewes fed the diet supplemented with Ca-OFA. Before weaning, the concentrate intake of ewes fed the diet supplemented with Ca-OFA was lower than that of ewes fed the basal diet. Therefore, ewes fed the diet supplemented with Ca-OFA did not compensate for reduced intake by consuming more roughage. Factors other than Ca-OFA supplementation can be cited, including nitrogen intake in the concentrate during this period, which was 12% lower for ewes fed the supplemented diet and the low nitrogen content of the roughage (1.23%).

After weaning, the lower roughage intake of ewes fed the diet supplemented with Ca-OFA (1.09 vs. 1.16 kg/d) could be explained as an effect of the higher intake of concentrates as much as an effect of the Ca-OFA itself. In fact, the substitution rate for the mean daily increase in intake of Ca-OFA concentrate of 0.28 kg per ewe after weaning compared with the mean daily intake of that concentrate before weaning was 0.46. The substitution rate for the mean daily increase of intake of basal concentrate of 0.11 kg per ewe was 1.28. Consequently, no clear negative effect of the supplement on voluntary intake of roughage could be detected. Bines et al. (5) found significantly reduced intakes of both concentrate and hay by dairy cows fed 0.54 kg/d of calcium salts of fatty acids (about 3% of fatty acids). The same result was reported by Jenkins and Jenny (15) for yellow grease at 5% of the diet but not for hydrogenated yellow grease at 3 or 5%. Palmquist (23) suggested that more work is needed on the effects of protected fats on the feed intake of ruminants. The type of fat (chain length and degree of saturation of the fatty acids), degree of protection, level of inclusion in the basal diet, and composition are probably aspects to consider.

Milk production tended to be lower for ewes fed the diet supplemented with Ca-OFA before weaning, because their energy and protein intakes were lower. These ewes produced 2.6% more energy in milk and had greater, but not significantly greater, BW gain during the preweaning period (Table 3). These results can be explained by a low estimation of the metabolizable energy content of the diet supplemented with Ca-OFA using the digestibility data from Experiment 1, by the higher efficiency of milk fat synthesis (29), or by both. Milk protein content after weaning was lower (5%) for ewes fed the diet supplemented with Ca-OFA ($P = 0.035$), which agrees with most results obtained with dairy cows. Wu and Huber (35) concluded that the reduction of the protein percentage in milk of dairy cows fed supplemental fats was caused by dilution because more milk was produced in the mammary gland when supplemental fats were fed, but protein production remained the same. Different researchers have avoided lower milk protein concentration when feeding fats by providing protected methionine and lysine (6, 8, 9) or soybean meal (26); their results support the conclusions of Wu and Huber (35). In our experiment, protein dilution did not appear to be the only explanation because protein production tended to be higher (4%; $P = 0.205$) in ewes fed the diet supplemented with Ca-OFA.

The reproductive performance of ewes in this study were not followed beyond 70 d after lambing, but the ovulation results were encouraging. The higher intake of energy from weaning to breeding by ewes fed the diet supplemented with Ca-OFA could have been related to ovulation. To achieve this greater energy intake without using Ca-OFA, the allowance of basal concentrate would probably need to increase, because
roughage was consumed ad libitum. An increase in the daily amount of basal concentrate would probably decrease the total daily intake because the substitution rate of basal concentrate was greater than 1. An increase in the basal concentrate under those conditions could also make the ewes prone to suffer rumen acidosis.

Changes in the pattern of milk fatty acids with Ca-OFA supplementation were probably not due to lower acetate production in rumen, because fiber digestibility was only slightly, and not significantly, less in ewes fed the diet supplemented with Ca-OFA in Experiment 1. Changes in milk fatty acids were not due to the dilution effect from inclusion of dietary long-chain fatty acids in milk fat, because fat content did not rise accordingly. The lower proportion of short-chain fatty acids was probably an effect of the inhibition of synthesis in the udder. Veen (31) reported that indications exist that trans isomers of long-chain fatty acids are responsible for inhibition. Actually, we detected trans-C18:1 among C18:1 isomers in milk fat, although they were not quantified. The total proportion of these C18:1 isomers increased drastically with Ca-OFA supplementation. A slight drop in C16:0 in milk fat was probably due to both inhibition of mammary synthesis and incorporation of dietary palmitic acid (9% in olive fatty acids). The proportion of C18:0 increased by direct incorporation of dietary stearic acid (3.8% in olive fatty acids) or dietary oleic acid hydrogenated in the rumen. Oleic acid rose less at 19 d than at 70 d after lambing, which probably reflected the lower intake of olive fatty acids at the beginning of the experiment. All of the observed changes in every fatty acid were smaller at the beginning of the experiment than after 70 d. Linoleic acid was reduced despite the 5.5% linoleic acid contained in olive fatty acids. Rumen biohydrogenation of dietary linoleic acid and less mobilization of adipose tissue should have been the cause of the reduction in the linoleic acid content in the milk of ewes fed the diet with supplemented Ca-OFA because mammary synthesis of linoleic acid does not take place. Partial biohydrogenation of dietary linoleic acid in the rumen could have been the origin of the strong increase in C18:1 isomers (13, 14, 17, 20).

CONCLUSIONS

The supplementation of Ca-OFA at 10% of the dietary DM did not significantly impair digestibility. The digestible energy value of the supplement found by difference was low, although the metabolizable energy value might have been a bit higher. The Ca-OFA supplement tended to improve milk production. Similarly, milk fat production and content were higher, although not significantly. Milk protein content was slightly, but significantly, decreased by supplementation, but milk protein production tended to be higher. The supplemental Ca-OFA decreased the proportion of every quantitatively important fatty acid in milk fat except C18:1 isomers and C18:0. Changes were clear at 7 d after the introduction of the supplement and more so at 58 d. The effects on ovulation were also clear and positive; therefore, dairy ewes may be a good model for use to study the effects of supplemental protected fat on early reproduction after parturition.

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