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Fatty acid profile of goat milk in diets supplemented with chia seed (*Salvia hispanica* L.)

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ABSTRACT

Chia seed (*Salvia hispanica* L.) is the greatest known plant source of n-3 α-linolenic acid. The present study evaluated the effects of 3 inclusion levels of chia seed [zero (control); low, 2.7% (CLow); and high, 5.5% (CHigh)] in diets of dairy goats on milk yield and fatty acid profile. Nine Saanen dairy goats in the last third of lactation period, live weight 38 ± 8.7 kg, housed in metabolic cages, were fed iso-proteic and iso-energetic (160 g of crude protein/d and 11 MJ of metabolizable energy/d) diets. Gas chromatography was used to analyze fatty acid profile and total conjugated linoleic acid (CLA). Silver ion HPLC was used to analyze the isomeric profile of CLA. The results were subjected to variance analysis using a Latin square design repeated 3 × 3. The CHigh treatment was higher for dry matter, neutral detergent fiber, and acid detergent fiber intake compared with CLow and control diets. Digestibility was not affected by the inclusion of chia seeds. The CHigh diet improved N intake with respect to the control and CLow diet. Milk yield and chemical composition were not affected by the treatment. The milk fatty acid profile of C18:0, C18:1, C18:2, and C:20 was higher for CHigh than the other treatments. The in vitro gas production (mL of gas/g of dry matter) was lower in CHigh than the control diet. In conclusion, the addition of chia seeds at the CHigh level in dairy goat diets negatively affected in vitro rumen fermentation, but increased the milk fatty acid profile of C18:0, C18:1n-9 *cis*, and C:20, monounsaturated fatty acids, and polyunsaturated fatty acids. The total CLA content increased from 0.33 to 0.73% with the supplementation of chia to the diet, as well as the isomers *cis*-9, *trans*-11, *trans*-7, *cis*-9, *trans*-11, *cis*-13, and *trans*-12, *trans*-14.
Key words: chia seed, in vitro gas production, conjugated linoleic acid, fatty acid, dairy goat

INTRODUCTION

Dairy foods have been proven to be an excellent source of beneficial metabolites, such as CLA, n-3 and n-6 fatty acids (**FA**), antioxidants, phenols, flavonoids, and bioactive peptides (Dewhurst et al., 2006; Hilario et al., 2010; Prandini et al., 2011). However, some studies have discouraged the consumption of foods of animal origin because of the potential negative health consequences of ingesting large amounts of SFA and cholesterol. Therefore, some indices have been developed to better describe the benefits and risks of foods for human consumption, calculated from the FA profile and SFA and cholesterol content (Connor et al., 1986; Ulbricht and Southgate, 1991; Chen et al., 2004).

Goat milk is of particular economic interest in certain areas of the world. The production of this type of milk can be considered an alternative for consumers who have some type of sensitivity or allergy to dairy cow products (Luna et al., 2008) as well as being used in the preparation of cheeses. One of the most important aspects of goat milk is its high content of C6:0, C8:0, and C10:0, which compose 18% of goat milk (Adlof, 2003). Medium-chain triglycerides normally reach a percentage of 36% in goat milk in comparison with 21% in cow milk, thereby reducing the synthesis of endogenous cholesterol (Haenlein, 2004).

Different FA have potential benefits for human health, such as rumenic acid (**RA**; *cis*-9,*trans*-11 C18:2), a principal isomer of CLA, with positive effects in the prevention of cancer and atherosclerosis (Aydin, 2005). The RA content may vary due to factors such as

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Table 1. Chemical composition (means ± SD) of the ingredients used in the diet (g/kg of DM, unless otherwise indicated) of dairy goats supplemented with chia seeds

Item	Chia seeds	Corn grain	Soybean meal	Barley hay	Corn silage
Ingredient					
DM (g/kg of fresh matter)	966 ± 58	932 ± 46	923 ± 48	920 ± 41	198 ± 10
OM	972 ± 41	919 ± 37	905 ± 37	879 ± 36	939 ± 38
CP	271 ± 12	995 ± 5	443 ± 22	106 ± 4	84 ± 4
Fat	291 ± 11	271 ± 10	12 ± 0.5	10 ± 0.4	17 ± 0.8
NDF	558 ± 33	46 ± 3	70 ± 4	565 ± 22	545 ± 32
ADF	285 ± 14	23 ± 1	37 ± 2	313 ± 15	322 ± 16
ME[1] (MJ/kg of DM)	15 ± 0.6	13 ± 0.6	13 ± 0.6	10 ± 0.4	11 ± 0.5
Fatty acid composition (%)					
C16:0	6.64 ± 0.29	13.02 ± 5.72	15.80 ± 0.79	35.73 ± 1.69	19.82 ± 0.82
C18:0	3.21 ± 0.14	2.56 ± 0.12	4.41 ± 0.19	5.28 ± 0.26	3.47 ± 0.16
C18:1n-9 *cis*	6.44 ± 0.34	33.92 ± 1.69	19.40 ± 0.87	39.30 ± 1.96	19.56 ± 0.93
C18:2n-6	24.49 ± 1.47	49.54 ± 2.97	52.93 ± 2.43	19.71 ± 1.08	48.80 ± 2.73
C18:3n-3	59.20 ± 2.29	0.95 ± 0.04	7.47 ± 0.41	0.00 ± 0.0	8.35 ± 0.45

[1]Calculated by Ewing (1997).

the production systems used and the supplementation of lipids to the diet with oils or oilseed, which contain PUFA, modifying the FA profile, principally those of long-chain UFA (Chilliard et al., 2007).

