

# Fatty acid profile of milk from dairy cows fed Mexican aster (*Cosmos bipinnatus* Cav.) silage in small-scale dairy systems in central Mexico

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## ABSTRACT

In the search for forage alternatives for the dry season in small-scale dairy systems, spontaneous vegetation such *Cosmos bipinnatus* Cav., the Mexican aster (cosmos), a weed in the central highlands, can be an option as silage. Cosmos has secondary metabolites that affect rumen fermentation, which may affect the fatty acid profile of milk and the content of beneficial acids. Given the interest of consumers, it is necessary to determine the fatty acid content in milk from cows fed cosmos. The objective was to determine the fatty acid profile of feed and milk from cows fed silage with a high proportion of cosmos. Nine multiparous Holstein cows, grouped in trios, were randomly assigned to treatments in a 3×3 Latin square design repeated three times. Treatments were 10 kg DM cow<sup>-1</sup> d<sup>-1</sup> silage containing 40% cosmos (CB40), 60% cosmos (CB60), or 10 kg DM cow<sup>-1</sup> d<sup>-1</sup> maize (*Zea mays* L.) silage (MZSL). Each cow also received 4.1 kg DM commercial concentrate. As for silages, maize silage had less content ( $p < 0.05$ ) of saturated fatty acids (SFA), and higher ( $p < 0.05$ ) monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, compared to CB40 and CB60 silages. However, in the milk profile, the treatment with the highest ( $p < 0.05$ ) PUFA content was CB60 (mean 3.83 g 100 g<sup>-1</sup>), 8% higher than MZSL; CB60 also had a lower content ( $p < 0.05$ ) of lauric and myristic fatty acids, detrimental for human health. The atherogenic index for all treatments was indicative of low cardiovascular disease risk. It was concluded that *C. bipinnatus* silages can be a viable alternative to be used in these systems with a health beneficial fatty acid profile in milk.

**Key words:** Alternative forage,  $\Delta 9$ -desaturase, milk quality, PUFA, weeds.

## INTRODUCTION

In developing countries, small-scale dairy systems can contribute to food security and improved livelihoods, and the reduction of rural poverty. However, small scale dairy systems (SSDS) are vulnerable to climatic and/or socioeconomic crises (Alders et al., 2021); consequently, production costs increase.

In the central highlands of Mexico, with a very marked dry season and altered rainfall patterns due to possible effects of climate change, forage alternatives are required given the scarcity in the quantity and quality of forage traditionally used in the area, such as maize for silage (Gómez-Miranda et al., 2023; Vega-García et al., 2023).

In terms of dire need, the use of secondary spontaneous vegetation such as cosmos (*Cosmos bipinnatus* Cav.), sometimes fed fresh in traditional family livestock farming systems (Martínez-Loperena et al., 2011), has been proposed as a viable forage alternative.

On the other hand, Hernández-Pineda et al. (2018) reported a reduction of 16% in methane emissions when 0.5 kg cosmos was included in the diet of dairy cows, and Vázquez-Carrillo et al. (2020) reported the reduction in methane yield when cosmos was included in the diet of intensively fed beef steers. These effects were probably modulated by the high secondary metabolite content of cosmos (Jang et al., 2008; Díaz-Medina et al., 2021). If cosmos does affect rumen fermentation expressed as reduced methane emissions, it may also affect the biohydrogenation processes and therefore, the lipid profile in milk as stated by Lourenço et al. (2008).

There is little nutritional information on cosmos as a forage resource, both in terms of cow performance as well as in the content of fatty acids (FA) and their possible effects on milk, since forages are the main source of polyunsaturated fatty acids (PUFA) for dairy cows, which are beneficial for human health.

Polyunsaturated fatty acids from forage, such as linoleic and linolenic acids, undergo microbial biohydrogenation at the rumen level, which results in the formation of intermediate fatty acids such as vaccenic and rumenic acids (Mejía-Urbe et al., 2022); where the latter influences the prevention and control of multiple diseases in humans. In addition, much importance has been given to the manipulation of the fatty acid contents of milk by changing the forage fed (Freitas et al., 2019).

