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# Feeding Cactus (*Opuntia stricta* [Haw.] Haw.) Cladodes as a Partial Substitute for Elephant Grass (*Pennisetum purpureum* Schum.) Induces Beneficial Changes in Milk Fatty Acid Composition of Dairy Goats Fed Full-Fat Corn Germ

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**Abstract:** The present study explored the combined effects of CC (*Opuntia stricta [Haw.] Haw.*) and full-fat corn germ (FFCG) as a source of supplementary PUFA on milk fatty acid (FA) composition of dairy goats fed elephant grass (EG). Twelve Saanen goats were used in a replicated 4 × 4 Latin square design in a 2 × 2 factorial arrangement of treatments (GC or FFCG as energy sources, and the inclusion or not of CC in the diet as a partial substitute for EG). The proportions of various milk FAs were influenced by CC, FFCG, or both. Significant interactions between CC and FFCG were noted for most *trans*-C18:1 and CLA isomers. Specifically, including CC in the FFCG-supplemented diet increased the levels of *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA in milk fat, whereas these isomers were unchanged or slightly reduced with CC in the GC diet. Similar patterns were observed for C18:2 n-6, while C16:0 increased with CC in the GC diet. Ratios of *trans*-C18:1/C18:0 and *trans*-11 C18:1/C18:0 were notably higher when CC was included in the FFCG-supplemented diet. These findings suggest that CC inhibits the last step of rumen biohydrogenation in dairy goats, enriching milk with *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA when supplementary PUFA is provided in the diet.

**Keywords:** rumen biohydrogenation; agro-industrial by-products; rumenic acid; milk fat; forage cactus; human health

## 1. Introduction

Ruminant milk fat is a natural source of health-promoting fatty acids (FAs), such as butyric acid, *cis*-9, *trans*-11 conjugated linoleic acid (CLA), odd- and branched-chain FA, *trans*-11 C18:1, and C18:3 n-3 [1]. Feeding fresh forages and lipid sources rich in polyunsaturated FA (PUFA) are effective strategies to manipulate the FA profile of milk, increasing the proportions of beneficial FA while decreasing those associated with potential adverse effects, such as C16:0 [2,3].

In a recent study from our research group, it was demonstrated that partially replacing sorghum silage with cactus (*Opuntia stricta* Haw. Haw.) cladodes in a soybean oil-supplemented diet greatly improved the FA profile with increased contents of *trans*-11 18:1, *cis*-9, *trans*-11 CLA and 18:2 n-6 in milk of cows [4]. This effect is associated with polyphenols or other components present in cactus cladodes (CCs) promotes an incomplete rumen biohydrogenation (BH) of dietary PUFA [4,5], increasing the rumen outflow of *trans*-11 C18:1 available to the mammary gland, where most of it is converted into *cis*-9, *trans*-11 CLA by the SCD-1 enzyme [6]. Furthermore, the phenolic compounds probably cause the inhibition of bacterial species responsible for PUFA lipolysis [7].

However, as reported in a subsequent study [8], the profile of *trans* C18:1 isomers appearing in milk from cows fed CC may vary depending on the type of forage associated with CC in the basal diet, with *trans*-10 C18:1 predominating over *trans*-11 C18:1 (referred to as the *trans*-10 shift) when moderate or high levels of full-fat corn germ (FFCG), a by-product rich in PUFA, are included in a basal diet containing sugarcane.

Contrary to what is commonly reported for dairy cows, goats have been shown to be less prone to the *trans*-10 shift and the associated milk fat depression induced by diet supplementation with plant oils rich in PUFA [9]. These particularities are related to lipid activity on ruminal metabolism, as well as the resistance of mammary lipogenesis to the inhibitory effects of the *trans*-10 configuration in the goats [10]. Accordingly, our research group showed [11] an increase in milk fat content of dairy goats when FFCG was included at 9.5% DM in diets containing either elephant grass as the sole forage or CC as a partial substitute for elephant grass, suggesting no significant increases in milk fat proportions of *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA. To address this question, the changes in milk FA composition of goats were examined in the present study, thereby complementing the results on production variables reported in our previous paper [11].

Additionally, the use of CC associated with a lipid source helps producers to produce milk and cheese with their own quality and characteristics beneficial to human health, making an important contribution to the local economy. We hypothesized that feeding CC as a partial substitute for elephant grass in a FFCG-supplemented diet would improve the milk FA profile of Saanen goats by increasing the proportions of *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA in milk fat, with no substantial changes in milk fat *trans*-10 C18:1 content.

## 2. Materials and Methods

The experiment was carried out at the Federal Rural University of Pernambuco (UFRPE) located in Recife, state of Pernambuco, Brazil. Animal handling practices were approved by the Ethics Committee on Animal Welfare of the UFRPE, license number #9253140220.

