Tropical and Subtropical Agroecosystems

CHEMICAL COMPOSITION AND PROPORTION OF PRECURSORS OF RUMENIC AND VACCENIC ACIDS IN ALTERNATIVE FORAGES FOR THE FEEDING OF RUMINANTS IN ARID ECOSYSTEMS

[COMPOSICIÓN QUÍMICA Y CONCENTRACIÓN DE PRECURSORES DE ÁCIDO RUMENICO Y VACCENICO EN FORRAJES ALTERNATIVOS PARA LA ALIMENTACIÓN DE RUMIANTES EN ECOSISTEMAS ÁRIDOS]

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SUMMARY

The nutrimental value of alfalfa hay (AH), two genotypes of cowpea [IT90K-277-2 (CG18) and Sesenteño (CG25)], a clone of Taiwan grass (TG), a local cultivar of prickly pear cactus in two presentations, green cladodes of 15 days of age or "nopalitos" (TN) and mature cladodes of 60 days of age (MN) and germinated seeds of maize (GM) of the genotype ASGROW 7573 was determined. The dry matter (DM), crude protein (CP), total lipids (TL), ash (A), crude fiber (CF) and gross energy (GE), as well as the concentration of linoleic acid (LA), α-linolenic acid (ALA), polyunsaturated fatty acids (PUFA) and *n-3* fatty acid. Results reveal that CG25 and CG18 showed the highest content of CP. With respect to TN, the genotype CG25 showed the highest amount followed by CG18 and AH. In turn, GM and TN showed the highest concentrations of LA; whereas CG18, CG25 and TG, had the highest levels of ALA. The forages that obtained the highest concentrations of *n*-3 fatty acids were CG18, CG25 and TG. These forages along with TN showed the highest concentration of PUFA. The forages CG25, CG18 and TG, had the highest content of ALA. Germinated seeds of maize showed the highest content of linolenic acid, followed by green cladodes. Both ALA and LA are precursors of rumenic acid and vaccenic acid in ruminants. Therefore, the use of these green forages in the feeding of ruminants is an alternative that could modify the proportions of the fatty acids of milk and meat for the purpose of increasing the PUFA, specifically, rumenic acid, as well as vaccenic acid
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Key words: Forages crops; PUFA; *n*-3; α-linolenic acid; linoleic acid.

RESUMEN

Se determinó el valor nutrimental de alfalfa henificada (AH), dos genotipos de fríjol yorimón [IT90K-277-2 (FYG18) y Sesenteño (FYG25)], un clon de pasto taiwán (PT), un cultivar local de nopal en dos presentaciones, pencas tiernas de 15 días de edad o "nopalitos" (NT) y pencas maduras (NM) de 60 días de edad y germinado de semillas de maíz (GM) del genotipo ASGROW 7573. Se cuantificó el contenido de materia seca (MS), proteína cruda (PC), lípidos totales (LT), cenizas (C), fibra cruda (FC) y energía bruta (EB), así como la concentración de ácido linoleico (LA), ácido α-linolénico (ALA), ácidos grasos poliinsaturados (PUFA) y ácidos grasos *n*-3. Los resultados revelan que los genotipos de frijol yorimón FYG25 y FYG18, mostraron el mayor contenido de PC. Respecto a la concentración de LT, el genotipo de yorimón FYG25 mostró la mayor cantidad seguido del FYG18 y AH. Los forrajes verdes YG25 y FYG18), así como el PT tuvieron el contenido mayor de ALA. Las semillas de germinado de maíz mostraron los contenidos mayores de LA seguido del nopal tierno. Ambos, ALA y LA, son precursores del ácido ruménico y del ácido vacénico en rumiantes. Por lo tanto, el uso de estos forrajes verdes en la alimentación de rumiantes es una alternativa que podría modificar las proporciones de ácido grasos en la leche y la carne con el propósito de incrementar los PUFA, específicamente el ácido ruménico así como el ácido vacénico.

Palabras clave: Cultivos forrajeros; PUFA; n-3; ácido α-linolénico; ácido linoleico.

INTRODUCTION

Considering the importance of farming activity in the arid and semiarid zones of Mexico, as well as the factors that limit forage production and thus the supply of feed for cattle, the incorporation of plant species with forage characteristics that show an efficient use of water is a priority for the sustainable development of the farming sector in these zones. In the present study an attempt is made to partially substitute the alfalfa hay used in the feeding of ruminants, with forage species of a high level of adaptability to the agroecological conditions of the arid and semiarid zones (Murillo-Amador *et al.*, 2000, 2002, 2003a,b). To this effect, it is important to know the chemical composition of forages that could be used as part of the diet of ruminants, in order to calculate their contribution of nutrients such as carbohydrates, protein, lipids, etc., and more specifically, of some fatty acids such as linoleic acid (LA; C18:2 *cis*-9, *cis*-12 or 18:2 *n*6) and α-linolenic acid (ALA; C18:3 *cis*-9, *cis*-12, *cis*-15 or 18:3 *n*3).

In plants, the most common fatty acids vary from 14 to 18 carbons, represented principally by the polyunsaturated fatty acids (PUFA) in a proportion of 70 to 80 % (Cabiddu *et al.*, 2006), with a high abundance of ALA and LA (Jenkins *et al.*, 2008). Plant cells are able to synthesize the LA from the oleic acid (18:1 *n*9) by desaturation of the Δ12-desaturase and the ALA acid from the linoleic acid by reaction of desaturation of the Δ15-desaturase. However, the Δ12 and the Δ15-desaturase are only present in plant cells, thus the acids LA and ALA are considered essential for animals (Christie, 1990). ALA is the precursor of the long chain PUFAS such as eicosapentanoic acid (EPA) and docosahexanoic acid (DHA).