Therefore, the objective was to determine the fatty acid profile of two silages with a high content of cosmos compared to maize silage, and their effect on the fatty acid profile of milk in small scale dairy systems in the central highlands of Mexico.

## MATERIAL AND METHODS

The study was undertaken in the central highlands of Mexico, in the Municipality of Aculco (20°00'-20°17' N, 99°40'-100°00' W; 2440 m a.s.l.), with a temperate sub-humid climate and a mean annual temperature of 13.2 °C, mean annual rainfall 850 mm (INEGI, 2010). The study was in collaboration with a small-scale dairy farmer through a participatory on-farm experiment following a livestock technology development approach (Conroy, 2005).

Silages with a high proportion of *Cosmos bipinnatus* Cav. were obtained from two plots 1.0 ha each sown with barley (*Hordeum vulgare* L.) 'Jennifer', the primary growth grazed by dairy cows, and the secondary growth left to accumulate for silage, evaluating their potential as a dual-purpose forage crop as has been reported for other small-grain cereals (Vega-García et al., 2023). No herbicides were used in the barley crops to reduce forage costs and improve the sustainability of the system, so that the fields were invaded with grasses and weeds, especially *C. bipinnatus*.

Botanical composition of the plots was determined before ensiling by cutting to ground level within six 0.5 m<sup>2</sup> square metal frames per plot and separating plant species by hand. Plot 1 had 43% *C. bipinnatus*, 33% barley and 24% other weeds; while Plot 2 had 62% cosmos, 4% barley, and 34% other weeds. These silages were termed CB40 and CB60 from their cosmos content respectively.

The control treatment was maize (*Zea mays* L.) silage, purchased locally by the participating farmer and made from a landrace *criollo* maize, with levels above 40% DM, which could indicate a late harvest as described by Gómez-Miranda et al. (2023). The high cosmos and the maize forages were ensiled in ground silos covered with black plastic sheet and soil and opened 128 d later.

### Animals

Nine multiparous late lactation Holstein cows were used; grouped in trios according to days in milk (DM), milk yield (MY), live weight (LW), and body condition score (BCS), as follows: A = MY18.4 ± 4.2 kg cow<sup>-1</sup> d<sup>-1</sup>; B = MY 9.0 ± 1.6 kg cow<sup>-1</sup> d<sup>-1</sup>, and C = MY 6.7 ± 1.4 kg cow<sup>-1</sup> d<sup>-1</sup>. Milking was by hand twice daily at 07:00 and 17:00 h, recording MY (kg milk cow<sup>-1</sup> d<sup>-1</sup>) with a clock scale during the measurement of variables days, taking a milk sample per cow that was kept refrigerated for later analysis (Radonjic et al., 2019). Milk fat, protein and lactose content were determined by an ultrasound milk analyzer (Ekomilk Bond, Ekomilk, Stara Zagora, Bulgaria) on aliquots of samples taken from each milking. Milk was expressed as 3.5% fat corrected milk.

The management of cows over the experiment as well as fieldwork with the collaborating farmer followed methods and practices accepted by Universidad Autónoma del Estado de México (DICARM-0221).

## Treatments

Treatments were: CB40 = 10 kg DM cow<sup>-1</sup> d<sup>-1</sup> silage with 40% Cosmos, CB60 = 10 kg DM cow<sup>-1</sup> d<sup>-1</sup> silage with 60% Cosmos, and MZSL = 10 kg DM cow<sup>-1</sup> d<sup>-1</sup> maize silage. Each cow also received 4.1 kg DM d<sup>-1</sup> of commercial 18% crude protein (CP) concentrate in two meals per day and water was freely available in the pen.

Table 1 shows the chemical composition of silages, where CB40 and CB60 had similar crude protein (CP) content to MZSL, but with higher acid detergent fiber (ADF) resulting in lower in vitro DM digestibility (IVDMD), and hence, lower estimated metabolizable energy content as the cosmos proportion increased.