Chia seed (*Salvia hispanica* L.), native to Mexico and Guatemala, has a high content of α-linolenic, linoleic, oleic, and stearic FA (Alvarez et al., 2008; Azcona et al., 2008) and could thus be an alternative in the diet of goats. The technique of in vitro gas production (Menke and Steingass, 1988; Theodorou et al., 1994) can be used to estimate the kinetics of fermentation of food in ruminant feed, in calculating its rumen degradation and interaction with rumen microorganisms. The objective of the present study was to determine the intake, digestibility, production, and composition of FA of goat milk, for goat diets supplemented with chia seed (*Salvia hispanica* L.).

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

The present study was carried out at the Animal Science farm of the School of Veterinary Medicine and Animal Science of the Universidad Autonoma del Estado de México. Nine Saanen dairy goats in the last third of lactation were used, with a live weight (**LW**) of 38 ± 8.7 kg; the goats were kept in metabolic cages (1.20 × 0.80 m). Three treatments were established: a control diet with no inclusion of chia seeds, and 2 levels of inclusion of chia seeds: low (**CLow**) with 2.7% and high (**CHigh**) with 5.5.

The diets were formulated to be iso-proteic and iso-energetic (160 g of CP/d and 11 MJ of ME/d) (NRC, 2007). The chemical composition of the ingredients is shown in Table 1. The diet was based on forage (barley hay and corn silage) and concentrate (corn grain and soybean meal) supplemented with vitamins and minerals (Malta Multitec) and different inclusion levels of chia seed (Table 2). The concentrate and the proportion of feed were mixed, and the animals had free access to drinking water. Animals were fed at 0800 and 1600 h daily. The adaptation period to the diets was 15 d followed by 5 d of sampling, recording feed intake and the amount of feces, urine, and milk excreted daily. Samples of feed, feces, and urine (10%) were frozen at −20°C until analysis.

Chemical Composition of the Diets

To determine the DM content of feed, refusals and feces, samples were dried in a forced-air oven (60°C, 48 h), and subsequently ground in a Wiley mill 3 mm diameter (Arthur H. Thomas, Philadelphia, PA). Organic matter was determined by incineration (550°C for 3 h), fat, total nitrogen (AOAC International, 2005), and CP content. The NDF and ADF were determined using the ANKOM technique (Van Soest et al., 1991) with α-amylase and uncorrected for ash. Milk samples were analyzed using a MilkoScan 133B (Foss Electric, Hillerød, Denmark) to obtain the values of protein, fat, TS, and SNF. Fatty acids of the dietary components were separated by the Soxhlet method (AOAC International, 2005).

In Vitro Gas Production

The in vitro gas production technique (Theodorou et al., 1994) was used to determine the kinetics of rumen fermentation. Three rumen cannulated lactating goats were used as donors of rumen fluid (LW of 40 ± 3 kg; ±SD) and fed the control diet. Equal amounts of ru-

Table 2. Ingredients (g/kg as fed, DM) and proportions used in formulating rations for dairy goats (g/kg of DM) of the control, 2.7% chia seed supplementation (CLow), and 5.5% chia seed supplementation (CHigh) dietary groups (means ± SD)

	Dietary group		
Item[1]	Control	CLow	CHigh
Ingredient			
Corn silage	350	350	350
Chia seeds	0	27	55
Corn grain	271	254	236
Barley hay	231	231	231
Soybean meal	114	104	94
MVS	34	34	34
Chemical composition			
DM (g/kg of fresh matter)	877 ± 42	879 ± 39	880 ± 46
OM	854 ± 41	856 ± 37	859 ± 44
CP	156 ± 7	159 ± 6	161 ± 7
Fat	17 ± 1	24 ± 1	32 ± 1
NDF	232 ± 11	254 ± 10	275 ± 10
ADF	127 ± 5	138 ± 6	149 ± 6
ME (MJ/kg of DM)	11.0 ± 0.3	11.0 ± 0.3	11.0 ± 0.4
Fatty acid composition (%)			
C16:0	20.5 ± 1.2	20.6 ± 1.0	20.1 ± 1.0
C18:0	3.6 ± 0.15	3.6 ± 0.16	3.6 ± 0.15
C18:1n-9 *cis*	27.3 ± 1.13	26.7 ± 1.23	26.1 ± 1.12
C18:2n-6 (LNA)	41.1 ± 2.13	40.4 ± 2.10	39.6 ± 2.05
C18:3n-3 (ALA)	4.0 ± 0.17	5.5 ± 0.23	7.1 ± 0.32

