

Fatty acid profile of forage shrubs in northern Mexico

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Abstract

Conjugated linoleic acid is considered a functional food since its consumption improves people's health. This compound is found in the milk of ruminant animals and is synthesized in the rumen and mammary gland using linoleic and α -linolenic acids as substrates, which are widely found in forage plants. Nevertheless, there is little information regarding the content of fatty acids in forages, particularly of native species. Therefore, the study aimed to evaluate the chemical composition and fatty acid profile of native forage shrubs that are consumed by goats under extensive grazing in northern Mexico in 2019. For this, forage shrubs consumed by goats were collected. The selected forages were determined: dry matter, organic matter, crude protein, neutral detergent fiber, acid detergent fiber, total lipids, and fatty acid profile. The forage shrubs consumed by the goats in the rangeland were huizache [*Vachellia farnesiana* (L.) Wild.], mesquite [*Prosopis laevigata* (Humb & Bonpl) Wild], gobernadora [*Larrea tridentata* (Moç. & Seseé DC)] and chaparro prieto [*Vachellia constricta* (Bentham Siegler & Ebinger Waif)]. Of these, mesquite (CP 148 g kg⁻¹ DM) and chaparro prieto (CP 147 g kg⁻¹ DM) had the best crude protein contents; likewise, mesquite had a high concentration in linoleic (14.21 g 100 g⁻¹ total fatty acids) and α -linolenic (36.22 g 100 g⁻¹ total fatty acids) fatty acids, so these forage shrubs can be considered in the diet of goats that aim to increase the concentration of conjugated linoleic acid in milk or meat of grazing goats.

Keywords:

conjugated linoleic acid, functional food, ruminant nutrition.

Introduction

The human population demands food of animal origin for its diet, and in recent years, a part of it has been concerned with acquiring foods that contribute to improving their health and preventing diseases (Verma and Srivastav, 2020). Therefore, the standards of nutritional quality and safety of food products in international markets have been increased (Godfray *et al.*, 2010).

In livestock production, to support the aforementioned trend, interest in modifying the lipid profile of the products generated has arisen. In particular, in milk, it is sought to increase the concentration of conjugated linoleic acid (CLA) (Siurana and Calsamiglia, 2016; Kim *et al.*, 2016) because two of its isomers have been of interest for their potential effects on human health, such as reduction of body fat, antiatherogenic, hypolipidemic, antidiabetogenic and immunomodulatory properties, among others (Haro *et al.*, 2006; Gómez-Cortés *et al.*, 2019). These isomers are cis-9, trans-11 and trans-10, cis-12, which together represent about 95% of the total CLA in ruminant milk, with cis-9, trans-11 being the ones present in greater proportion (90%) (Grinari *et al.*, 2000).

Ruminant milk is the food in nature with the highest amount of CLA (Ritzenthaler *et al.*, 2001), synthesized through the incomplete biohydrogenation of linoleic (C18:2 *n*-6) and α -linolenic (C18:3 *n*-3) fatty acids (FAs) in the rumen. However, only about 10% of the CLA in milk is synthesized by this route, and the remaining 90% is synthesized endogenously in the mammary gland by the action of the Δ 9-desaturase enzyme, which synthesizes CLA from vaccenic acid (VA) (Mosley *et al.*, 2006), which is another intermediate of ruminal biohydrogenation (Kim *et al.*, 2008).

On the other hand, diet is the factor that most influences the concentration of CLA in ruminant milk (Siurana and Calsamiglia, 2016), and grazing is the most effective and economical nutritional strategy to increase the CLA content (Lahlou *et al.*, 2014). This is because fresh forages have a high concentration of linoleic acid (C18:2 *n*-6) and α -linolenic acid (C18:3 *n*-3) (Boufaïed *et al.*, 2003), precursors of CLA and vaccenic acid (VA) in rumen. This causes a higher rate of escape of CLA and VA to the mammary gland, and consequently, there is greater availability of substrate for the Δ 9-desaturase enzyme to act (Lahlou *et al.*, 2014), which is responsible for synthesizing about 90% of total CLA in ruminant milk (Mosley *et al.*, 2006).