**Table 1.** Chemical composition of silages (g kg<sup>-1</sup> DM). CB40: Diet silage with 40% *Cosmos bipinnatus*; CB60: diet silage with 60% *C. bipinnatus*; MZSL: diet with maize silage.

	Treatments		
	CB40	CB60	MZSL
Dry matter, g kg <sup>-1</sup>	443	416	457
Organic matter, g kg <sup>-1</sup> DM	888	886	899
Crude protein, g kg <sup>-1</sup> DM	98	97	103
Neutral detergent fiber, g kg <sup>-1</sup> DM	571	570	540
Acid detergent fiber, g kg <sup>-1</sup> DM	377	382	282
In vitro dry matter digestibility, g kg <sup>-1</sup> DM	566	496	611
Estimated metabolizable energy, MJ kg <sup>-1</sup> DM	8	7	9

## Experimental design and statistical analyses

The experiment used a 3×3 Latin square design repeated three times, with randomization in the sequence of treatments in the first and third frames, while in the second frame the allocation was mirrored to minimize residual treatment effects as reported in previous work (Gómez Miranda et al., 2023). The duration of the experiment consisted of 42 d divided into three 14 d experimental periods, with 10 d adaptation to the diet and the last 4 d of measurement (productive response, refusals and sampling). Short periods in experiments in milk production systems are well established and accepted (Miguel et al., 2014; Vega-García et al., 2023; Gómez-Miranda et al., 2023). The mathematical model used was as follows:

$$Y_{ijkl} = \mu + S_i + C_j(S_i) + P_k + T_l + e_{ijklm}$$

where  $Y_{ijkl}$  is response variable;  $\mu$  is population mean;  $S_i$  is effect of squares,  $i$  is A, B, C;  $C_j(S_i)$  is effect of cows within squares,  $j$  is 1, 2, ... 9;  $P_k$  is effect of experimental periods,  $k$  is 1, 2, 3;  $T_l$  is effect of treatment,  $l$  is CB40, CB60, MZSL;  $e_{ijklm}$  is residual variation. Data were analyzed by ANOVA; and if significant differences were detected among means, the Tukey's test was applied ( $p \leq 0.05$ ).

## Fatty acid profile on feeds and milk

Fatty acids (FA) in feed were determined by the technique described by Sukhija and Palmquist (1988) with modifications by Palmquist and Jenkins (2003), using 10% methanolic hydrochloric acid and hexane as organic solvent. For the analysis of FA in milk, fat was extracted by the ultracentrifugation method described by Feng et al. (2004) and methylated according to the methods described by Christie (1982) with modifications by Chouinard et al. (1999).

Methyl esters were separated and quantified using a gas chromatograph equipped with an autosampler (Perkin Elmer Clarus 500), with a 100 m × 0.25 mm × 0.20 μm capillary column (SUPELCO-2560), using N<sub>2</sub> as carrier gas (Limón-Hernández et al., 2019). The detector and injector were kept at 260 °C, the oven started with a temperature of 140 °C for 5 min, increasing 4 °C per minute until 240 °C was reached. Identification of FA in the chromatogram was performed using the retention times of methyl ester standards (Supelco 37 Component FAME Mix, *trans*-vaccenic acid and conjugated linoleic acid from SIGMA-Aldrich). Fatty acids are reported in g 100 g<sup>-1</sup> total FA.

Calculation of the atherogenic index was from the equation by Chen and Liu (2020), derived from lauric (C12:0), myristic (C14:0), and palmitic (C16:0) FA, divided by the unsaturated FA content.

## RESULTS AND DISCUSSION

Factors as the proportion of forage in the diet, or the presence of secondary metabolites are able to modify both the rumen microbiota that affects ruminal biohydrogenation, as well as the amount of precursors of beneficial fatty acids (FA) to be secreted in milk.