The fresh or green biomass of some forages has a higher proportion of ALA than the seeds, because ALA forms part of the digalactosil diglycerides (DGDG) associated with the tylachoidal membranes of the chloroplasts, being the most predominant of the polyunsaturated fatty acids in land plants (Sinclair *et al.*, 2002).

When ruminants consume green forages, ALA and LA are the principal substrate for biohydrogenation by the microorganisms of the rumen (Carriquiry *et al.*, 2008; Jenkins *et al.*, 2008), producing isomers of biological importance such as conjugated linoleic acid CLA *cis*-9, *trans*-11, also known as rumenic acid (RA) and vaccenic acid (*trans*-11 C18:1; Palmquist, 1988). The intermediaries that escape from complete biohydrogenation in the rumen reach the small intestine, are absorbed and transported to the mammary gland, which uses the vaccenic acid as substrate for the synthesis of the most important amount of CLA *cis*-9, *trans*-11 in the milk (Kay *et al.*, 2004). Prior to 1987, scientific interest in the conjugated linoleic acids was limited to the rumen microbiologists, who studied the isomer CLA *cis*-9, *trans*-11 as an intermediary in the biohydrogenation of linoleic acid. This changed when Ha *et al.* (1987) reported that CLA *cis*-9, *trans*-11 was an effective inhibitor of neoplasia induced by benzopyrene in mice. Since then it has gained considerable attention as a nutrient that exerts effects in experimental animals and in humans such as the inhibition of carcinogenesis, inhibition of arteriosclerosis induced by cholesterol, reduction of the accumulation of body fat and the increase in the immune response, among others (Tanaka, 2005). Under these considerations, it is interesting to identify among some alternative forages for the arid ecosystems, those that can be used in the diet of ruminants and that due to their higher content of LA and ALA, finally contribute to the obtainment of animal based foods (meat and milk) with a higher content of fatty acids that improve the health of the consumers. Therefore, the objective of the present study was to know the chemical composition and the concentration of fatty acids in different forage species that can be used in the complementation of the diet of ruminants during the dry season, in substitution of alfalfa hay. The proposed hypothesis refers to the assumption that the chemical composition is different among the proposed green forages (cowpea, Taiwan grass, nopal cactus and corn seed sprouts) and alfalfa hay and particularly, the content of precursors of CLA *cis*-9, *trans*-11 and vaccenic acid (LA and ALA) is lower in alfalfa hay than in the abovementioned green forages.

MATERIALS AND METHODS

Study site

The present work of investigation was carried out in the Centro de Investigaciones del Noroeste, S.C. (CIBNOR), located in an arid zone of the State of Baja Califonia Sur (B.C.S.), 17 km from the city of La Paz, Mexico (24º08'N, 110º24'W). The climate of the region is BW and BS, desertic and dry according to the classification of Köppen (García, 1973), with maximum temperatures 33.9º C, minimum 10.7º C and mean of 22º C.

Plant material (forages) used

Five different plant species were used, of which alfalfa hay (*Medicago sativa*) was treated as control, while the other four were considered as alternative forage species for arid and semiarid zones (Murillo-Amador *et al.*, 2000, 2002, 2003a,b; Agredano-Hernández,

2007), emphasizing two genotypes (G-18: IT90K-277-2 and G-25: Sesenteño) of cowpea [*Vigna unguiculata* (L.) Walp.], a local cultivar of nopal cactus (*Opuntia* spp.), a clone of Taiwan grass (*Pennisetum purpureum*) and germinated corn seed (*Zea mays*) genotype ASGROW 7573.

Samples of forages

The samples of the five forage species were taken randomly in the following manner: a sample consisting of alfalfa hay (AH) from a commercial plot under conventional management (INIFAP, 1986), whose origin was the Valle de Santo Domingo, B.C.S. The cowpea samples (CG18 and CG25), cultivated under conventional management (Murillo-Amador *et al.*, 2003a) were taken from a field located in the Valle de "El Carrizal", municipality of La Paz, B.C.S., 58 days after sowing, in the flowering stage and manually cut with a knife at 5 cm from the soil surface. For cactus, tender cladodes "nopalitos" (TN) were taken of approximately 15 days of age and mature cladodes (MN) of approximately two months of age, both collected in a commercial plantation under conventional management (Murillo-Amador *et al.*, 2002, 2006), located in the community of El Centenario, municipality of La Paz, B.C.S. For the Taiwan grass (TG) a composed sample was obtained by collecting leaves of five different plants whose age fluctuated between 70 and 80 days, which were taken from a plot under conventional management (Agredano-Hernández, 2007), located in the Valle de "El Carrizal", municipality of La Paz, B.C.S. In addition, a sample was taken comprised of germinated maize seed (GM), which was collected from a greenhouse located in the installations of the experimental field of the CIBNOR, 14 days after sowing, in the phenological stage of shoot. All of the collected samples were placed in plastic bags, which were introduced in a plastic box with ice (cooler) and transported to the proximal chemical analysis laboratory.

Proximal chemical analysis

The samples in the laboratory were divided into two parts: one part was used for the determination of dry matter (DM), which was subjected to a temperature of 105º C for four hours in the drying oven (Terlab®). The other part of the samples was used for the determination of chemical analyses, which were dried at 70º C during a period of 24 to 72 hours in a furnace (HTP-80®). The ashes (A) were determined by combustion at 600 º C during five hours, using the muffle (Thermolyne 6000®). The crude protein (CP) was determined in the Foss Kjeltec 2300 distillator during four minutes per sample and in the Foss Kjeltec 2040 digestor during 25 minutes, by the microkjeldahl method. The crude fiber (CF) was quantified by the method of successive hydrolysis in an extraction multiunit (Fiber Tec M6 Tecator®) and the gross energy (GE) was determined with PARRI261® calorimeter (AOAC, 2005).