### *2.1. Animals, Experimental Design, and Treatments*

Twelve multiparous Saanen goats producing 2.1 $\pm$ 0.4 kg of milk/day, with an average body weight of 54 $\pm$ 5.0 kg and 45 $\pm$ 7 days in milk, were used in the study. The animals were housed in individual stalls equipped with feeders and water troughs.

The experimental design was a triple 4 $\times$ 4 Latin square with 21-day experimental periods, with the first 14 days used for adaptation to experimental diets and the last 7 days for data and sample collection. Goats were assigned to the following dietary treatments in a 2 $\times$ 2 factorial arrangement, with factors including energy source [ground corn (GC) vs. full-fat corn germ (FFCG)] and forage source [elephant grass (EG) vs. 50:50 mixture of elephant grass and cactus cladodes (EGCC)]: T1) EG+GC: elephant grass (EG) as the sole forage source (fresh cut) and ground corn as the energy source (GC); T2) EG+FFCG: EG as the sole forage source and full-fat corn germ as the energy source (FFCG); T3) EGCC+GC: cactus cladodes (CCs) as a partial substitute for EG and GC as the energy source; T4) EGCC+FFCG: CC as a partial substitute for EG and full-fat corn germ (FFCG) as the energy source. Ingredients used in the experimental diets and their chemical composition are shown in Table 1.

**Table 1.** Chemical composition (g/kg DM, unless stated otherwise) of feedstuffs used in the experimental diets.

| Item | Elephant Grass | Cactus Cladodes | Ground Corn | FFCG [1] | Corn Gluten Feed | Soybean Meal |
|---|---|---|---|---|---|---|
| Dry matter (g/kg as-fed) | 189.0 | 102.7 | 857.9 | 897.4 | 844.9 | 873.1 |
| Crude protein | 73.8 | 44.9 | 95.0 | 122.5 | 238.4 | 506.1 |
| Organic matter | 907.6 | 867.7 | 983.9 | 990.1 | 926.6 | 925.5 |
| Ash | 92.4 | 132.4 | 16.1 | 9.90 | 73.4 | 87.3 |
| Neutral detergent fiber | 702.0 | 314.0 | 136.6 | 240.0 | 379.0 | 140.0 |
| Non-fiber carbohydrates [2] | 114.2 | 496.9 | 699.6 | 148.5 | 285.9 | 258.2 |
| Total fatty acids (FAs) | 20.6 | 14.3 | 43.1 | 485.1 | 18.64 | 25.9 |
| FA (g/100 g of total FA) | | | | | | |
| C12:0 | 0.65 | 1.89 | nd [3] | nd | 0.01 | nd |
| C14:0 | 0.71 | 2.15 | 0.09 | 0.04 | 0.07 | 0.27 |
| C16:0 | 36.4 | 35.9 | 18.2 | 13.2 | 29.3 | 21.8 |
| C18:0 | 2.78 | 4.57 | 1.42 | 2.18 | 2.95 | 2.40 |
| C18:1 n-9 | 4.82 | 11.0 | 26.4 | 34.3 | 19.1 | 12.2 |
| C18:2 n-6 | 25.6 | 26.9 | 50.7 | 47.4 | 43.7 | 56.9 |
| C18:3 n-3 | 23.7 | 9.06 | 1.89 | 0.90 | 1.83 | 4.48 |

[1] Full-fat corn germ. [2] Calculated using the equation proposed by NRC [12]. [3] Not detected.

The experimental diets (Table 2) were formulated to be isonitrogenous and to meet the energy and nutrient requirements of lactating goats with average milk production of 2 kg/day with 4.0% milk fat [13]. Previously, feeding practices have already been detailed [11].

**Table 2.** Ingredient proportions and chemical composition (g/kg DM, unless stated otherwise) of experimental diets.

| Ingredient | Experimental Diets [1] | | | |
|---|---|---|---|---|
| | EG+GC | EG+FFCG | EGCC+GC | EGCC+FFCG |
| Elephant grass | 620.0 | 620.0 | 310.0 | 310.0 |
| Cactus cladodes | 0.00 | 0.00 | 310.0 | 310.0 |
| Ground corn | 95.0 | 0.00 | 95.0 | 0.00 |
| Full-fat corn germ | 0.00 | 95.0 | 0.00 | 95.0 |
| Corn gluten feed | 135.0 | 135.0 | 132.0 | 132.0 |
| Soybean meal | 130.0 | 130.0 | 130.0 | 130.0 |
| Urea | 0.00 | 0.00 | 3.00 | 3.00 |
| Salt | 5.00 | 5.00 | 5.00 | 5.00 |
| Mineral blend [2] | 15.0 | 15.0 | 15.0 | 15.0 |
| Chemical composition [3] | | | | |
| Dry matter (g/kg as-fed) | 268.7 | 269.1 | 196.2 | 196.4 |
| Organic matter | 901.6 | 902.2 | 889.4 | 890.0 |
| Crude protein | 152.7 | 155.3 | 151.5 | 154.0 |