Silages of plants with secondary metabolites have shown to affect the FA profile in milk (Rufino-Moya et al., 2022). Also, FA profile of forages has a direct effect on the formation of precursors of beneficial FA that will be in the milk; which is directly related to the origin of FA in the forage, as well as the biohydrogenation process occurring in the rumen (Djordjevic et al., 2019), which can be affected by the nutritional composition of the forages including secondary metabolites such as is the case of *Cosmos bipinnatus* (Díaz-Medina et al., 2021).

However, as there is no information on the lipid profile of cosmos, fresh or preserved, so this research sheds light on its lipid composition and how it can influence FA profile of milk.

### Fatty acid profile of feeds

Short chain fatty acids (SCFAs) in silages had a significant difference ( $p < 0.05$ ), with 40% cosmos silage (CB40) having 60% more than 60% cosmos silage (CB60) or maize silage (MZSL); while CB60 had significantly ( $p < 0.05$ ) 17% and 30% higher medium chain FA (MFA) compared to CB40 and MZSL respectively. The content of long chain FA was up to 30% higher in MZSL than in the cosmos silages (Table 2).

**Table 2.** Fatty acid (FA) profile (g 100 g<sup>-1</sup> FA) of forage and concentrate. Values with different letters are significantly different at  $p < 0.05$ . CB40: Silage with 40% *Cosmos bipinnatus*; CB60: silage with 60% *C. bipinnatus*; MZSL: maize silage; SEM: standard error of the mean; CC: commercial concentrate; SFA: saturated FA; PUFA: polyunsaturated FA; MUFA: monounsaturated FA; UFA: unsaturated FA; n3: omega 3; n6: omega 6.

Fatty acid	Silages			SEM	p-value	CC
	CB40	CB60	MZSL			
Butyric (C4:0)	6.89 <sup>a</sup>	3.03 <sup>ab</sup>	1.94 <sup>b</sup>	0.735	0.027	0.75
Lauric (C12:0)	0.50	0.50	0.57	0.011	0.607	2.61
Myristic (C14:0)	2.31 <sup>a</sup>	2.74 <sup>a</sup>	1.21 <sup>b</sup>	1.029	0.012	1.47
Pentadecanoic (C15:0)	0.45 <sup>b</sup>	0.59 <sup>a</sup>	0.2 <sup>c</sup>	0.056	0.002	0.17
Palmitic (C16:0)	37.19 <sup>b</sup>	44.48 <sup>a</sup>	27.01 <sup>c</sup>	2.481	0.000	24.13
Palmitoleic (C16:1)	0.55 <sup>b</sup>	0.75 <sup>b</sup>	1.61 <sup>a</sup>	0.159	0.000	0.31
Stearic (C18:0)	8.01 <sup>b</sup>	11.09 <sup>a</sup>	5.01 <sup>c</sup>	0.860	0.002	3.43
Oleic (C18:1 n-9)	12.66 <sup>b</sup>	10.36 <sup>b</sup>	24.97 <sup>a</sup>	2.221	0.003	27.04
Linoleic (C18:2 n-6)	17.50 <sup>b</sup>	10.98 <sup>b</sup>	29.6 <sup>a</sup>	2.671	0.002	35.44
Araquidic (C20:0)	3.89 <sup>ab</sup>	4.77 <sup>a</sup>	1.2 <sup>b</sup>	0.526	0.042	0.4
Linolenic (C18:3 n-3)	5.55	6.37	4.75	0.125	0.70	1.21
Behenic (C22:0)	1.46	1.98	0.74	0.176	0.253	0.20
Tricosanoic (C23:0)	0.86	0.53	0.27	0.084	0.431	0.10
Others	2.13	1.78	0.89	0.259	0.324	2.82
Chain length						
Short chain	9.14 <sup>a</sup>	3.54 <sup>b</sup>	2.88 <sup>b</sup>	0.973	0.016	5.49
Middle chain	40.89 <sup>b</sup>	49.74 <sup>a</sup>	30.56 <sup>c</sup>	2.715	0.000	26.24
Long chain	49.96 <sup>b</sup>	46.71 <sup>b</sup>	66.55 <sup>a</sup>	3.009	0.000	68.27
Saturation degree						
SFA	62.9 <sup>b</sup>	69.8 <sup>a</sup>	38.8 <sup>c</sup>	4.60	0.000	35.4
PUFA	23.1 <sup>b</sup>	17.4 <sup>b</sup>	34.4 <sup>a</sup>	2.44	0.009	36.8
MUFA	14.1 <sup>b</sup>	12.8 <sup>b</sup>	26.8 <sup>a</sup>	2.19	0.004	27.7
Category						
Total of n3	5.55	6.37	4.75	0.190	0.703	27.1
Total of n6	17.5 <sup>b</sup>	10.9 <sup>b</sup>	29.6 <sup>a</sup>	2.226	0.002	35.4
Relations						
UFA/SFA	0.59 <sup>b</sup>	0.43 <sup>c</sup>	1.57 <sup>a</sup>	0.175	0.0001	1.80
n3/n6	0.31	0.59	0.16	0.052	0.222	0.76