Lipids in forages range from 30 to 100 g kg⁻¹ DM, which are mostly found in chloroplasts (León *et al.*, 2011). The lipid content in chloroplasts varies between 22 and 25% of DM. Of this lipid fraction, five fatty acids (FAs) are mainly found in most grasses, and approximately 95% are composed of linoleic acid (C18:3 *n*3), linolenic acid (C18:2 *n*3), and palmitic acid (C16:0). Fresh forages contain a high proportion (50-75%) of fatty acids in the form of C18:3 *n*3, and this content varies with environmental factors such as state of maturity, seasonality, and light intensity (Elgersma *et al.*, 2004).

Nonetheless, information on the profile of FAs in forages is limited, particularly in forage shrubs native to northern Mexico. Therefore, it is necessary to generate such information to develop sustainable management schemes since these shrubs are widely used in small-scale goat production systems. In addition, knowledge of the lipid value of these shrub species could help to develop schemes of production of foods of high nutritional value (Núñez-Domínguez *et al.*, 2016) in a sector of the population of high marginalization, adding value to their products (meat or milk), which would provide the opportunity to improve their income (Granados-Rivera *et al.*, 2020) and ensure a sustainable livelihood system for these communities (Bernahu and Beyene, 2015).



Based on this background, this study aimed to evaluate the chemical composition and fatty acid profile of native forage shrubs that are consumed by goats under extensive grazing in northern Mexico.

Materials and methods

The study was carried out in 2019 in the region of La Comarca Lagunera, located between coordinates 24° 22' and 26° 23' north latitude and 102° 22' and 104° 47' west longitude, at 1 100 masl. The climate corresponds to BWhw, which is characterized by being desertic, semi-warm with a cool winter, and average annual rainfall of 240 mm; the average annual temperature in the shade is 25 °C, with ranges from -1 °C in winter to 44 °C in summer (García, 2004).

The collection of shrub samples followed the methodology proposed by Toyas-Vargas *et al.* (2013), which consisted of carrying out a tour with at least two groups of people who go behind the goats, writing down and collecting samples of plant species they consume. When most goats choose a plant species to consume ($N > 75\%$), six samples of approximately 300 g of the part consumed by the goat are taken manually, approximately at the height where they consumed them.

Forage samples were placed in paper bags and then transferred to the proximate chemical analysis laboratory. The plants chosen by the animals were adult plants with green foliage in a state of physiological maturity. The collected samples were weighed fresh and then dried in a forced-air oven at a temperature of 50 °C until reaching a constant weight (approximately 72 h). The contents of dry matter (DM), organic matter (OM), crude protein (CP), total lipids (TL) (AOAC, 2019), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were analyzed (Van Soest *et al.*, 1991).

FA extraction was performed according to the methodology of Folch *et al.* (1957). Subsequently, to determine the FA profile, 50 μ l of the extracted lipids was taken and placed in polypropylene tubes, and 3 ml of sodium methoxide (0.5 M in methanol to protect the isomerization process of unsaturated FAs) was added and stirred for 1 min with vortex. The tubes were then placed in a beaker with distilled water at 50 °C for 10 min, then removed from the beaker and cooled for 5 min. Then, 3 ml of 5% methanolic hydrochloric acid was added to extract the total fat and stirred for 1 min with vortex.

The tubes were placed inside the beaker with distilled water at 80 °C for 10 min, then removed and left to cool for 10 min, 3.5 ml of hexane was added to dissolve and extract only the fat and 5 ml of 6% potassium carbonate to saponify and release the FAs, which were stirred with vortex for 1 min and centrifuged for 5 min at 2 500 rpm. The hexane fraction, located at the top of the tube, was then extracted, and deposited in polypropylene tubes, which contained 0.5 g of sodium to remove excess moisture and 0.1 g of activated carbon to remove impurities, then stirred with vortex and centrifuged at 1 500 rpm for 5 min.