Analysis of total lipids and fatty acids

The green forages were lyophilized in a Virtis 5L® lyophilizer during 24 hours, then they were pulverized in a morter, and the total lipids (TL) were extracted with a mixture of solvents of water chloroform:methanol (1:2:1.8) based on the technology of Bligh and Dyer (1959). A fraction of the lipids was used to quantify by the calcination method (Marsh and Weinstein, 1966) and the other fraction was dried with gassy N_2 and 2.5 mL of hydrochloric-methanol acid HCl:MeOH (5:95). These were heated at 85º C for 2.5 hours to methylize the lipids based on the method of Sato and Murata (1988); they were left to cool at room temperature and then 1.5 mL of hexane was added, mixing with the vortex to separate the upper phase, which was placed in a clean test tube with a cover (No. 99447) which had been previously labeled. Then 1.5 mL of hexane was added to the original sample and the upper phase was separated once again, and stored at -20º C for 24 h. Later, they were removed from the freezer and dried in the Pierce Reactivap III® evaporizer with gassy N_2. To each sample the hexane necessary to obtain a concentration of fatty acids within the linear range of gas chromatograph of mass spectrometry (Sato and Murata, 1988), adding a teaspoon of anhydrous sodium sulphate with the purpose of eliminating any residue of water (Christie, 2003).

The methyl esters were separated by means of the BPX70MOD.M method uzing the model GCD 1800 B gas chromatograph, with mixture of 37 standards (Supelco 47885-U) Column DB23 60 m*0.25 µm (catalogue agilent 122-2362). The helium was used as gas carrier with flow of 1 mL/minute. The initial temperature of the column was 110 º C, which was maintained for 3 minutes to be later increased to a rate of 30 º/minute until reaching 16 5º C, and maintained at this temperature during 2 minutes. Next, it was increased again to a rate of 10 º/minute until reaching 210 º C, maintained for 2 minutes. Finally, the temperature was increased to a rate of 3 º/minute until reaching 240 º C, and maintained for 10 minutes. The temperature of the injector and of the detector was 250 º C (Folch *et al.*, 1956). The fatty acids present in the samples were identified by the comparison of the mass spectra, through confirmation by interpretation of the spectra of methyl-esters of fatty acids according to McLafferty and Turecek (1993), as well as the

comparison of the retention times of the peaks in the sample, with the retention times of a commercial pattern of 37 methyl-esters of fatty acids. To calculate the concentration of the fatty acids present in the samples, the area below the peaks was integrated and was interpolated with a calibration curve that relates five known concentrations (5, 10, 20, 40 and 80 µg/mL) of each one of the 37 standards of esterified methyl fatty acids, with their respective areas below the peak, this being the area directly proportional to the mass of the system (GC-DM). To calculate the concentration of each fatty acid in g/100 g, the µg/mg were considered as 100%. Saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, *n*-3, linoleic acid and α-linolenic acid were principally determined.

Statistical analysis

The data are shown with the averages and the standard error of the mean. To determine the differences among the dependent variables (chemical concentration: dry matter, ash, crude protein, crude fiber, energy, total lipids; composition of fatty acids: saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, *n*-3, *n*-6, linoleic acid and α-linolenic acid) of the five different types of forages (independent variables), the data were analyzed through analysis of variance (ANOVA) of a factor or path (forages) with four replicates. When significant statistical differences were found ($P \leq 0.05$), the Tukey comparison of means test was used (P =0.05). To satisfy the assumptions of the analysis of variance (Sokal and Rohlf, 1981), the data shown in g/100 g were previously transformed by means of arcosene. All of the statistical analyses were made with the program SAS (SAS Institute, 2001).

RESULTS AND DISCUSSION

The forages showed significant differences ($P < 0.0001$) among the response variables evaluated (DM, CP, A, TL, CF and GE). The content of DM was higher in the GM, followed by MN, TN, AH, TG, CG18 and CG25. The TN had a higher amount of DM than the TG, CG25 and CG18, but was similar to the AH. On the other hand, the AH captured a higher amount of DM than the genotypes of cowpea (CG25 and CG18), but similar to TG, whereas CG25 and CG18 did not present significant differences (Table 1). Recently, Lee *et al.* (2008) studied alfalfa hay to determine the concentration of DM, observing values similar to those obtained in the genotypes of cowpea (CG18 and CG25), also determining lower amounts of DM with respect to GM, TG, TN, MN and AH. Another study (Atti *et al.*, 2006) showed that oat hay and cactus [*Opuntia ficus-indica* (L.) *ficus-inermis* (Web.)] obtained lower values in DM with respect to those obtained in the present investigation. Lower amounts of DM with respect to those shown in the present study were reported by Cabiddu *et al.* (2006) when evaluating the green forages *Daisy forb, Lolium rigidum, Medicago polymorpha* and *Hedysarum coronarium* during the vegetative phase. Values similar to those obtained in DM in the cowpea genotypes (CG18 and CG25) were reported in the different stages of maturity (anthesis and post-anthesis phase) in the stem of cowpea (Baloyi *et al.*, 2008). In an experiment conducted by Agredano-Hernández (2007), lower values of DM are reported in the leaf of TG with respect to those reported in the TG of the present work. According to the results of the present study and the reports of other studies, it is evident that the values in the concentration of DM in the different forages will show a differential of response according to the genetic material used and with the agro ecological conditions of evaluation.