In the degree of saturation, saturated fatty acids (SFA) in the silages showed significant differences ( $p < 0.05$ ), where CB60 had 44% higher SFA than MZSL and 9.8% higher than CB40, related with the contents of unsaturated FA in CB60 (Table 2). Forages are usually the main source of polyunsaturated fatty acids (PUFA), particularly conjugated linolenic acid (Prieto-Manrique et al., 2016), which is why, in terms of animal feed, attempts have been made to increase their content through the diet.

In an in vitro experiment, Limón-Hernández et al. (2019) used *Brassica napus* as an alternative forage in microsilos to increase the PUFA content of rations, obtaining a content of up to 54.1% PUFA; much higher contents than those reported in the silages with a high proportion of *Cosmos bipinnatus* or maize in this work.

It is important to know the nutritional characteristics of forages, such as their FA profile, as this allows us to understand more fully their relationship with productive responses. Studies have reported that native forage species increase PUFA values (Pérez-Reverón et al., 2024).

Given the interest of consumers in the quantity and quality of fats in their diets, wild plants of the same subfamily (*Asteroidae*) have therapeutic applications. In this regard, butyric acid has been considered as an important inhibitor of cancer cells (Techeira et al., 2023). Regarding this FA, in this experiment there was a large concentration found in silages with the presence of cosmos, even higher (up to 5 times more in CB40) than those previously reported in annual cereal silages in the same region (Vega-García et al., 2020; Colín-Navarro et al., 2021).

This can be related to the type of fermentation given by each organic substrate in the silage and the time of harvesting, the part of the plant analyzed and its processing are three of the multiple factors that change the composition of FA in plants (Kokten et al., 2023).

In addition, it should be considered that there is a loss unsaturated FA in the transition from fresh to ensiled forage due to oxidative losses of PUFA during the cutting process and at the end of the aerobic phase (Rufino-Moya et al., 2022). As mentioned by Acosta et al. (2022) there is great variation in the FA profile due to the forage species, especially in the concentrations of linoleic and  $\alpha$ -linolenic acids, which are related to the differences found between the treatments in this work.

### Fatty acid profile of milk

The productive response showed no differences between treatments ( $p > 0.05$ ) for any variable as shown in Table 3, although high fat and low protein levels were observed during the experiment.

Table 4 shows the lipid profile of milk, where the contents of FA C8:0 and C10:0 were 9.6% lower in CB60 compared to MZSL, similar case as for C12:0 (7.8%), where CB40 showed no differences; C14:0 in CB60 presented 4.5% less; and C15:0 was 11% higher in MZSL compared to CB40 ( $p < 0.05$ ).

In terms of unsaturated FA, C15:1 was 5.2% higher in MZSL and C16:1 was 8.3% lower in CB40 ( $p < 0.05$ ); and a 4.3% higher content of linoleic acid (C18:2n6t) was observed in CB40 ( $p = 0.05$ ).