The first phase of hexane was then extracted and filtered through an acrodisc (Thermo Scientific, titan 44513-NN, 17 mm green filter and 0.45 μ m nylon membrane, to ensure an impurity-free sample) and placed in a vial where it was stored at -5 °C until analysis by gas chromatography. The FA methyl esters were determined through gas chromatography, using a Hewlett Packard 6890 chromatograph with flame ionization detector (FID) with automatic injector (Supelco, series 53308-02), for which it was necessary to use a fused-silica capillary column (SP-2560, 100 m \times 0.25 mm \times 0.2 μ m film thickness) and a Supelco FAME Mix C4-C24 standard, Cat. No. 18919-1AMP.

Helium was used as carrier gas. 0.1 μ l of the methylated fat sample was injected and the temperature ramp used started at 140 °C for 2.95 min, then increased by 1 °C min^{-1} to reach 210 °C, then increased by 0.7 °C min^{-1} to reach 235 °C. Statistical analysis was performed with the statistical package SAS (2002). The information was analyzed using Anova under a completely randomized design with six replications, considering the species as a source of variation. The comparison of least squares means was performed through the adjusted Tukey test ($\alpha = 0.05$).

Results and discussion

The forage shrubs that were selected by the goats in the rangeland were huizache (*Vachellia farnesiana*), mesquite (*Prosopis laevigata*), gobernadora (*Larrea tridentata*), and chaparro prieto (*Vachellia constricta*) (Table 1). In Mexico, these shrubs have been identified as preferred by goats in the rangelands of Oaxaca (Mandujano *et al.*, 2019), Tamaulipas (Alva-Pérez *et al.*, 2019), Nuevo León (Armenta-Quintana *et al.*, 2011), Coahuila (Maldonado-Jáquez *et al.*, 2017) and Baja California Sur (Toyes-Vargas *et al.*, 2013), so they are important forages in the diet of goats, particularly during the dry season (Zapata-Campos and Mellado-Bosque, 2021), when they can represent more than 80% of the diet of grazing goats (Armenta-Quintana *et al.*, 2011).

Table 1. Chemical composition (g kg⁻¹ DM) of forage shrubs consumed by grazing goats.

Variable	Forage shrubs				p
	Huizache	Mezquite	Gobernadora	Chaparro prieto	
	(g kg ⁻¹ de MS)				
Organic matter	947	917	962	927	0.368
Crude protein	122 b	148 a	134 ab	147 a	0.026
Total lipids	49 b	24 c	67 a	62 a	0.014
Neutral detergent fiber	468 b	427 bc	603 a	412 c	0.011
Acid detergent fiber	381 b	371 b	519	389 b	0.017

a, b y c= different letters among columns indicate a statistical difference (Tukey; $\alpha=0.05$); huizache [*Vachellia farnesiana* (L.) Wild.]; mesquite [*Prosopis laevigata* (Humb & Bonpl) Wild]; gobernadora [*Larrea tridentata* (Moç. & Seseé DC.)] and chaparro prieto [*Vachellia constricta* (Bentham Sieglér & Ebinger Waif)].

Likewise, the rangelands have a high population of shrub species, representing about 25% of the total vegetation of the rangeland (Estell *et al.*, 2010), so there is high availability for goats; however, the consumption of shrubs is influenced by age (Hai *et al.*, 2014), sex (Ferretti *et al.*, 2014; Manousidis *et al.*, 2016) and physiological state (Mellado *et al.*, 2011; Cardozo-Herrán *et al.*, 2019) of the goat.

Regarding CP, mesquite, gobernadora, and chaparro prieto had similar values, and the lowest content was found in huizache ($p=0.026$). These values were lower than other reports for the same species in northern Mexico (Guerrero *et al.*, 2010; Toyes-Vargas *et al.*, 2013). In this regard, CP contents of 200 g kg⁻¹ (Foroughbakhch *et al.*, 2013) and 170 g kg⁻¹ have been reported for mesquite, and values of 188 g kg⁻¹ for huizache (Toyes-Vargas *et al.*, 2013). The CP content of forage shrubs among species varies according to the time of year and is closely related to precipitation distribution (Chimphango *et al.*, 2020).

This may explain the lower CP values found in the present study since the sampling of the shrubs studied was carried out in the dry season, which is when precipitation is minimal or null in the region (Isidro-Requejo *et al.*, 2019). In addition, the CP content in the species studied can vary between 100 and 330 g kg⁻¹ (Quiroz-Cardoso *et al.*, 2015; Carvalho *et al.*, 2017; Santos *et al.*, 2017), which is within the range found in the shrub species studied and is sufficient to promote optimal growth of rumen microflora (NRC, 2007).