[1]LNA = linoleic acid; ALA = α-linolenic acid; MVS = mineral-vitamin supplement: Malta Multitec (1.0 kg of DM; MaltaCleyton, Texcoco, Estado de México) containing 25 mg of antioxidant, 4.5 g of calcium carbonate, 6 g of salt, 30 g of ionophore, 50 g of zinc oxide, 6 g of sodium bicarbonate, 6 g of copper sulfate, 20 g of ferrous sulfate, 125 g of sodium sulfate, and 18,000 IU of vitamin E, 3,000,000 IU of vitamin A, 3,750,000 IU of vitamin D, 140 g of potassium chloride, 0.500 g of ethylene diamine, 0.090 g of cobalt carbonate, 500 mg of magnesium oxide, 36 g of manganese oxide, and 0.090 g of selenium.

men fluid were collected and filtered through 4 layers of cheesecloth. The buffer solution was prepared according to the technique of Menke and Steingass (1988), in which 0.800 g of DM of each ingredient and each diet mixture were incubated in glass flasks of 125 mL, to which 90 mL of buffer solution and 10 mL of rumen fluid were added, to make 3 bottles per sample. Two additional bottles without substrate were also prepared as blanks to adjust for the potential contribution of other solubles in the extracts on overall gas production and to correct readings of substrate, including bottles from self-fermentation of rumen inocula. The bottles were incubated in a water bath at 39°C. The gas volume was recorded at 3, 6, 9, 12, 24, 36, 48, 72, and 96 h of incubation in 3 series of incubation. Gas production was registered by means of a pressure transducer (Delta Model HD 8804, Caselle di Selvazzano, Italy). After incubation, the samples were filtered and dried (48 h, 65°C) to measure the proportion of dry matter disappearance (**DMd**). Gas production at 96 h was correlated with DMd to calculate relative gas production (**RGP**, mL gas/g of DMd).

The kinetic parameters of gas production (GP) were estimated through an iterative procedure of nonlinear regression analysis (PROC NLIN, 2002, version 8.2, SAS Institute Inc., Cary, NC) according to Krishnamoorthy et al. (1991), calculated as

$$GP = B(1 - e^{-C(t - l)}),$$

where GP is the volume of gas (mL of gas/g of DM) at time t, B is the asymptotic GP (mL/g of DM), C is the fractional rate of GP (g/h), and l (h) is the discrete lag time before gas production.

Fatty Acids in Milk

Milk fat was extracted using the procedure described by Frank et al. (1975); 250 mL of milk sample was placed in a volumetric flask of 500 mL, and 250 mL of detergent solution [50 g of sodium hexametaphosphate and 24 mL of Triton X-100 (Hycel, México) dissolved in 1 L of water] was added. The flask was agitated vigorously and placed in a water bath at 90°C. The flask was inverted every 15 min until a clear suspension of the fatty material was obtained in the neck of the flask. The extracted fat was filtered at 50°C through number 4 Whatman paper in the presence of anhydrous sodium sulfate, and conserved in glass tubes at −20°C until analysis.

Chromatographic Analysis for Fatty Acids

The preparation of the FAME was carried out according to the procedure ISO-IDF (2002). These were analyzed in a gas chromatograph with flame ionization detector, Shimadzu GC-2010 Plus (Shimadzu, Kyoto, Japan), with auto-injector, Split (1:100). A capillary column CP-Sil 88 Supelco (SPTM^{2560}, Fused Silica, Cat. No. 24056; Supelco, Bellefonte, PA) was used with 100 m × 0.25 mm (di) × 0.20 μm film thickness, using a temperature gradient program. Nitrogen was used as gas carrier; the temperatures of the injector and detector were 250 and 270°C, respectively. The initial temperature of the oven was 140°C, which was maintained for 5 min. The temperature was then increased by 5°C/min to 195°C and maintained for 1 min. It was increased by 6°C/min until reaching 220°C and was maintained at that temperature for 20 min; then it was increased by 5°C/min until reaching 249°C and was maintained at that temperature for 5 min. The run time was 50.17 min.

Butterfat was used (reference material CRM 164; European Community Bureau of Reference, Brussels, Belgium) to determine the response factor of the FA individually. A standard of 37 components was also used for the identification of the FA (37 Component FAME Mix analytical standard, Supelco No. Cat. 47885-U. 33; Luna et al., 2008; Chilliard et al., 2009).

Isomers of the CLA were analyzed in a HPLC, Hitachi model Elite Chrom, UV/Vis detector (Hitachi High Technologies Corporation, Japan), at a wavelength of 233 nm, using the software EZChrom Elite-Enterprise (Agilent Technologies, Santa Clara, CA) for the integration of the chromatograms. The column used was stainless steel with 4.6 mm internal diameter × 250 mm in length and 5 μm particle size (ChromoSpher 5 lipid column, Varian Chrompack, Varian, Lake Forest, CA). The mobile phase for separation of isomers of the CLA was acetonitrile 0.1% in hexane, operated isocratically at a flow rate of 1 mL/min. The injection volume was 30 μL. For the identification of the different isomers, a mixture of the methyl esters of the CLA (*cis*-9,*trans*-11; *trans*-9,*cis*-11; *trans*-10,*cis*-12; *cis*-10,*cis*-12; *trans*-9,*trans*-11 and small amounts of other isomers *cis* and *trans* C18:2; linoleic acid, conjugated methyl ester Sigma catalog no. 05632–250 mg, Sigma, St. Louis, MO) was used, and injected with reference material to compare the order of elution of the isomers with that from the existing literature (Adlof, 2003). The amounts of the other isomers of the CLA were calculated from the areas relating to the area of the principal isomer, *cis*-9,*trans*-11 (Luna et al., 2008). Results were expressed in milligrams per gram of fat.