**Table 2.** *Cont.*

| Ingredient | Experimental Diets [1] | | | |
|---|---|---|---|---|
| | **EG+GC** | **EG+FFCG** | **EGCC+GC** | **EGCC+FFCG** |
| Ether extract | 21.8 | 62.3 | 20.0 | 60.5 |
| Neutral detergent fiber | 517.6 | 527.4 | 396.2 | 406.0 |
| Non-fiber cabohydrates [4] | 209.0 | 157.1 | 327.2 | 274.9 |
| Metabolizable energy (Mcal/kg DM) | 2.3 | 2.3 | 2.3 | 2.3 |
| C18:0 | 2.6 | 2.6 | 3.1 | 3.2 |
| C18:1 n-9 | 9.7 | 10.4 | 11.5 | 12.3 |
| C18:2 n-6 | 33.9 | 33.7 | 34.3 | 33.9 |
| C18:3 n-3 | 15.7 | 15.6 | 11.2 | 11.1 |

[1] (1) EG+GC: elephant grass (EG) as the sole forage source (fresh cut) and ground corn as the energy source (GC); (2) EG+FFCG: EG as the sole forage source and full-fat corn germ as the energy source (FFCG); (3) EGCC+GC: cactus cladodes (CCs) as a partial substitute for EG and GC as the energy source; (4) EGCC+FFCG: CC as a partial substitute for EG and full-fat corn germ (FFCG) as the energy source. [2] Composition of commercial mineral blend (per kg): 205 g Ca, 60 g P, 15 mg Co, 700 mg Cu, 10 mg Cr, 700 mg Fe, 40 mg I, 1.600 mg Mn, 19 mg Se, 2.500 mg Zn, 600 mg F, 400,000 IU vitamin A, 2400 IU vitamin E, and 1000 mg monensin. [3] Estimated based on chemical composition of feed ingredients (Table 1) and their relative proportions in the diets. [4] Calculated using the equation proposed by NRC [12].

### 2.2. Sample Collection

Samples of CC, EG and concentrate feeds were collected from the 15th to the 21st day of each experimental period. At the end of the experimental periods, the samples were pooled, stored at −20 °C and subsequently analyzed for chemical composition.

Goats were manually milked twice a day at 7:00 am and 3:00 pm, and composite milk samples from morning and afternoon milking were collected on the 21st day of each experimental period, immediately stored at −20 °C and analyzed for milk FA profile.

### 2.3. Analytical Procedures

Individual feed samples were analyzed for dry matter (DM), organic matter (OM), ash, crude protein (CP), ether extract (EE), and neutral detergent fiber (NDF) as described in Galeano et al. [11]. In addition, a second set of feed samples was freeze-dried, ground through a 1 mm sieve, and analyzed for FA composition by gas chromatography (GC) as described by Gama et al. [4].

For the analysis of milk FA composition, milk samples were thawed at room temperature, and a volume of 1 mL was used for lipid extraction according to the AOAC Official Method 989.05 [14], followed by alkaline-catalyzed transesterification using sodium methoxide in methanol [15].

The fatty acid methyl esters (FAMEs) were separated and quantified using a 1.0 µL sample on a gas chromatograph (GC model 7820A, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector and fitted with a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm × 0.2 µm film thickness; Varian, Mississauga, ON, Canada). A detailed description of sample preparation procedures and GC operating conditions used in the analysis of milk FA composition can be found in Gama et al. [4].

### 2.4. Statistical Analysis

The experiment was designed as a replicated 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments (GC or FFCG as energy sources, and the inclusion or not of CC in the diet as a partial substitute for EG). Data were analyzed through the PROC MIXED procedure of SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA), using the following model:

$$\hat{Y}_{ijkl} = \mu + S_i + G_{j(i)} + P_k + ES_l + CC_m + (ES \times CC)_{lm} + E_{ijklm}.$$

where $\hat{Y}_{ijkl}$ = observation; μ = overall mean; S = fixed effect of square "i" = 1, 2, 3; G = random effect of animal "j" within a square "i" = 1, . . . 4; P = fixed effect of experimental period "k" = 1, . . . 4; ES = fixed effect of energy source "l" = 1, 2; CC = fixed effect of cactus cladodes inclusion "m" = 1; ES∗CC = effect of the interaction between ES and CC; and E = residual error. The effects of dietary inclusion of CC, FFCG, and their interaction were considered statistically significant when $p < 0.05$. $p$-values greater than 0.05 and less than or equal to 0.10 ($p \leq 0.10$) were considered to indicate a trend.

## 3. Results

Changes in milk fat proportions of individual FAs are presented in Tables 3 and 4, while effects on selected FA groups and FA ratios are shown in Table 5.