The TL content differed between species ($p=0.014$); the highest values were observed in gobernadora and chaparro prieto. These values are within the expected range for lipid content in forage plants, which can be up to 10% of the DM of forage (Bauchart *et al.*, 1984). On the other hand, the TL content was higher than that reported by Toyes-Vargas *et al.* (2013), which could be related to the forage harvest season since the low water content in plants can cause a higher concentration of the solid fraction in shrub species (Suárez-Paternina *et al.*, 2015).

Differences were found between species for the values of neutral detergent fiber (NDF) ($p=0.011$) and acid detergent fiber (ADF) ($p=0.017$). These values are similar to those reported for the same plant species (Maldonado-Jáquez *et al.*, 2017; Zapata-Campos and Mellado-Bosque, 2021). In this respect, FDN and ADF are reference values that allowed the determination of the nutritional quality of a forage; that is, it made it possible to know if an ingredient has low or high energy content and if it is bulky or has higher density.

For example, forages with low NDF contents ($< 400 \text{ g kg}^{-1} \text{ DM}$) are of higher nutritional quality than those containing high concentrations ($> 600 \text{ g kg}^{-1} \text{ DM}$) of this compound (Van Soest *et al.*, 1991). Based on the above, except for gobernadora, the three shrub species evaluated have adequate nutritional characteristics for ruminant feeding. In this regard, similar results were reported for the semi-arid region of northern Mexico, where it is indicated that native shrub species have nutritional characteristics superior to other plant strata, such as trees and grasses, particularly during the dry season (Guerrero *et al.*, 2010).

Regarding the concentration of fatty acids (FAs), there were differences in saturated FAs ($p<0.001$), monounsaturated FAs ($p<0.001$), and polyunsaturated FAs ($p<0.001$) (Table 2). These results differ from those reported by Toyés-Vargas *et al.* (2013), who found no difference for the concentration of saturated FAs and monounsaturated FAs in five forage species in northern Mexico; nevertheless, it was similar in polyunsaturated FA content.

Table 2. Fatty acid (FA) profile ($\text{g } 100 \text{ g}^{-1} \text{ FA}$) of forage shrubs selected by grazing goats.

Fatty acid	Forage shrubs				p-value
	Huizache	Mezquite	Gobernadora	Chaparro prieto	
C14:0	1.29 ab	0.76 b	2.7 a	0.68 b	0.003
C15:0	0.41	0.32	0.29	0.42	0.429
C16:0	33.16 a	25.4 ab	23.08 b	17.85 c	<0.001
C16:1	2.08 b	2.54 b	1.19 c	3.33 a	<0.001
C17:0	1.16	0.91	0.83	1.21	0.776
C18:0	9.39 ab	10.27 a	8.52 b	8.5 b	0.028
C18:1 <i>n</i> -9	4.31	3.95	3.84	4.19	0.095
C18:2 <i>n</i> -6	16.05 b	14.21 b	22.36 a	24.93 a	0.012
C18:3 <i>n</i> -3	26.25 c	36.22 a	29.27 bc	32.87 ab	<0.001
C18:3 <i>n</i> -5	0.47 b	0.54 b	0.7 ab	0.97 a	<0.001
C20:0	1.4	1.7	1.56	1.68	0.161
C20:1 <i>n</i> -11	0.33	0.53	0.46	0.3	0.824
C20:1 <i>n</i> -9	0.07 c	0.36 b	0.79 a	0.01 c	0.028
C21:0	0.15	0.13	0.16	0.12	0.93
C22:0	1.48 ab	0.97 b	2.3 a	1.72 ab	<0.001
C23:0	0.83 a	0.07 b	0.73 a	0.04 b	0.006
C24:0	1.16	1.09	1.2	1.17	0.361
SFAs	51.42 a	41.65 b	42.68 b	34.8 c	<0.001
MUFAs	7.02 a	7.39 a	4.99 b	8.63 a	<0.001
PUFAs	41.56 b	50.96 a	52.33 a	56.57 a	<0.001