Calculations and Experimental Statistical Design. Fat-corrected milk was calculated at 3.5%, FCM (kg/d) = [milk (kg/d) × 0.432] + [fat (kg/d) × 16.216], and ECM was calculated as ECM = [milk (kg/d) × 0.327] + [fat (kg/d) × 12.86] + [protein (kg/d) × 7.65] (Dairy Records Management Systems, 2006). The feed efficiency (FE) was calculated using the following formula: FE = milk yield (kg/d)/DMI (kg/d); adjusted FE = 3.5% FCM (kg)/DMI (kg).

The in vivo experimental data were subjected to ANOVA using the general linear model (GLM) procedure (SAS 8.2, 2002, SAS Institute Inc.) in a Latin square design repeated 3 × 3.

$$Y_{ijk} = \mu + P_i + A_j + T_{(k)} + E_{ijk},$$

where Y_{ijk} = response variable in period i, animal j, and treatment k; μ = overall mean; P_i = effect of period i; A_j = effect of the animal; $T_{(k)}$ = effect of treatment; and E_{ijk} = random error.

The in vitro GP data were subjected to ANOVA using the GLM (PROC GLM, 2002, SAS Institute Inc.). The Tukey test ($P < 0.05$) was used to interpret any significant difference between the mean values.

RESULTS AND DISCUSSION

Chemical Composition of the Diets

No differences were observed ($P > 0.05$) among the weights of the animals (15.2 ± 0.48 kg of LW$^{0.75}$); with respect to the feed intake (g/kg of LW$^{0.75}$), CHigh exhibited a higher ($P < 0.05$) intake of DM (168.5), OM (155.4), NDF (51.7), and ADF (32.5) compared with CLow and the control diet (DM, 145 ± 13; OM, 134 ± 12; NDF 43 ± 5; ADF 27 ± 0.3 g/kg of LW$^{0.75}$, respectively; Supplemental Table S1, https://doi.org/10.3168/jds.2017-12785). Bernard (1990) and Economides (1998) included sunflower oil or flaxseed, respectively, in diets for goats and found no differences ($P > 0.05$) in DMI among treatments, in contrast to the present study, where it is shown that DMI for CHigh was higher compared with the other treatments.

Digestibility

No significant differences among treatments were observed ($P > 0.05$) for DM (704 ± 21 g/kg), OM (763 ± 7 g/kg), NDF (605 ± 12 g/kg), and ADF (513 ± 23 g/kg) digestibility (Supplemental Table S2, https://doi.org/10.3168/jds.2017-12785). Silva et al. (2007) observed no significant differences ($P > 0.05$) in NDF digestibility at different inclusion levels of soybean oil

Table 3. In vitro gas production of the ingredients used in diets[1]

Item	b	c	Lag time	DMd_{96h}	RGP
Ingredient					
Barley hay	156[c]	0.04[c]	5.86[a]	72.3[bc]	214[b]
Chia seed	75[d]	0.05[a]	3.18[b]	38.5[d]	243[b]
Corn grain	250[a]	0.05[ab]	3.17[b]	86.3[ab]	304[b]
Soybean meal	213[b]	0.05[a]	2.41[b]	89.3[a]	245[b]
Corn silage	264[a]	0.04[bc]	2.52[b]	62.7[c]	438[a]
SEM	6.37	0.002	0.300	3.76	27.6
P-value	0.001	0.001	0.001	0.001	0.001

[a–d]Different superscript letters in the same column indicate significant differences between values.

[1]b = total gas production (mL/g of DM incubated); c = fermentation time (h-1/2); lag time = lag; DMd_{96h} = proportion of missing DM (%); RGP = relative gas production (mL of gas/g of DM disappearance).

in the diets of dairy goats. The inclusion of soybean oil in the diet reduced total NDF digestibility by 10%, with an observed decrease in DMI over the control diet. Karalazos et al. (1992) observed that NDF digestibility increased in diets containing 17.0, 35.5, and 53.0% of cottonseed inclusion when compared with the control treatment.

Nitrogen Balance

Nitrogen intake was higher ($P < 0.001$) for CHigh (38.3 g N/d) compared with CLow and control diets (32.7 ± 3.3; Supplemental Table S3, https://doi.org/10.3168/jds.2017-12785). The N excretion in feces and urine was similar ($P > 0.05$) among treatments (16.6 ± 2.5 and 8.8 ± 0.96 g of N/d, respectively), being positive in all cases.