**Table 3.** Milk fatty acid profile (g/100 g of total FA—from C4:0 to *cis*-9 C17:1) of dairy goats fed elephant grass (EG) or cactus cladodes (CCs) as a partial substitute for EG, and ground corn (GC) or full-fat corn germ (FFCG) as energy sources.

| Fatty Acid | Diet [1] | | | | | *p*-Value [2] | | |
|---|---|---|---|---|---|---|---|---|
| | EG+GC | EG+FFCG | EGCC+GC | EGCC+FFCG | SEM | CC | FFCG | CC × FFCG |
| C4:0 | 2.540 | 2.730 | 2.450 | 2.730 | 0.07 | 0.48 | <0.001 | 0.40 |
| C5:0 | 0.023 | 0.021 | 0.023 | 0.242 | 0.07 | 0.33 | 0.33 | 0.33 |
| C6:0 | 2.101 | 1.690 | 2.462 | 2.210 | 0.08 | <0.001 | <0.001 | 0.25 |
| C7:0 | 0.018 | 0.016 | 0.024 | 0.024 | 0.08 | 0.31 | 0.34 | 0.33 |
| C8:0 | 2.140 | 1.401 | 2.853 | 2.180 | 0.11 | <0.001 | <0.001 | 0.75 |
| C9:0 | 0.027 | 0.020 | 0.048 | 0.036 | 0.01 | <0.001 | 0.007 | 0.43 |
| C10:0 | 6.320 | 3.460 | 9.530 | 5.930 | 0.42 | <0.001 | <0.001 | 0.35 |
| *cis*-9 C10:1 | 0.180 | 0.090 | 0.230 | 0.130 | 0.01 | 0.001 | <0.001 | 0.67 |
| C11:0 | 0.051 | 0.030 | 0.092 | 0.050 | 0.01 | <0.001 | <0.001 | 0.08 |
| C12:0 | 2.830 | 1.500 | 4.330 | 2.430 | 0.19 | <0.001 | <0.001 | 0.11 |
| *cis*-9 C12:1 | 0.046 | 0.019 | 0.080 | 0.030 | 0.01 | <0.01 | <0.001 | 0.10 |
| C13:0 | 0.079 | 0.047 | 0.110 | 0.137 | 0.02 | 0.08 | 0.96 | 0.37 |
| *iso*-14:0 | 0.130 | 0.080 | 0.120 | 0.090 | 0.01 | 0.86 | <0.001 | 0.15 |
| C14:0 | 8.340 | 4.670 | 10.850 | 7.140 | 0.44 | <0.001 | <0.001 | 0.97 |
| *cis*-9 C14:1 | 0.130 | 0.060 | 0.160 | 0.080 | 0.01 | 0.01 | <0.001 | 0.59 |
| *iso*-C15:0 | 0.290 | 0.190 | 0.310 | 0.240 | 0.01 | 0.02 | <0.001 | 0.34 |
| *anteiso*-C15:0 | 0.470 | 0.270 | 0.520 | 0.370 | 0.03 | 0.01 | <0.001 | 0.28 |
| C15:0 | 0.970 | 0.670 | 1.010 | 0.770 | 0.04 | 0.12 | <0.001 | 0.55 |
| *iso*-C16:0 | 0.230 | 0.150 | 0.220 | 0.160 | 0.02 | 0.70 | <0.001 | 0.54 |
| C16:0 | 21.920 | 20.540 | 24.290 | 20.370 | 0.37 | 0.01 | <0.001 | <0.01 |
| *trans*-9 C16:1 | 0.150 | 0.170 | 0.140 | 0.330 | 0.02 | 0.01 | <0.001 | <0.001 |
| *trans*-12 C16:1 | 0.470 | 0.460 | 0.450 | 0.420 | 0.02 | 0.07 | 0.10 | 0.43 |
| *cis*-9 C16:1 | 0.750 | 0.620 | 0.680 | 0.490 | 0.05 | 0.02 | <0.001 | 0.51 |
| *iso*-C17:0 | 0.560 | 0.470 | 0.580 | 0.490 | 0.02 | 0.22 | <0.001 | 0.97 |
| *anteiso*-17:0 | 0.580 | 0.420 | 0.570 | 0.420 | 0.02 | 0.75 | <0.001 | 0.84 |
| C17:0 | 0.800 | 0.610 | 0.760 | 0.560 | 0.03 | 0.16 | <0.001 | 0.80 |
| *cis*-9 C17:1 | 0.450 | 0.350 | 0.400 | 0.290 | 0.03 | 0.18 | 0.018 | 0.94 |

[1] (1) EG+GC: elephant grass (EG) as the sole forage source (fresh cut) and ground corn as the energy source (GC); (2) EG+FFCG: EG as the sole forage source and full-fat corn germ as the energy source (FFCG); (3) EGCC+GC: cactus cladodes (CCs) as a partial substitute for EG and GC as the energy source; (4) EGCC+FFCG: CC as a partial substitute for EG and full-fat corn germ (FFCG) as the energy source. [2] Effects of CC, FFCG, and CC × FFCG were considered statistically significant at $p < 0.05$.