SFAs= saturated fatty acids; MUFAs= monounsaturated fatty acids; PUFAs= polyunsaturated fatty acids; a, b y c= different literals among columns indicate a statistical difference (Tukey; $\alpha=0.05$); huizache [*Vachellia farnesiana* (L.) Wild.]; mesquite [*Prosopis laevigata* (Humb & Bonpl) Wild]; gobernadora [*Larrea tridentata* (Moç. & Seseé DC.) and chaparro prieto [*Vachellia constricta* (Bentham Sieglér & Ebinger Waif)].

The present study confirmed that the three FAs with the highest concentration in forage species are C18:3 *n*-3, C18:2 *n*-6, and C16:0; this was reported by other research groups (Boufaïed *et al.*, 2003; Elgersma *et al.*, 2004; León *et al.*, 2011; Toyés-Vargas *et al.*, 2013; Prieto-Manrique *et al.*,

2016). This can be explained by the fact that plant cells are the only ones capable of synthesizing C18:3 *n*-3 and C18:2 *n*-6 from oleic acid through the action of enzymes $\Delta 15$ and $\Delta 12$ desaturase, respectively (Ursin, 2003).

These FAs are present in high concentrations in green forages, where they can represent up to 75% of total lipids (Clapham *et al.*, 2005) since they are part of the digalactosyl diglycerides that are associated with thylakoid membranes in chloroplasts, for this reason, they are the FAs that predominate in terrestrial plants (Sinclair *et al.*, 2002).

Concerning FA C16:0, a concentration higher than that reported in the literature was found for forage grasses such as alfalfa, orchard grass, and ryegrass (Boufaïed *et al.*, 2003); (Elgersma *et al.*, 2004). Nevertheless, it coincides with the results of Toyés-Vargas *et al.* (2013), who explain that this is probably a mechanism of adaptation of plants to areas with high temperatures because a reduction in the fluidity of the membranes in the plant would help reduce evapotranspiration, this could be achieved by incorporating a higher concentration of a saturated FA such as C16:0 into the plant membrane.

Regarding the concentration of the FAs C18:3 *n*-3 ($p < 0.001$) and C18:2 *n*-6 ($p = 0.012$), there were differences between species. Mesquite and chaparro prieto had the highest concentration together. This is important as they are precursors of vaccenic acid. In particular, C18:3 *n*-3 is isomerized to C18:3 *cis*-9, *trans*-11, *cis*-15, then hydrogenated to C18:2 *trans*-11, *cis*-15, to be hydrogenated again to C18:1 *trans*-11, which is vaccenic acid. Meanwhile, C18:2 *n*-6 is first isomerized to C18:2 *cis*-9 *trans*-11 and then hydrogenated to C18:1 *trans*-11 acid (vaccenic) (Griinari *et al.*, 2000).

The importance of vaccenic acid lies in the fact that it is a precursor of conjugated linoleic acid (CLA), and the latter can be synthesized endogenously in ruminant tissues from vaccenic acid (C18:1 *trans*-11) by the action of the $\Delta 9$ -desaturase enzyme (Mosley *et al.*, 2006). Finally, CLA is an FA with high relevance in the production of foods of animal origin due to its potential anticancer effect and its reduction of fat in adipose and muscle tissue (Yang *et al.*, 2015).

Conclusions

The four shrubs evaluated have nutritional characteristics suitable for use in the diet of grazing goats. In particular, mesquite (*Prosopis laevigata*) stands out due to its high CP content and low NDF content and also had the highest concentration of FA C18:3 *n*-3. Chaparro prieto (*Vachellia constricta*) also stands out, which had a high concentration of CP and high contents of vaccenic acid precursors (C18:3 *n*-3 and C18:2 *n*-6). These forage shrubs can be considered in diets that aim to increase the concentration of CLA in milk or meat from grazing goats.

Finally, it is advisable to carry out complementary studies with these and other native forage species that evaluate their nutritional changes, as well as the FA profile throughout the year, considering the phenological stages and the response to controlled conditions (agronomic management) in order to obtain information on their sustainable use.

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