In Vitro Gas Production

The parameters of the in vitro gas production of the ingredients used in the diets are presented in Table 3. Differences in gas production for each ingredient ($P < 0.001$) were observed, being higher ($P < 0.001$) in corn silage and corn grain, and lower in chia seed. The fractional rate of degradation (c) was higher ($P < 0.001$) for soybean and corn grain, followed by barley hay and corn silage. The DMd at 96 h was higher ($P < 0.001$) for soybean and corn grain, followed by barley hay and corn silage, with DMd being lower for chia seed. The RGP was higher ($P < 0.05$) for corn grain than for the other ingredients. Gas production is an indirect measure of the degradation of substrates, particularly from carbohydrates. Furthermore, it is a good estimator for the production of short-chain FA (Blümmel et al., 1999; Liu et al., 2002).

The parameters of the in vitro gas production of the diets were different among treatments ($P < 0.002$; Table 4). Increased gas production and the c fraction were higher for the control treatment than CHigh, where the inclusion of chia seeds affected the fermentation rate. Higher levels of dietary fat (>6% fat as a percentage of DM) have negative effects on rumen fermentation, which are associated with the inhibition of microbial activity, particularly microorganisms with cellulolytic and methanogenic activity (Johnson and McClure, 1973; Devendra and Lewis, 1974). The DMd at 96 h was higher ($P < 0.0221$) for CLow with respect to CHigh, and the RGP was higher for CHigh ($P < 0.05$) followed by control and CLow. Silva et al. (2016) compared flaxseed and chia seed in in vitro cultures, both of which increased the flows of C18:3n-3, C20:4n-6, and total PUFA ($P < 0.01$). Both chia seed and flaxseed treatments had higher rumen concentrations of C18:0,

Table 4. In vitro gas production of the diets with different inclusion levels of chia seed[1]

Item	b	c	Lag time	DMd_{96h}	RGP
Dietary group[2]					
Control	239[a]	0.05[a]	3.35[a]	73[ab]	343
CLow	219[ab]	0.05[b]	2.63[b]	80[a]	282
CHigh	199[b]	0.04[c]	3.41[a]	62[b]	347
SEM	6.89	0.0012	0.201	4.29	27.47
P-value	0.002	0.001	0.020	0.022	0.197

[a–c]Different superscript letters in the same column indicate significant differences between values.

[1]b = total gas production (mL/g of DM incubated); c = fermentation time (h-1/2); DMd_{96h} = proportion of DM disappearance (mg/100 mg) at 96 h; RGP = relative gas production (mL of gas/g of DM disappearance).

[2]CLow = 2.7% chia seed supplementation; CHigh = 5.5% chia seed supplementation.

indicating that both chia seed and flaxseed FA were extensively biohydrogenated in the rumen.

Production and Chemical Composition of Milk

When the different levels of chia seed were included in the diet, no differences ($P > 0.05$) were observed among treatments in milk production (0.715 ± 0.015 kg/d) and FCM 3.5% (0.646 ± 0.018 kg/d; Supplemental Table S4, https://doi.org/10.3168/jds.2017-12785). Mir et al. (2000) fed dairy goats with 4 inclusion levels of canola oil without observing any effect on milk production. Similarly, Chilliard et al. (2003) and Lock et al. (2008) reported that the supplementation of fats in diets of dairy goats had no effect on milk production. Fernández (1994) observed no changes in milk production after utilizing 2 levels of soybean oil in lactating cows. In contrast, Lu (1993) found a decrease in milk production with the supplementation of animal fat (5%) in lactating goats; FE was similar ($P > 0.05$) among treatments (0.276 ± 0.16), with respect to the protein content in milk (3.5 ± 0.23 g/100 g), no differences were observed among treatments ($P > 0.05$). The addition of fat leads to a reduction of rumen fermentable OM, the reduction of the precursors of glucose and the synthesis of microbial protein, and thus later the "pool" of AA available for the synthesis of proteins in the milk. This explains why a lower amount of protein in the milk is observed with respect to the fat content. Some studies have reported a reduction of protein in milk with diets supplemented with fat (Christensen et al., 1994), which depends on the amount supplied. Bartocci et al. (1988) reported that in dairy goats, with an inclusion of cottonseed of 18%, they found a higher percentage of milk fat without affecting the concentration of protein. However, in the present study the fat content was lower than expected (2.9 ± 0.09 g/100 g), which was possibly due to the fact that the animals were in the final third of lactation. The results observed by other authors have shown that the increase in the concentration of fat in the diet does not alter the production of milk fat (Grant and Weidner, 1992; Lu, 1993; Pires et al., 1996). For lactose milk content, no differences were observed ($P > 0.05$) among the treatments; Luna et al. (2008) reported differences in goat milk when the diets were supplemented with whole linseed (1.84%) and sunflower oil (0.81%) compared with the control diet. Ayeb et al. (2016) found no differences in the lactose content of goat milk with the inclusion of dry olive leaves in the diet. The TS in the diet were similar ($P > 0.05$) among treatments (12 ± 0.12 g/100); Ayeb et al. (2016) observed an increase of the TS when dry olive leaves were included in the diet.