Table 1. Chemical composition (Mean ± E.E; g/100 of DM) of alfalfa hay and four alternative forages for arid ecosystems.

Variable	GM	CG18	CG25	TG	TN	MN	AH
DM	96.9±0.08^{a}	91.3±0.23^{e}	91.2±0.21^{e}	92.2±0.25^{d}	93±0.49^{c}	94.2±0.06^{b}	92.3±0.05^{c,d}
CP	14.5±0.36^{d}	19.3±0.45^{b}	23.3±0.75^{a}	8.1±0.15^{f}	9.7±0.39^{e}	5.8±0.37^{g}	17.6±0.41^{c}
A	3.2±0.32^{e}	11±0.18^{c}	11.4±0.45^{c}	9.7±0.42^{d}	22.5±0.25^{a}	26.4±0.38^{b}	10.7±0.51^{c,d}
CF	9.3±0.15^{c}	16.5±0.98^{b}	14.3±0.59^{b}	29.5±0.27^{a}	6.5±0.44^{d}	9.9±1.52^{c}	29.9±0.68^{a}
GE[1]	4.42±0.004^{a}	3.93±0.05^{c}	3.91±0.07^{c}	4.01±0.03^{b,c}	3.25±0.06^{d}	2.85±0.08^{e}	4.13±0.05^{b}
TL	3.41±0.13^{c}	4.93±0.19^{b}	5.97±0.59^{a}	3.13±0.37^{c}	2.13±0.04^{d}	2.20±0.13^{d}	4.32±0.10^{b}
18:2*n*6c	1.95±0.19^{a,b}	0.41±0.16^{d}	0.50±0.02^{c,d}	0.50±0.05^{c,d}	0.89±0.09^{b,c,d}	1.36±0.42^{a,b,c}	1.96±0.22^{a}
18:3*n*3α	0.82±0.16^{c}	4.52±0.72^{a}	3.66±0.32^{a}	2.60±0.69^{a,b,}	1.21±0.05^{b,c}	3.33±0.92^{a,b}	4.72±0.33^{a}

^{a,b,c,d,e,f,g} Different literals among rows indicate inequality ($P \leq 0.0001$). GM=Germinated maize; CG18 = Cowpea G18; CG25 = Cowpea G25; TG = Taiwan grass; TN = Tender nopal; MN = Mature nopal; AH = Alfalfa hay. [1]Mcal/kg.

The analysis of variance for CP showed significant differences among the forages ($P < 0.0001$), where the cowpea (CG25) showed the highest concentration, followed by CG18, AH, GM, TN, TG and MN (Table 1). Similar values to those obtained in the present study in CP in AH were reported by Lee *et al.* (2008). In an experiment carried out with cactus [*Opuntia ficus-indica* (L.) *ficus-inermis* (Web.)], Atti *et al.* (2006) observed higher amounts of CP with respect to MN, but lower with respect to TN and to the rest of the forages evaluated in the present work. The results of TN showing higher values of CP with respect to MN coincide with those of Flores *et al.* (1995), who in a study done in 20 varieties of cactus and analyzing stems (suberificated), mature cladodes (annual leaf) and young cladodes (shoots), conclude along with Pimienta (1990), that the protein content is higher in the shoots or new growth. To this respect, Muñoz de Chávez *et al*. (1995) point out that as with other garden vegetables, cactus has an average of 1.7 % of CP. Other studies (Baloyi *et al.*, 2008) have revealed that leaves of cowpea harvested in the month of February, show higher values in the concentration of CP with respect to the values obtained for the genotype CG18; however, as the stage of maturity advances, this concentration tends to decrease. In a study carried out with TG (Agredano-Hernández, 2007), higher values of CP were obtained than those reported in TG in the present study. In general terms, the values of CP obtained in some forages evaluated, such as in cowpea (CG18 and CG25), show that this species contains sufficient protein to satisfy the needs of ruminants for relatively high production levels (Minson, 1977), including the grain of this legume, which was demonstrated by Singh *et al.* (2006) by including grains of cowpea in the mixture of a feed concentrate, reporting a positive associative effect in the absorption of fiber, in the balance of nitrogen, in the environment of the rumen and in the performance of the growth of lambs, concluding that the grains of cowpea can completely substitute the principal source of protein (peanut) that is used as mixture in the concentrate for lambs in growth.

The results also reveal significant differences ($P < 0.0001$) in the concentration of ashes among the forages, where the TN presented the highest concentration, followed by MN, CG25, AH, TG and GM (Table 1). The results that TN showed the highest content of ashes with respect to MN, do not coincide with those obtained by Tegegne (2002), who in an essay made in Ethiopia, demonstrated that the shoots or new growth of nopal cactus showed a lower content of ash than the stems and leaves; this variation is due to the series of compounds and elements that comprise the ash and to the close relationship of these with the soil chemistry and to the complex phenomena of the availability of its elements for the plant (Bravo, 1978). Rodríguez-Félix and Cantwell (1988) indicate that the chemical composition of the fresh young cladodes showed values of 1.3 % of ash, of which 90% is calcium. The content of ashes in CG18, CG25 and AH was similar, but they obtained higher concentrations than the GM. However, the TG was similar to the AH but different from the two genotypes of cowpea. In a study done by Lee *et al.* (2008), alfalfa hay was evaluated, obtaining higher concentrations of ashes than the GM; however, in the rest of the forages evaluated the values of ashes were lower than those reported in this work. In one study, Baloyi *et al.* (2008) reported values of ashes in leaves of cowpea, which were higher than those obtained in the CG18 and CG25. The fact that the values of ashes differ within the same species indicates that these depend on the variety, the age of the plants, the growth conditions of the crop, among other inherent factors both of the genotypic differential and its interaction with the environmental factors (Tarawali *et al.*, 1997).