**Table 4.** Milk fatty acid profile (g/100 g of total FA—from *iso*-C18:0 to C24:0) of dairy goats fed elephant grass (EG) or cactus cladodes (CCs) as a partial substitute for EG, and ground corn (GC) or full-fat corn germ (FFCG) as energy sources.

| Fatty Acid | Diet [1] | | | | | *p*-Value [2] | | |
|---|---|---|---|---|---|---|---|---|
| | EG+GC | EG+FFCG | EGCC+ GC | EGCC+ FFCG | SEM | CC | FFCG | CC × FFCG |
| *iso*-C18:0 | 0.096 | 0.067 | 0.083 | 0.059 | 0.01 | 0.19 | <0.01 | 0.76 |
| C18:0 | 8.940 | 15.380 | 6.370 | 11.820 | 0.57 | <0.001 | <0.001 | 0.30 |
| *trans*-4 C18:1 | 0.022 | 0.035 | 0.016 | 0.046 | 0.01 | 0.35 | <0.001 | <0.01 |
| *trans*-5 C18:1 | 0.018 | 0.026 | 0.015 | 0.036 | 0.01 | 0.06 | <0.001 | <0.001 |
| *trans*-6+7+8 C18:1 | 0.150 | 0.230 | 0.120 | 0.370 | 0.02 | <0.01 | <0.001 | <0.001 |
| *trans*-9 C18:1 | 0.230 | 0.310 | 0.190 | 0.410 | 0.02 | 0.03 | <0.001 | <0.001 |
| *trans*-10 C18:1 | 0.260 | 0.310 | 0.240 | 0.530 | 0.03 | <0.01 | <0.001 | 0.001 |
| *trans*-11 C18:1 | 1.370 | 1.360 | 1.290 | 3.230 | 0.21 | <0.001 | <0.001 | <0.001 |
| *trans*-12 C18:1 | 0.170 | 0.260 | 0.140 | 0.460 | 0.02 | <0.001 | <0.001 | <0.001 |
| *trans*-13+14 C18:1 | 0.290 | 0.370 | 0.230 | 0.400 | 0.05 | 0.73 | 0.05 | 0.52 |
| *cis*-9 C18:1 | 28.070 | 33.640 | 20.450 | 25.460 | 1.42 | <0.001 | <0.001 | 0.80 |
| *cis*-11 C18:1 | 0.840 | 0.800 | 0.720 | 0.660 | 0.05 | <0.01 | 0.22 | 0.86 |
| *cis*-12 C18:1 | 0.180 | 0.190 | 0.160 | 0.260 | 0.01 | 0.06 | <0.001 | <0.001 |
| *cis*-13 C18:1 | 0.065 | 0.069 | 0.053 | 0.076 | 0.01 | 0.37 | <0.001 | 0.002 |
| *trans*-16 C18:1 | 0.120 | 0.240 | 0.090 | 0.260 | 0.01 | 0.93 | <0.001 | 0.035 |
| C18:2 n-6 | 1.900 | 1.820 | 1.990 | 2.290 | 0.07 | <0.001 | 0.08 | <0.01 |
| *trans*-9, *trans*-12 C18:2 | 0.030 | 0.020 | 0.021 | 0.03 | 0.01 | 0.73 | 0.63 | 0.18 |
| *cis*-9, *trans*-12 C18:2 | 0.043 | 0.052 | 0.037 | 0.06 | 0.01 | 0.29 | <0.001 | <0.01 |
| *trans*-9, *cis*-12 C18:2 | 0.026 | 0.020 | 0.026 | 0.030 | 0.01 | 0.05 | 0.68 | 0.05 |
| *cis*-9, *trans*-11 CLA | 1.000 | 0.920 | 0.980 | 1.960 | 0.08 | <0.001 | <0.001 | <0.001 |
| *trans*-9, *cis*-11 CLA | 0.031 | 0.029 | 0.031 | 0.048 | 0.01 | <0.001 | <0.001 | <0.001 |
| *trans*-10, *cis*-12 CLA | 0.022 | 0.015 | 0.021 | 0.023 | 0.01 | 0.09 | 0.25 | 0.05 |
| C18:3 n-6 | 0.029 | 0.032 | 0.028 | 0.031 | 0.01 | 0.59 | 0.13 | 0.86 |
| C18:3 n-3 | 0.171 | 0.106 | 0.171 | 0.130 | 0.01 | 0.08 | <0.001 | 0.08 |
| C20:0 | 0.201 | 0.275 | 0.168 | 0.262 | 0.01 | 0.01 | <0.001 | 0.21 |
| *cis*-11 C20:1 | 0.036 | 0.031 | 0.034 | 0.032 | 0.01 | 0.75 | <0.01 | 0.17 |
| C20:2 n-6 | 0.016 | 0.013 | 0.017 | 0.016 | 0.01 | 0.03 | 0.09 | 0.11 |
| C20:3 n-6 | 0.060 | 0.063 | 0.054 | 0.061 | 0.01 | 0.34 | 0.27 | 0.67 |
| C20:4 n-6 | 0.180 | 0.133 | 0.191 | 0.153 | 0.01 | 0.06 | <0.001 | 0.53 |
| C20:5 n-3 | 0.021 | 0.018 | 0.019 | 0.017 | 0.01 | 0.30 | 0.20 | 0.76 |
| C21:0 | 0.041 | 0.030 | 0.041 | 0.031 | 0.01 | 0.91 | <0.001 | 0.90 |
| C22:0 | 0.020 | 0.015 | 0.021 | 0.023 | 0.01 | <0.01 | 0.17 | 0.01 |
| C22:5 n-3 | 0.058 | 0.042 | 0.057 | 0.041 | 0.01 | 0.78 | <0.001 | 0.91 |
| C23:0 | 0.029 | 0.024 | 0.022 | 0.023 | 0.01 | 0.25 | 0.48 | 0.36 |
| C24:0 | 0.024 | 0.017 | 0.027 | 0.022 | 0.01 | 0.04 | <0.01 | 0.45 |