Composition of Fatty Acids in Milk

The inclusion of chia seeds at 2 levels in the diets of the animals resulted in significant changes ($P < 0.05$) in the profile of FA in the goat milk (Table 5). A decrease ($P < 0.05$) of 3.4% was observed in the SFA with the inclusion of chia seed (CHigh) with respect to the control diet, mainly due to the decrease in the contents of medium-chain FA (C10:0, C12:0, C14:0, and C16:0) in the milk. Ollier et al. (2009) also observed a reduction of these FA with the inclusion of whole rapeseed at 14.6% to a diet high in forage, and Bernard et al. (2005) with the inclusion of protected linseed and sunflower oil. This reduction of the medium-chain FA may be due to the fact that they are principally synthesized de novo in the mammary gland and the *trans* FA produced in the rumen may inhibit the synthesis of FA (Chilliard et al., 2001; Bernard et al., 2005). The above is of interest for human health, given that the FA C14:0 and C16:0 raise the level of cholesterol and are associated with an increase in the incidence of atherosclerosis and coronary diseases (Williams, 2000).

The concentrations of short-chain FA (4:0, 6:0, and 8:0) remained unchanged in the goat milk ($P > 0.05$). Similar results were found in goat milk by Luna et al. (2008) with diets supplemented with whole linseed and sunflower oil, and by Chilliard et al. (2003) for diets supplemented with the oil and seeds of linseed, sunflower oil, lupine, and soybean.

The content of C18:0 increased ($P < 0.05$) in goat milk from diets with a higher content of chia seed, due to the complete biohydrogenation of the linoleic and linolenic acids in the rumen to C18:0, given that the release of the lipids in the seeds is slower than in the form of oil (Chilliard et al., 2003; Ollier et al., 2009). Similarly, the content of the MUFA increased, which is principally related to the FA C18:1n-9 *cis*, coming partially from the biohydrogenation in the rumen of the FA such as linoleic acid (Sanz-Sampelayo et al., 2007) and in the mammary gland from the action of the enzyme desaturase Δ^9 on stearic acid C18:0 produced in the rumen (Bauman and Griinari, 2003).

Linoleic acid presented the highest concentration of PUFA, although no significant differences ($P > 0.05$) were observed among treatments. This may be due to the biohydrogenation of these FA in the rumen (Chilliard et al., 2007), given that the 18:3n-3 and the 18:2n-6 disappear in the rumen at an average of 93 and 85%, respectively (Bernard et al., 2009). The PUFA content increased ($P < 0.05$) in the diets with chia seed with respect to the control diet.

The inclusion of chia seed increased ($P < 0.05$) the CLA content compared with the control diet. When

the diets are supplemented with oil seeds or vegetable oils, these FA can be produced in the rumen through the action of anaerobic bacteria by the isomerization of the linoleic acid to form *cis*-9,*trans*-11 C18, or RA and other isomers such as *trans*-9,*cis*-11 C18:2; *trans*-10,*cis*-12 C18:2, and through biohydrogenation from the *trans*-vaccenic fatty acid (**TVA**) *trans*-11, C18:1 (Chilliard and Ferlay, 2004; Chilliard et al., 2007), all of which pass to the blood stream and are absorbed in the mammary gland. The TVA is reduced by the Δ^9 desaturase enzymes and forms the FA *cis*-9,*trans*-11 C18:2, among other isomers. It has been estimated that between 64 and 97% of the RA present in milk comes from the endogenous synthesis of the TVA in the mammary gland. These enzymes can also act on myristic, palmitic, and stearic acids (Chilliard et al., 2007; Manso et al., 2016). These results correspond to the desaturation index (C14:1/C14), an estimator of the activity of the enzyme Δ^9 desaturase (Renna et al., 2012), which increased ($P < 0.05$) with the inclusion of the chia seed in the present study, which indicates that the activity of the stearol-CoA desaturase (**SCD**) in the mammary gland did not decrease. This is in contrast to the results of Luna et al. (2008), who observed a decrease in the activity of the enzyme with the inclusion of whole linseed and sunflower oil in the diets. Bernard et al. (2005) supplemented diets with protected linseed and sunflower seed oils, and observed a decrease in the activity of the enzyme SCD, suggesting a negative regulation of SCD by a diet containing PUFA and long-chain FA and by the products of the biohydrogenation in the rumen, such as *trans* C18:1 or *trans* C18:2. This difference may have been due to the fact that the release of the lipids in the chia seeds is slower than in the form of oil, and therefore a complete biohydrogenation of the PUFA occurs. The results of ratios compared with those obtained by Chilliard et al. (2009) in cow milk with diets that included raw and extruded linseed and linseed oil showed that the ratios of C14:1 *cis*-9/C14:0 + C14:1 *cis*-9 and C16:1 *cis*-9/

Table 5. Effect of addition to the diet of chia seeds on the fatty acid profile (g/100 g of FAME) of goat milk