The lipid profile by experimental periods showed an overall significant increase in period 2, with a significant decrease in C18:2n6c ( $p < 0.0001$ ), and an increase in C18:2c9t11 ( $p < 0.0001$ ).

Short chain FA were 9.6% higher in MZSL compared to CB60, reflected in a 3% decrease in long chain FA ( $p < 0.05$ ); and polyunsaturated FA (PUFA) were 8% higher in CB60 compared to MZSL, with CB40 with intermediate values ( $p > 0.05$ ). Results for the experiment by periods showed a decrease of total n3 progressively by 20% over time; the opposite was the case with the atherogenicity index, which increased by up to 8% (Table 4).

It has been mentioned that milk fat is one of the most complex edible fats as it harbors more than 400 FA of various categories with unique physicochemical and biological properties, several of them with important properties for human health as they contribute between 16%-26% of the omega-3 FA in the human diet (Djordjevic et al., 2019; Mollica et al., 2021). The FA profile in milk is a determining factor in determining its nutritional value, being of great interest the contents of conjugated linoleic acid (CLA) given its beneficial effects on human health (Minieri et al., 2020).

Literature review the response in FA of milk from diets based on diverse vegetation with a higher content of short-chain FA, but lower than medium-chain FA and higher in long-chain FA than reported in this work (Elgersma, 2015; Timlin et al., 2023); which can be attributed to the methods of forage conservation (silage) and the amount of secondary metabolites of each plant; which may be due as Kalač and Samková (2010) mentioned, to the effect of lipolysis by the ensiling process.

**Table 3.** Milk fatty acid (FA) profile (g 100 g<sup>-1</sup> FA) by treatment and experimental period. Values with different letters are significantly different at  $p < 0.05$ . CB40: Silage with 40% *Cosmos bipinnatus*; CB60: silage with 60% *C. bipinnatus*; MZSL: maize silage; SEM: standard error of the mean.