[1] (1) EG+GC: elephant grass (EG) as the sole forage source (fresh cut) and ground corn as the energy source (GC); (2) EG+FFCG: EG as the sole forage source and full-fat corn germ as the energy source (FFCG); (3) EGCC+GC: cactus cladodes (CCs) as a partial substitute for EG and GC as the energy source; (4) EGCC+FFCG: CC as a partial substitute for EG and full-fat corn germ (FFCG) as the energy source. [2] Effects of CC, FFCG, and CC × FFCG were considered statistically significant at $p < 0.05$.

As expected, significant interactions between CC and FFCG were observed for *trans* C18:1 ($p < 0.001$) (expect *trans*-13+14 C18:1) and CLA isomers ($p < 0.001$). Increased proportion of *trans*-9, *cis*-11 CLA (0.048 g/100 g of total FA) ($p < 0.001$) was also observed in milk fat from goats fed the EGCC+FFCG diet, but *trans*-10, *cis*-12 CLA was not altered across treatments, varying from 0.015 to 0.023 g/100 g of total FA (Table 4).

Except for C4:0, which increased in goats fed FFCG ($p < 0.001$), milk fat proportions of many other de novo synthesized FA were decreased by FFCG ($p < 0.001$). There was an increase in C16:0 concentrations for EGCC+GC ($p < 0.01$; Table 3).

There was an increase in the proportion of C18:2 n-6 in milk fat ($p < 0.01$) with the inclusion of FFCG in the EGCC diet (Table 4).

FFCG reduced the proportions of OBCFA in milk fat ($p < 0.001$), whereas CC tended to increase the proportions of OBCFA ($p = 0.08$). Overall, EGCC+FFCG markedly increases the proportions of PUFA in milk fat ($p < 0.001$; Table 5).

The proportion of C18:3 n-3 in milk fat was reduced by FFCG, particularly in the EG diet, with a trend ($p = 0.08$) towards interaction between CC and FFCG (Table 4). There was an increase in the n-6/n-3 ratio when FCCG was added ($p < 0.001$; Table 5).

**Table 5.** Proportions (g/100 g of total FA) of major fatty acid (FA) groups and FA ratios in milk fat of dairy goats fed elephant grass (EG) or cactus cladodes (CCs) as a partial substitute for EG, and ground corn (GC) or full-fat corn germ (FFCG) as energy sources.