The analysis of variance showed significant differences ($P < 0.0001$) among the forages for CF, where AH and TG were the highest values (Table 1). CG18 and CG25 were similar ($P > 0.05$) to each other but obtained higher concentrations of CF than GM and MN, which showed values that were statistically equal to each other, but obtained higher concentrations than the TN. The results regarding the content of CF in cowpea in the present study, agree with those mentioned by Tarawali *et al.* (1997), who indicate that the nutrimental values of cowpea can be compared very well with other forages with respect to the content of crude protein, digestibility and mineral content, but not with that of crude fiber, given that it is in general lower compared with other forages. Other studies (Atti *et al.*, 2006) have revealed that the content of CF in nopal [*Opuntia ficus-indica* (L.) *ficus-inermis* (Web.)] is lower to what was reported in this investigation in AH and TG, but was higher than what was obtained in TN, MN, GM, CG18 and CG25. Fuentes-Rodríguez (1997*a*,*b*) reported average values of the content of CF of 10.2 to 17.1 of various species of nopal, which are higher than those shown in the present study. Similarly, Pimienta (1990) and Flores *et al.* (1995) concluded that the content of crude fiber increases with the age of the cladode, reaching 16.1 % in the suberified stems, but close to 8.0 %, on the average, in the new growth; this was also observed by Tegegne (2002) in an essay with nopal cactus in Ethiopia. However, Muñoz de Chávez *et al.* (1995) point out that on the average, the nopal cactuses present values of 3.5 % of CF, whereas Rodríguez-Félix and Cantwell (1988) indicate that in the chemical composition of the fresh cladodes (nopalitos), values of 1.1 % of the fiber content were determined, value

which makes it comparable to the fiber content of spinach.

The analysis of variance also indicated significant differences ($P < 0.0001$) among the forages for the variable GE, where the GM showed the highest concentration with respect to the rest of the forages, followed in descending order by AH, TG, CG18, CG25, TN and MN (Table 1). The AH showed a higher amount than the forages CG25, CG18, TN and MN, but obtained concentrations similar to TG. In turn, the TG showed concentrations similar to the forages CG25 and CG18, but obtained higher concentrations than the forages TN and MN. These two showed the lowest amounts of GE. To this respect, Atti *et al.* (2006) report values of 0.75 FU/kg of DM in nopal [*Opuntia ficus-indica* (L.) *ficus-inermis* (Web.)].

The analyses reveal significant differences ($P < 0.0001$) in the concentration of TL among the forages, being CG25 the one that showed the highest concentration, followed in descending order by CG18, AH, GM, TG, MN and TN. CG18 and AH were similar to each other but obtained higher concentrations than the forages GM, TG, MN and TN. The GM and TG were similar to each other but obtained higher concentrations than the MN and TN, the latter two showing the lowest values of TL. These results coincide with those presented by Muñoz de Chávez *et al.* (1995), who point out that as with other garden vegetables, the nopal cactus cladodes have a high water content (90.1 %) and low content of lipids. Similar results were obtained by Rodríguez-Félix and Cantwell (1988), who indicate that the chemical composition of the fresh cladodes (nopalitos) is mainly water (91 %), 1.5% proteins and 0.2 % lipids. Recent studies in alfalfa hay show results similar to the forages TN and MN and lower concentrations than the forages TG, GM, AH, CG18 and CG25 (Lee *et al.*, 2008).

The forages showed significant differences ($P <0.0001$) in the concentration of saturated fatty acids, monosaturated fatty acids, polyunsaturated fatty acids, omega three and omega six (Table 2). The MN showed the highest content of saturated fatty acids (SFA) followed by AH and GM, which showed statistical equality with AH, following in descending order TG, followed by TN, CG18 and CG25, these three being statistically equal among each other (Table 2). To this respect, Biondi *et al.* (2008) reported differential values in the content of saturated fatty acids in a mixture of cut grasses, faba bean hay and wheat straw.

With respect to the concentration of polyunsaturated faty acids (PUFA), the forages CG25 and TG showed the highest concentration, followed in descending order and with statistical equality by the forages CG18 and TN, whereas the fodders that showed a lower concentration and statistical equality among each other were the MN, AH and GM (Table 2). Recent reports (Biondi *et al.*, 2008) have demonstrated the variability in the concentration of polyunsaturated acids in cut grasses, faba bean hay and wheat straw, of which the cut grass even showed concentrations higher than those obtained in the forages evaluated in the present study, where the faba bean hay showed concentrations similar to AH and MN and lower than the GM, but higher than TN, CG18, CG25 and TG. However, the wheat straw showed concentrations lower than all of the forages evaluated. Other studies (Atti *et al.*, 2006) have demonstrated that the use of nopal in the feeding of goats is associated with a higher content of C18:2, to conjugated linoleic acid (CLA), as well as to a higher proportion of polyunsaturated acids (PUFA) and to the proportion PUFA:SFA, all with respect to the forage used as control, concluding that the use of cactus cladodes in the feeding of goats maximizes the proportion of CLA, PUFA and the relationship of PUFA:SFA. The above represents a comparative advantage for the nopal cactus used in the present study as alternative forage, given that the TN showed higher values of PUFA with respect to the conventional forage (AH) commonly used in the feeding of the species of highest importance (cattle and goats) in the state of Baja California Sur (INEGI, 2006).