	Treatments			SEM	<i>p</i> -value
	CB40	CB60	MZSL		
Butyric (C4:0)	2.07	2.25	2.61	0.021	0.070
Caproic (C6:0)	1.70	1.78	1.87	0.006	0.220
Caprylic (C8:0)	0.87 <sup>ab</sup>	0.83 <sup>b</sup>	0.92 <sup>a</sup>	0.003	0.040
Capric (C10:0)	1.70 <sup>ab</sup>	1.60 <sup>b</sup>	1.77 <sup>a</sup>	0.006	0.020
Lauric (C12:0)	2.02 <sup>ab</sup>	1.89 <sup>b</sup>	2.05 <sup>a</sup>	0.006	0.050
Myristic (C14:0)	9.56 <sup>a</sup>	9.14 <sup>b</sup>	9.58 <sup>a</sup>	0.019	0.030
Myristoleic (C14:1)	0.62	0.60	0.63	0.001	0.060
Pentadecanoic (C15:0)	0.70 <sup>b</sup>	0.71 <sup>ab</sup>	0.79 <sup>a</sup>	0.003	0.020
<i>cis</i> -10-Pentadecanoic (C15:1)	1.35 <sup>a</sup>	1.34 <sup>a</sup>	1.28 <sup>b</sup>	0.003	0.030
Palmitic (C16:0)	31.19	31.12	31.12	0.003	0.990
Palmitoleic (C16:1)	1.54 <sup>b</sup>	1.65 <sup>a</sup>	1.68 <sup>a</sup>	0.005	0.010
Heptadecanoic (C17:0)	0.89	0.87	0.84	0.001	0.200
Stearic (C18:0)	15.00	14.86	14.37	0.026	0.090
Vaccenic (C18:1t11)	1.73	1.66	1.52	0.008	0.250
Oleic (C18:1c9)	23.94	24.56	24.09	0.025	0.460
Linolelaidic (C18:2n6t)	0.23 <sup>a</sup>	0.22 <sup>b</sup>	0.22 <sup>b</sup>	0.000	0.050
Linoleic (C18:2n6c)	2.20	2.30	2.10	0.007	0.080
Linolenic (C18:3n3)	0.27	0.26	0.24	0.001	0.140
Rumenic (C18:2c9t11)	0.93	1.01	0.91	0.004	0.080
Others	1.41	1.28	1.31	0.005	0.100
	Periods			SEM	<i>p</i> -value
	1	2	3		
Butyric (C4:0)	2.16 <sup>b</sup>	2.8 <sup>a</sup>	1.98 <sup>b</sup>	0.034	0.002
Caproic (C6:0)	1.68	1.82	1.85	0.007	1.777
Caprylic (C8:0)	0.82	0.89	0.90	0.003	0.072
Capric (C10:0)	1.54 <sup>b</sup>	1.75 <sup>a</sup>	1.79 <sup>a</sup>	0.010	0.000
Lauric (C12:0)	1.85 <sup>b</sup>	2.04 <sup>a</sup>	2.07 <sup>a</sup>	0.006	0.002
Myristic (C14:0)	9.01 <sup>b</sup>	9.50 <sup>a</sup>	9.77 <sup>a</sup>	0.030	0.000
Myristoleic (C14:1)	0.64 <sup>a</sup>	0.59 <sup>b</sup>	0.62 <sup>ab</sup>	0.001	0.009
Pentadecanoic (C15:0)	0.65 <sup>b</sup>	0.77 <sup>a</sup>	0.78 <sup>a</sup>	0.005	0.001
<i>cis</i> -10-Pentadecanoic (C15:1)	1.38 <sup>a</sup>	1.28 <sup>b</sup>	1.31 <sup>b</sup>	0.003	0.005
Palmitic (C16:0)	31.38	31.16	30.88	0.019	0.700
Palmitoleic (C16:1)	1.65	1.61	1.61	0.001	0.465
Heptadecanoic (C17:0)	0.93 <sup>a</sup>	0.81 <sup>b</sup>	0.85 <sup>b</sup>	0.004	0.000
Stearic (C18:0)	14.70 <sup>ab</sup>	15.35 <sup>a</sup>	14.16 <sup>b</sup>	0.040	0.001
Vaccenic (C18:1t11)	1.44 <sup>b</sup>	1.56 <sup>b</sup>	1.91 <sup>a</sup>	0.019	0.001
Oleic (C18:1c9)	25.16 <sup>a</sup>	24.11 <sup>ab</sup>	23.33 <sup>b</sup>	0.710	0.003
Linolelaidic (C18:2n6t)	0.23	0.22	0.22	0.000	0.370
Linoleic (C18:2n6c)	2.17 <sup>ab</sup>	2.38 <sup>a</sup>	2.05 <sup>b</sup>	0.013	0.001
Linolenic (C18:3n3)	0.32 <sup>a</sup>	0.26 <sup>b</sup>	0.19 <sup>c</sup>	0.005	0.000
Rumenic (C18:2c9t11)	0.79 <sup>c</sup>	0.95 <sup>b</sup>	1.11 <sup>a</sup>	0.012	0.000
Others	1.41	1.25	1.34	0.006	0.064

**Table 4.** Milk fatty acid (FA) profile according to category. Values with different letters are significantly different at  $p < 0.05$ . CB40: Silage with 40% *Cosmos bipinnatus*; CB60: silage with 60% *C. bipinnatus*; MZSL: maize silage; SEM: standard error of the mean; SFA: saturated FA; PUFA: polyunsaturated FA; MUFA: monounsaturated FA; UFA: unsaturated FA; n3: omega 3 FA; n6: omega 6 FA; CLA: conjugated linoleic acid.