| Item [2] | Diet [1] | | | | | *p*-Value [3] | | |
|---|---|---|---|---|---|---|---|---|
| | EG+GC | EG+FFCG | EGCC+ GC | EGCC+ FFCG | SEM | CC | FFCG | CC × FFCG |
| Σ SCFA | 13.100 | 9.280 | 17.290 | 13.060 | 0.61 | <0.001 | <0.001 | 0.65 |
| Σ MCFA | 33.110 | 26.710 | 39.480 | 29.940 | 0.83 | <0.001 | <0.001 | 0.04 |
| Σ LCFA | 0.320 | 0.350 | 0.290 | 0.600 | 0.04 | 0.01 | <0.001 | 0.01 |
| Σ OBCFA | 4.420 | 3.140 | 4.540 | 3.930 | 0.19 | 0.08 | 0.001 | 0.20 |
| Σ OLCFA | 2.040 | 1.480 | 2.140 | 2.090 | 0.26 | 0.13 | 0.19 | 0.26 |
| Σ BCFA | 2.380 | 1.660 | 2.410 | 1.830 | 0.09 | 0.14 | <0.001 | 0.29 |
| Σ SFA | 59.810 | 54.820 | 67.900 | 59.070 | 1.46 | <0.001 | <0.001 | 0.06 |
| Σ MUFA | 34.030 | 39.650 | 25.930 | 34.010 | 1.42 | <0.001 | <0.001 | 0.24 |
| Σ PUFA | 3.590 | 3.290 | 3.650 | 4.890 | 0.11 | <0.001 | 0.010 | <0.001 |
| Σ n-3 | 0.250 | 0.170 | 0.250 | 0.190 | 0.01 | 0.31 | <0.001 | 0.17 |
| Σ n-6 | 2.190 | 2.060 | 2.290 | 2.550 | 0.05 | <0.001 | 0.30 | 0.01 |
| Σ *trans*-C18:1 | 3.790 | 4.220 | 3.470 | 7.910 | 0.47 | <0.001 | <0.001 | <0.001 |
| Σ *trans* FA − (VA+RA) | 1.420 | 1.940 | 1.200 | 2.710 | 0.17 | 0.05 | <0.001 | 0.001 |
| FA ratios | | | | | | | | |
| Σ *trans* C18:1/C18:0 | 0.290 | 0.210 | 0.370 | 0.490 | 0.02 | <0.001 | 0.49 | 0.001 |
| *trans*-11 C18:1/C18:0 | 0.150 | 0.090 | 0.200 | 0.280 | 0.01 | <0.001 | 0.77 | <0.01 |
| n-6:n-3 FA | 9.020 | 12.610 | 9.280 | 14.180 | 0.16 | 0.05 | <0.001 | 0.16 |

[1] (1) EG+GC: elephant grass (EG) as the sole forage source (fresh cut) and ground corn as the energy source (GC); (2) EG+FFCG: EG as the sole forage source and full-fat corn germ as the energy source (FFCG); (3) EGCC+GC: cactus cladodes (CCs) as a partial substitute for EG and GC as the energy source; (4) EGCC+FFCG: CC as a partial substitute for EG and full-fat corn germ (FFCG) as the energy source. [2] VA = *trans*-11 C18:1 (vaccenic acid); RA = *cis*-9, *trans*-11 CLA (rumenic acid); SCFA = short-chain fatty acid; MCFA = medium-chain fatty acid; LCFA = long-chain fatty acid; OBCFA = sum of odd- and branched-chain fatty acid, except 13:0, iso 17:0, and *anteiso* 17:0 as these co-eluted with *cis*-9 12:1, *trans*-9 16:1, and *cis*-9 16:1, respectively; OLCFA = odd- and long-chain fatty acid; BCFA = branched-chain fatty acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid. [3] Effects of CC, FFCG, and CC × FFCG were considered statistically significant at $p < 0.05$.

## 4. Discussion

To better understand the discussion of this paper, the diets with EGCC and FFCG showed greater intake ($p \leq 0.04$) of DM, NDF, and metabolizable energy (1843.50 g/day; 672.6 g/day and 4.2 Mcal/day, respectively), and total and fat-corrected milk yields (2.85 and 2.41 kg/day, respectively). The diets with cactus cladodes provided greater digestibility of dry matter and OM (640 and 632 g/kg, respectively). The remaining data were previously detailed in Galeano et al. [11].

The present study was designed to test the hypothesis that feeding EGCC with FFCG in the diet of dairy goats would increase the contents of *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA in goat milk, with no substantial changes in milk fat *trans*-10 C18:1.

The proportions of *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA in milk fat increased 2.5-fold and 2-fold, respectively, when FFCG was included in the EGCC diet. However, no changes were observed when FFCG was added to the EG diet. A similar response pattern was observed for the other *trans*-18:1 isomers, which increased to a greater extent (2 to 3-fold) when FFCG was included in the EGCC diet. Moreover, goats fed FFCG had higher proportions of C18:0 in milk fat than those fed GC, whereas C18:0 was reduced by EGCC. The marked increase in milk fat C18:0 with FFCG indicates that the oil present in FFCG was largely available for rumen BH.

A reduction in milk fat C18:0 content with cactus feeding was first observed in dairy cows [4], suggesting that CCs inhibit the last step of rumen biohydrogenation (BH) favoring the supply of *trans*-11 C18:1 to mammary gland, which was confirmed in subsequent studies with dairy cows fed FFCG-supplemented diets [8,16]. This idea of an incomplete rumen BH of dietary PUFA induced by CC is further supported by the interaction between CC and FFCG observed for *trans*-C18:1/C18:0 and *trans*-11 C18:1/C18:0 ratios in milk fat (Table 5). Although the polyphenols present in CC are expected to modulate the rumen microbiota [5], further research is needed to elucidate the mechanisms underlying the effects of CC on rumen BH.