The forages CG25, CG18 and TG showed statistical equality among each other, as well as the highest concentration of *n*-3 with respect to the rest of the forages, followed in descending order by MN, AH, TN and GM. In turn, the forages MN, AH and TN showed statistical similarity and obtained a higher concentration of *n*-3 than the GM (Table 2). The fact that the forages proposed in the present study as alternatives, CG25, CG18 and TG showed a higher concentration of *n*-3 comparatively with respect to the conventional forage (AH), represents an advantage, given that it has been demonstrated that to obtain quality meat in cattle, the fatty acids should be increased, in particular the *n*-3, in the diet supplied to the animals (Scollan *et al.*, 2001). Similarly, other studies have indicated that lambs fed with grass presented better nutritional characteristics than the meat of lambs raised under a system of intensive production (Demeyer and Doreau, 1999; French *et al.*, 2000; Wood *et al.*, 1999).

Table 2. Concentration of fatty acids (Mean ± E.E; g/100 g of fatty acids) of alfalfa hay and four alternative forages for arid ecosystems.

Fatty Acids	GM	CG18	CG25	TN	MN	TG	AH	*P*
12:0	—	—	—	0.21 ±0.10^{a}	2.03 ±0.29^{b}	1.14 ±0.30^{b}	—	<0.0011
14:0	0.18 ±0.01^{d}	0.27 ±0.03^{c,d}	0.38 ±0.02^{b,c,d}	0.59 ±0.17^{b,c,d}	3.49 ±0.23^{a}	0.88 ±0.20^{b}	0.65 ±0.06^{b,c}	<0.0001
15:0	0.40 ±0.06^{b}	0.61 ±0.07^{a,b}	0.76 ±0.04^{a}	0.51 ±0.07^{a,b}	0.68 ±0.01^{a}	0.53 ±0.05^{a,b}	—	0.0061
15:1*n*-8	—	—	—	0.30 ±0.04^{a}	0.16 ±0.03^{b}	—	—	0.0480
16:0	20.97 ±0.76^{a,b}	18.17 ±1.23^{b,c}	16.17 ±0.26^{c,d}	17.46 ±0.25^{b,c}	17.30 ±0.65^{b,c}	12.70 ±1.03^{d}	23.79 ±1.42^{a}	<0.0001
16:1*n*-9	0.24 ±0.01^{b,c}	7.63 ±3.51^{a}	3.42 ±0.22^{a}	0.14 ±0.01^{c}	0.16 ±0.02^{c}		2.37 ±0.10^{a,b}	<0.0001
16:1*n*-7	0.23 ±0.01^{b,c}	0.15 ±0.02^{c}	0.22 ±0.01^{b,c}	0.28 ±0.02^{b,c}	0.33 ±0.01^{b}	1.60 ±0.17^{a}	—	<0.0001
17:0	0.38 ±0.02^{b}	0.22 ±0.02^{b}	0.26 ±0.00^{b}	0.46 ±0.10^{b}	1.21 ±0.06^{a}	0.47 ±0.24^{b}	0.66 ±0.16^{a,b}	0.0002
ISO17:0	—	—	—	0.31 ±0.13^{a}	0.50 ±0.02^{a}	—	—	0.1834
17:1	0.04 ±0.01^{a}	—	—	—	—	—	0.10 ±0.02^{a}	0.0588
18:0	3.71 ±0.11^{a,b}	2.71 ±0.19^{b,c}	2.40 ±0.06^{c}	2.23 ±0.15^{c,d}	2.70 ±0.13^{b,c}	1.39 ±0.11^{d}	4.82 ±0.69^{a}	<0.0001
18:1*n*-9c	15.72 ±1.61^{a}	1.40 ±0.45^{c}	1.46 ±0.11^{c}	7.70 ±0.88^{b}	5.93 ±0.27^{b}	1.73 ±0.10^{c}	6.76 ±2.18^{b}	<0.0001
18:1*n*-7c	0.82 ±0.08^{a}	0.20 ±0.05^{b}	0.28 ±0.06^{b}	0.24 ±0.02^{b}	0.61 ±0.05^{a}	—	—	<0.0001
18:2*n*-6c	36.25 ±2.37^{a}	5.02 ±1.74^{d}	8.53 ±0.54^{c,d}	26.53 ±2.70^{a,b}	16.32 ±1.92^{b,c}	12.51 ±2.41^{c}	16.57 ±0.43^{b,c}	<.0001
18:3*n*-6	—	0.44 ±0.03^{a,b}	0.50 ±0.01^{a}	0.20 ±0.01^{c}	0.25 ±0.01^{c}	0.45 ±0.01^{a,b}	0.37 ±0.03^{b}	<0.0001
18:3*n*-3α	15.85 ±3.89^{c}	59.98 ±3.91^{a}	61.12 ±1.26^{a}	36.30 ±1.22^{b}	38.62 ±0.60^{b}	55.26 ±4.42^{a}	37.44 ±4.48^{b}	<0.0001
20:0	1.10 ±0.03^{a,b}	0.46 ±0.10^{b}	0.52 ±0.01^{b}	1.26 ±0.42^{a,b}	2.23 ±0.41^{a}	1.92 ±0.31^{a}	1.84 ±0.10^{a}	0.0003

Table 2. Concentration of fatty acids (Mean ± E.E; g/100 g of fatty acids) of alfalfa hay and four alternative forages for arid ecosystems.