Fatty acid[1]	Dietary group[2]			SEM	*P*-value
	Control	CLow	CHigh		
C4:0	2.53 ± 0.17	2.6 ± 0.29	2.65 ± 0.44	0.0819	0.618
C6:0	2.79 ± 0.24	2.75 ± 0.35	2.89 ± 0.31	0.077	0.457
C8:0	3.38 ± 0.30	3.21 ± 0.46	3.34 ± 0.30	0.0932	0.395
C10:0	13.81 ± 0.93^{a}	12.89 ± 1.13^{b}	12.97 ± 0.85^{ab}	0.269	0.024
C11:0	0.55 ± 0.13	0.58 ± 0.25	0.46 ± 0.08	0.044	0.151
C12:0	7.31 ± 1.55	6.87 ± 1.0	6.42 ± 1.5	0.342	0.190
C14:0	14.48 ± 0.97^{a}	13.51 ± 1.88^{ab}	12.91 ± 1.16^{b}	0.326	0.001
C14:1	0.34 ± 0.04	0.33 ± 0.10	0.34 ± 0.04	0.016	0.891
C15:0	1.19 ± 0.27^{ab}	1.31 ± 0.40^{b}	1.03 ± 0.18^{a}	0.081	0.047
C15:1	0.22 ± 0.02	0.22 ± 0.10	0.24 ± 0.04	0.015	0.511
C16:0	30.08 ± 2.10^{a}	30.7 ± 2.43^{a}	27.61 ± 1.45^{b}	0.664	0.001
C16:1	0.17 ± 0.03	0.14 ± 0.05	0.14 ± 0.02	0.009	0.089
C17:0	0.37 ± 0.07^{ab}	0.42 ± 0.15^{b}	0.30 ± 0.03^{a}	0.027	0.005
C17:1	0.56 ± 0.10^{ab}	0.62 ± 0.19^{b}	0.46 ± 0.06^{a}	0.037	0.008
C18:0	4.72 ± 1.49^{a}	5.36 ± 2.10^{ab}	6.9 ± 1.14^{b}	0.545	0.008
C18:1 *trans* total	0.77 ± 0.31^{a}	0.94 ± 0.21^{ab}	1.04 ± 0.16^{b}	0.073	0.027
C18:1n-9 *cis*	14.34 ± 0.97^{a}	15.4 ± 1.46^{a}	17.45 ± 1.07^{b}	0.499	<0.001
C20:0	0.14 ± 0.02^{a}	0.15 ± 0.08^{a}	0.36 ± 0.06^{b}	0.032	<0.001
C18:2n-6 *cis* (LNA)	1.31 ± 0.23	1.31 ± 0.21	1.34 ± 0.23	0.056	0.459
C18:3n-3 (ALA)	0.33 ± 0.06	0.29 ± 0.02	0.32 ± 0.05	0.025	0.385
C18:2 *cis*-9,*trans*-11 (CLA)	0.33 ± 0.04^{a}	0.52 ± 0.02^{b}	0.73 ± 0.05^{c}	0.048	<0.001
ΣSFA	81.34 ± 1.68^{a}	80.36 ± 4.09^{a}	77.84 ± 1.49^{b}	0.609	<0.001
ΣMUFA	16.58 ± 1.10^{a}	17.47 ± 1.78^{a}	19.68 ± 1.05^{b}	0.542	<0.001
ΣPUFA	1.97 ± 0.15^{a}	2.12 ± 0.27^{a}	2.40 ± 0.14^{b}	0.079	0.005
ATI	5.13 ± 0.45^{a}	4.82 ± 1.14^{a}	3.89 ± 0.36^{b}	0.26	0.001
C14:1 *cis*-9/(C14:0 + C14:1 *cis*-9)	0.023 ± 0.003	0.025 ± 0.009	0.026 ± 0.004	0.002	>0.05
C16:1 *cis*-9/(C16:0 + C16:1 *cis*-9)	0.006 ± 0.001	0.005 ± 0.002	0.0052 ± 0.001	0.0005	>0.05
C18:1 *cis*-9/(C18:0 + C18:1 *cis*-9)	0.76 ± 0.055	0.76 ± 0.085	0.72 ± 0.025	0.022	>0.05
CLA *cis*-9,*trans*-11/(C18:1 *trans*-11 + CLA *cis*-9,*trans*-11)	0.35 ± 0.07^{a}	0.56 ± 0.16^{b}	0.50 ± 0.08^{b}	0.004	<0.001

[a–c]Different letters in the same row indicate significant differences between values.

[1]LNA = linoleic acid; ALA = α-linolenic acid; ATI = atherogenic index, (C12 + 4C14 + C16)/(PUFA + MUFA)(C12 + 4C14 + C16)/(PUFA + MUFA) (Ulbricht and Southgate, 1991).

[2]CLow 2.7% chia seed supplementation; CHigh = 5.5% chia seed supplementation.