Increased levels of *trans*-10 C18:1 in goat milk are normally observed when the lipid supplements rich in PUFA are added to low-fiber/high-starch diets [17–19]. In the present study, CC replaced half of the EG in the EGCC diets, increasing NFC content (Table 2), which may have contributed to increasing the milk fat *trans*-10 C18:1 content in goats fed the EGCC+FFCG diet. However, the content of *trans*-10 C18:1 found in goat milk in the present study is lower than that found in cows fed increasing levels of FFCC in diets containing CC associated with sugarcane [8] or sugarcane bagasse [16], which supports the idea that ruminal BH pathways are more stable and less prone to *trans*-10 shift in goats [17].

The proportions of these two CLA isomers (*trans*-9, *cis*-11 and *trans*-10, *cis*-12), which have been associated with MFD in cows and to a lesser extent in goats [20], were also lower than those reported by Toral et al. [17] in goats fed a high-starch/high-sunflower oil diet (0.064 and 0.053 g/100 g of total FA for *trans*-10, *cis*-12 and *trans*-9, *cis*-11 CLA, respectively). In most dietary scenarios, diet-induced increases in *trans*-10 C18:1, *trans*-10, *cis*-12 CLA, and *trans*-9, *cis*-11 CLA have little impact on milk fat synthesis in dairy goats, strengthening the idea that goat mammary glands are less sensitive to the anti-lipogenic effects of *trans*-10 *cis*-12 CLA and other MFD-related isomers.

Therefore, the FFCG (by-product rich in PUFA) has the ability to decrease de novo FA synthesis, regardless of the inclusion of cactus cladodes (CCs). These results are consistent with previous studies involving cows fed different levels of FFCG [8,16,21]. Furthermore, an interaction effect was observed for milk fat C16:0 content, which increased when CCs were included in the GC diet, but not when added to the FFCG-supplemented diet. Hence, associating supplemental PUFA with CC appears to be a promising strategy to increase the milk proportions of health-promoting FAs such as *cis*-9, *trans*-11 CLA while preventing elevated C16:0 contents usually associated with CC feeding. In addition, as can be seen in Table 3, the levels of C12:0 and C14:0 are higher when CC is used, but when combined with the FFCG, a significant reduction in the levels of these respective isomers is observed.

The independent effects of FFCG and CC on individual milk OBCFA (Table 5) suggest that both treatments affected the rumen microbial population, since OBCFAs are largely derived from bacteria leaving the rumen [22]. Overall, FFCG markedly reduced the proportions of OBCFA (notably BCFA) in milk fat, whereas CC tended to increase the proportions of OBCFA (Table 5). The toxic effects of PUFA on rumen bacteria and the accompanying changes in milk OBCFA contents have been long recognized [23], but only recently have the CC effects on milk OBCFA been reported by Gama et al. [4], which may be partially attributed to the action of phenolic compounds present in CC on rumen microbiota.

The inclusion of FFCG in the EGCC diet increased the proportion of C18:2 n-6 in milk fat (Table 4), which is in accordance with findings of [8,16]. As suggested by Gama et al. [4], this effect may be associated with the modulation of rumen BH by polyphenols present in CC. In addition, this effect can typically be observed in response to the increase in the concentration of long-chain fatty acids (especially C18:2 n-6) originating from FFCG (Table 1).

The linear reduction in the proportion of $\alpha$-linolenic acids (C18:3 n-3) in milk fat was also observed in goats fed soybean oil added to low- or high-forage diets [24], as well as in goats fed sunflower oil in diets based on grassland hay, but not when sunflower oil was included in a maize silage-based diet [18]. The reason for this effect is not clear, since the replacement of corn by FFCG in our study is expected to increase the intake of C18:3 n-3 given the much higher FA content of FFCG (Table 2), and the similar DM intake across treatments [11].

The increase in the n-6/n-3 ratio is not desirable (Table 5), since it is a parameter used to evaluate the nutritional quality of fats, oils, and diets; foods with lower n-6/n-3 values are more desirable from a human-health perspective [25].

Most interactions, mainly of PUFA, CLA and SCFA, occurred when EGCC+FFCG were included in the diet. The combination of CC with a lipid source was always associated with significant improvements in the results. This combination improves all beneficial fatty acids and has a lower adverse impact compared to the absence of CC.

## 5. Conclusions

Feeding CC as a partial substitute for EG in dairy goats by favoring the outflow of *trans*-11 C18:1 at the expense of C18:0 to the mammary gland enriches milk with *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA when supplementary PUFA is provided by FFCG. These results corroborate previous findings with dairy cows showing that the combination of CC with plant oils rich in PUFA is a promising strategy to improve the nutritional value of milk fat.

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