Fatty Acids	GM	CG18	CG25	TN	MN	TG	AH	*P*
20:1	—	0.05 ±0.00^{b}	0.07 ±0.00^{b}	—	—	—	0.16 ±0.03^{a}	0.0040
20:1*n*-9	0.22 ±0.01^{a}	—	—	0.16 ±0.01^{a,b}	0.12 ±0.01^{b}	—	—	0.0046
20:1*n*-11	—	0.15 ±0.04^{a}	0.05 ±0.01^{a}	—	—	—	—	0.0913
20:2	0.11 ±0.01	—	—	—	—	—	—	—
20:3*n*-3	—	0.11 ±0.02^{a}	0.13 ±0.04^{a}	—	—	—	—	0.7560
21:0	—	—	—	0.22 ±0.02^{a}	0.51 ±0.00^{b}	—	—	0.0002
22:0	1.11 ±0.10^{b,c}	0.36 ±0.03^{c}	0.52 ±0.02^{c}	2.21 ±0.21^{a,b}	2.79 ±0.59^{a}	3.96 ±0.63^{a}	1.06 ±0.28^{b,c}	<0.0001
23:0	0.18 ±0.01^{b}	0.26 ±0.10^{b}	0.18 ±0.04^{b}	0.42 ±0.04^{a,b}	0.42 ±0.00^{a,b}	0.25 ±0.04^{b}	0.99 ±0.38^{a}	0.0023
24:0	1.62 ±0.07^{a}	0.84 ±0.06^{b}	1.24 ±0.06^{a,b}	1.25 ±0.07^{a,b}	1.70 ±0.21^{a}	1.49 ±0.26^{a}	1.62 ±0.10^{a}	0.0020
25:0	0.15 ±0.01^{b}	0.17 ±0.05^{b}	0.27 ±0.11^{a,b}	0.31 ±0.02^{a,b}	0.44 ±0.02^{a}	0.18 ±0.03^{a,b}		0.0182
26:0	0.53 ±0.02^{a,b}	0.35 ±0.06^{b}	0.81 ±0.07^{a}	0.36 ±0.06^{b}	0.93 ±0.17^{a}	0.49 ±0.10^{a,b}	0.56 ±0.06^{a,b}	0.0015
28:0	0.09 ±0.04^{d}	0.37 ±0.02^{b,c,d}	0.63 ±0.17^{b}	0.26 ±0.03^{b,c,d}	0.47 ±0.04^{b,c}	2.99 ±0.45^{a}	0.15 ±0.02^{c,d}	<0.0001
SFA	30.46 ±0.78^{a,b,c}	24.83 ±1.72^{c}	24.19 ±0.61^{c}	28.12 ±0.99^{c}	37.45 ±1.99^{a}	28.55 ±2.52^{b,c}	36.19 ±2.24^{a,b}	<0.0001
MUFA	17.30 ±1.69^{a}	9.61 ±3.61^{a,b}	5.52 ±0.32^{b}	8.83 ±0.86^{a,b}	7.34 ±0.21^{b}	3.34 ±0.24^{b}	9.41 ±2.13^{a,b}	0.0003
PUFA	52.22 ±2.12^{c}	65.55 ±3.32^{a,b}	70.29 ±0.91^{a}	63.03 ±1.53^{a,b,c}	55.20 ±2.19^{b,c}	68.23 ±2.36^{a}	54.39 ±4.09^{b,c}	0.0001
n-3	15.85 ±3.89^{c}	60.10 ±3.91^{a}	61.24 ±1.22^{a}	36.30 ±1.22^{b}	38.62 ±0.60^{b}	55.26 ±4.42^{a}	37.44 ±4.48^{b}	<0.0001
n-6	36.25 ±2.37^{a}	5.45 ±1.72^{d}	9.04 ±0.54^{c,d}	26.73 ±2.69^{a,b}	16.58 ±1.94^{c}	12.96 ±2.40^{c}	16.94 ±0.44^{b,c}	<0.0001

Table 2. Concentration of fatty acids (Mean ± E.E; g/100 g of fatty acids) of alfalfa hay and four alternative forages for arid ecosystems.

Fatty Acids	GM	CG18	CG25	TN	MN	TG	AH	*P*
n-7	1.06 ±0.08^{a}	0.36 ±0.07^{a}	0.51 ±0.06^{a}	0.52 ±0.05^{a}	0.95 ±0.06^{a}	1.19 ±0.37^{a}	—	0.0180
n-8	—	—	—	0.30 ±0.04^{a}	0.16 ±0.03^{b}	—	—	0.0480
n-9	16.20 ±1.61^{a}	9.04 ±3.62^{a,b}	4.88 ±0.26^{b,c}	8.01 ±0.88^{a,b}	6.22 ±0.24^{b,c}	1.73 ±0.10^{c}	9.14 ±2.10^{a,b}	<0.0001
n-11	—	0.15 ±0.04^{a}	0.05 ±0.01^{a}	—	—	—	—	0.0913
n-6:*n*-3	2.68 ±0.55^{a}	0.09 ±0.03^{c}	0.15 ±0.01^{c}	0.74 ±0.10^{b}	0.42 ±0.05^{b,c}	0.25 ±0.07^{b,c}	0.47 ±0.07^{b,c}	<0.0001
n-3:*n*-6	1:2.68^{a}	1:0.09^{c}	1:0.15^{c}	1:0.74^{b}	1:0.42^{b,c}	1:0.25^{b,c}	1:0.47^{b,c}	<0.0001

Means with different superindex (a-d) among rows differ significantly ($P < 0.05$).
GM = Germinated maize; CG18 = Cowpea genotype G18; CG25 = Cowpea genotype G25; TG = Taiwan grass; TN = tender nopal; MN = Mature nopal; AH = Alfalfa hay;
SFA = saturates; MUFA = monosaturates; PUFA = polyunsaturates;
n-3 = omega three; *n*-6 = omega six; *n*-7 = omega seven; *n*-8 = omega eight; *n*-9 = omega nine;
n-11 = omega eleven.
Empty cells indicate undetected fatty acids.