Table 6. Conjugated linoleic acid isomers (mg/g of CLA) as determined by silver ion HPLC

	Dietary group[1]				
Item	Control	CLow	CHigh	SEM	*P*-value
Isomer					
trans-12,*trans*-14	0.035[a]	0.0137[b]	0.067[c]	0.0067	<0.001
trans-11,*trans*-13	0.004[a]	0.0219[a]	0.068[b]	0.0092	<0.001
trans-10,*trans*-12	0.020[a]	0.029[a]	0.054[b]	0.0082	0.007
trans-9,*trans*-11	0.015[a]	0.020[a]	0.055[b]	0.0078	<0.001
trans-8,*trans*-10	0.020[a]	0.041[b]	0.029[ab]	0.0044	0.002
trans-7,*trans*-9	0.030[a]	0.020[a]	0.064[b]	0.0089	0.001
Σ *trans*,*trans*	0.124[a]	0.146[a]	0.339[b]	0.0296	<0.001
trans-11,*cis*-13	0.018[a]	0.044[a]	0.171[b]	0.0289	<0.001
10,12 (*cis*/*trans*)[2]	0.003[a]	0.016[a]	0.062[b]	0.0105	<0.001
9,11 (*cis*/*trans*)[2]	3.049[a]	4.694[b]	6.461[c]	0.4413	<0.001
7,9 (*cis*/*trans*)[2]	0.165[a]	0.428[b]	0.358[ab]	0.0667	0.013
Σ *cis*/*trans*	3.238[a]	5.184[b]	7.053[c]	0.4677	<0.001

[a–c]Different superscript letters in the same row indicate significant differences between values.

[1]CLow 2.7% chia seed supplementation; CHigh = 5.5% chia seed supplementation.

[2]*cis*/*trans* = *cis*-*trans* + *trans*-*cis*.

C16:0 + C16:1 *cis*-9 were lower and similar to the ratio of C18:1 *cis*-9/C18:0 + C18:1 *cis*-9, CLA/C18:1 *trans* + CLA.

It is observed that the atherogenicity index decreases with the inclusion of chia by as much as 25%. Renna et al. (2012), varying the proportion of forage in goat diets, found a decrease of 40% in the atherogenicity index with the inclusion of sunflower seed in the diet. In Chilliard et al. (2003), goats fed a low-forage diet supplemented with linseed and sunflower seeds had the atherogenicity index decreased 45 and 50%, respectively.

Isomers of CLA

The distribution of the content of the isomers of CLA is shown in Table 6. The most abundant isomer is *cis*-9,*trans*-11 C18:2 (87 to 90% of the total CLA), from the rumen and the endogenous synthesis of the *trans*-11 C18:1, by action of the enzyme Δ^9 desaturase in the mammary gland.

The isomer *trans*-7,*cis*-9 C18:2 represents, after RA, the second most representative CLA isomer in milk fat (Secchiari et al., 2003) and is synthesized by the action of the enzyme Δ^9 desaturase in the mammary gland on *trans*-7 C18:1 (Rego et al., 2009). It has been found that this isomer is increased when the diets of goats are supplemented with high-oleic sunflower oil (Ferlay et al., 2003). In our case, with the inclusion of chia seeds, values between 5 and 8% of the total of CLA were reached. Rego et al. (2009) found in the fat of cow milk percentages of 8.4% of this isomer with the inclusion of rapeseed oil in the diet and 3% for diets supplemented with oil of sunflower and linseed. The content of the isomer *trans*-10,*cis*-12 C18:2 in the milk increased with the inclusion of chia seed. This isomer is formed in the rumen through the action of anaerobic bacteria; the enzymes present carry out the isomerization of the linoleic acid found in the seeds (Griinari and Bauman, 1999). This FA has been associated with a higher risk of coronary diseases and also with negative effects on the productive yields of milk in the animals, associated with the syndrome of milk fat depression (Manso et al., 2016). In our results, the content of this FA was lower than 1% of the total of the CLA isomers. These results agree with Chilliard et al. (2007), Lock et al. (2008), and Luna et al. (2008). It has been found that when there is an increase in the diets in the amount of concentrate, which includes easily degraded carbohydrates and seeds rich in PUFA, the bacterial population of the rumen increases, which produces significant amounts of these 2 FA, the isomer *trans*-7,*cis*-9, and *trans*-10,*cis*-12 C18:2 (Piperova et al., 2000).

The isomer *trans*-11,*cis*-13 also increased by as much as 2.3% with the inclusion of chia seed (CHigh) in the diet. Bernard et al. (2009) found that the incorporation of seeds or seed oil containing linolenic acid increases this isomer.

The isomer *trans*-9,*trans*-11 C18:2 inhibits the enzyme Δ^9 desaturase in bovine (Perfield et al., 2007). The amount of this isomer increased in the diets that contained chia seed, although the values were lower than those found by Luna et al. (2008) and Bernard et al. (2009) with the incorporation of sunflower or linseed oils in goat diets. The sum of these *trans*-*trans* isomers was less than 5% of the total CLA. Similar values were found by Luna et al. (2008); most of these isomers increased with the inclusion of chia (CHigh), with *trans*-12,*trans*-14 and *trans*-11,*trans*-13 showing the highest increase, due to the presence of linolenic

acid in the chia seed incorporated in the diet (Collomb et al., 2004; Sanz-Sampelayo et al., 2007).

CONCLUSIONS

Adding chia seed to diets for dairy goats negatively affected in vitro rumen fermentation compared with the control diet. The inclusion of 5.5% of chia seed diminished the C14:0 and C16:0 FA in milk and improved the C18:1, C18:2, and C20:0 content in milk fat, increasing the PUFA concentration up to 20% and all the isomers of CLA, being healthier for the human consumption, without affecting milk yield production and chemical composition.

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