The GM showed the highest concentration of *n*-6, followed by TN, which showed statistical equality with GM, followed in descending order by AH, MN, TG, CG25 and CG18, the latter forage showing the lowest concentration of *n*-6. The fact that the TN showed a higher concentration of *n*-6 with respect to MN, coincides with Dewhurst *et al.* (2006), who point out that the genetic differences in the concentration of fatty acids in grasses will be more apparent in young plants with respect to the more mature grasses in the stages of flowering or senescence destined for conservation such as silage or hay. On the other hand, a considerable number of research have studied the genetic variation in the levels of fatty acids in forage grasses and legumes commonly consumed by ruminants (Dewhurst *et al.*, 2006). These studies have provided evidence of the genetic effects; however, large effects of the environmental factors have also been identified, such as sunlight (Dewhurst and King, 1998), the intervals in the cuts, the season of the year, the fertilization regimen, among others.

The forages showed significant differences with respect to linoleic acid (LA), α-linolenic acid and the proportion *n*3:*n*6 (Table 2), where the GM showed the highest concentration of LA, with statistical similarity the TN, followed by the forages AH, MN, TG, CG25 and CG18, the cowpea genotypes showing the lowest content of LA. With respect to the content of α-linolenic acid (ALA), the forages CG CG25, CG18 and TG showed the highest content of this acid, followed in descending order by the forages MN, AH, TN and GM, the latter showing the lowest content of ALA. Differential responses in the content of LA and ALA have been reported in different plant species used as feed in different domestic species. To this respect, Lourenco *et al.* (2007) carried out a study in which he used three mixtures of different forages (mixture of grasses, mixture of forages of legumes and a mixture of ryegrass), where he found that the content of LA was higher in the grass mixture, whereas the ryegrass mixture had the highest content of ALA. Atti *et al.* (2006), in an experiment made with nopal cactus [*Opuntia ficus-indica* (L.) *ficus-inermis* (Web.)] observed higher contents of LA than the GM;

however, contents lower than the forages CG25, CG18, TG, MN and AH were registered, but concentrations similar to the TN. In another experiment Steinshamn *et al.* (2007) evaluated during two consecutive years, the concentration of LA and ALA in the silage of *Trifolium repens* L. and *Trifolium pratense* L., both of the second cut, observing that the contents of both acids between the two species, did not show significant differences, but when comparing the contents of LA with those obtained in the present study, it is observed that the content of LA of both species is higher than that of the forages CG18, CG25 and TG, but lower than the forages GM, TN, MN and AH, while the content of ALA of both species only surpassed that of GM. On the other hand, Cabiddu *et al.* (2006) used different green forages in vegetative phase (*Lolium rigidum* Gaudin, *Hedysarum coronarium* L., *Medicago polymorpha* L., *Chrysanthemum coronarium* L.) during the winter and spring periods, observing differences among the forages in the content of LA and ALA in both seasons of the year, with lower contents than those found in the forages in the present study.

The content of LA and ALA in the AH obtained in the present study, was lower than that was reported by Lee *et al.* (2008), who carried out an experiment with alfalfa hay; however, they reported lower values of LA and ALA to the GM and TN, but higher than the rest of the forages evaluated. Biondi *et al.* (2008) evaluated a mixture of straw of cut grasses, faba bean hay and wheat straw, finding different contents of LA and ALA among the forages evaluated. Furthermore, the contents reported of both acids were similar to what was obtained in CG25, but higher than the forages GM, TN, MN, TG and AH and lower than CG18. Recent studies (Buccioni *et al.*, 2008) developed with a mixture of dry forages comprised of natural grass, second cut alfalfa, corn flour, soybean flour, barley flour, total fat of soybean and supplement of vitamins and minerals, showed contents of LA similar to CG18 and lower than CG25, GM, TN, MN, TG and AH, as well as lower values in the content of ALA with respect to the forages evaluated in the present study. On the other hand, Flowers *et al.* (2008) reported contents of ALA in a forage comprised of alfalfa hay, Festuca, clover and weeds (50:20:20:10), higher than those shown by the forages GM, AH, TN and MN in the present study.

Once the ruminant has consumed forage, it has been observed that the ruminal bacteria *Butyrivibrio fibrisolvens* converts the linoleic acid through isomerization into conjugated acid C18:2 possibly CLA *cis*-9, *trans*-11 or rumenic acid followed by the hydrogenation of the C18:1 *trans*-11 or vaccenic acid (Kepler *et al.*, 1966; Paillard *et al.*, 2007; Jenkins *et al.*, 2008). In another study it was observed that the goat kids that consumed nopal cactus obtained a higher concentration of C18:2 and CLA, indicating that the nopal cactus used as green forage produces higher quality meat in nutritional terms (Atti *et al.*, 2006).

With respect to the proportion *n*-3:*n*-6, the GM showed the highest value, followed by TN, AH, MN, TG, CG25 and CG18 (Table 2). On the other hand, the proportion obtained by the TN was higher than the forages CG25 and CG18, but showed statistical equality to MN, TG and AH. Finally, CG8, CG25, MN, TG and AH showed similar statistical proportions.

CONCLUSIONS

The green forages cowpea (CG25 y CG18), as well as Taiwan grass, had the highest content of α-linolenic acid. Germinated seeds of maize showed the highest content of linolenic acid, followed by green cladodes. Both polyunsaturated fatty acids (ALA and LA) are precursors of rumenic acid and vaccenic acid in ruminants. Therefore, the use of these green forages in the feeding of ruminants is an alternative that could modify the proportions of the fatty acids of milk and meat for the purpose of increasing the PUFA, specifically, rumenic acid, as well as vaccenic acid.

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