	Treatments			SEM	p-value
	CB40	CB60	MZSL		
Chain length					
Short chain	8.75 <sup>ab</sup>	8.66 <sup>b</sup>	9.58 <sup>a</sup>	0.039	0.030
Middle chain	46.09	45.72	46.19	0.019	0.660
Long chain	45.15 <sup>a</sup>	45.61 <sup>a</sup>	44.22 <sup>b</sup>	0.055	0.050
Others	1.41	1.28	1.31	0.005	0.100
Saturation degree					
SFA	68.23	67.505	68.31	0.035	0.360
PUFA	3.71 <sup>ab</sup>	3.83 <sup>a</sup>	3.52 <sup>b</sup>	0.011	0.010
MUFA	29.63	30.27	29.67	0.027	0.470
Category					
Total of n3	0.27	0.26	0.24	0.001	0.140
Total of n6	2.44	2.52	2.33	0.007	0.090
Relations					
UFA/SFA	0.49	0.50	0.49	0.001	0.350
n3/n6	0.11	0.10	0.10	0.001	0.290
Atherogenicity index	2.158	2.06	2.177	0.004	0.130
Δ9-desaturase product/substrate ratio					
C14:0/C14:1	0.06	0.06	0.06	0.000	0.667
Palmitic/Palmitoleic	0.04	0.05	0.05	0.000	0.087
Stearic/Oleic	1.60	1.69	1.72	0.004	0.072
CLA/Vaccenic	1.81	1.67	1.70	0.005	0.593
	Periods			SEM	p-value
Category	1	2	3		
Chain length					
Short chain	8.41 <sup>b</sup>	9.64 <sup>a</sup>	8.94 <sup>ab</sup>	0.048	0.000
Middle chain	45.93	46.03	46.03	0.004	0.970
Long chain	45.61	45.15	44.22	0.05	0.070
Saturation degree					
SFA	67.28	68.08	68.68	0.055	0.090
PUFA	3.56 <sup>b</sup>	3.85 <sup>a</sup>	3.65 <sup>ab</sup>	0.001	0.018
MUFA	30.79 <sup>a</sup>	29.61 <sup>ab</sup>	29.17 <sup>b</sup>	0.065	0.021
Category					
Total of n3	0.32 <sup>a</sup>	0.26 <sup>b</sup>	0.19 <sup>c</sup>	0.005	0.000
Total of n6	2.41 <sup>ab</sup>	2.60 <sup>a</sup>	2.28 <sup>b</sup>	0.012	0.002
Relations					
UFA/SFA	0.50	0.49	0.48	0.001	0.060
n3/n6	0.13 <sup>a</sup>	0.10 <sup>b</sup>	0.08 <sup>c</sup>	0.002	0.000
Atherogenicity index	2.04 <sup>b</sup>	2.14 <sup>ab</sup>	2.21 <sup>a</sup>	0.006	0.020
Δ9-desaturase product/substrate ratio					
Myristic-Myristoleic	0.06 <sup>a</sup>	0.05 <sup>b</sup>	0.05 <sup>b</sup>	0.0001	0.000
Palmitic/Palmitoleic	0.05	0.05	0.05	0.0001	0.583
Stearic/Oleic	1.70 <sup>a</sup>	1.70 <sup>a</sup>	1.50 <sup>b</sup>	0.006	0.010
CLA/Vaccenic	1.76	1.64	1.78	0.005	0.590

On the other hand, it is also recognized that the amount of secondary metabolites in forages can alter the rumen microbiota or interfere with biohydrogenation as in the case of *C. bipinnatus*, resulting in differences in the lipid profile between treatments. The increase in short chain FA may be related to rumen microbial fermentation.

The lipid profile of treatments CB40 and CB60 were lower than reported by Delgadillo-Puga et al. (2014) and Villeneuve et al. (2013), but similar to that reported by Schwendel et al. (2017) in organic production systems. According to Markiewicz-Kęszycka et al. (2013), the proportion of mono-unsaturated fatty acids (MUFA) in cow's milk should be around 20%-35%, in addition to containing around 24% oleic acid, and small amounts of other MUFA such as myristoleic acid (1%), palmitoleic (1.5) and vaccenic acid (1.5%-5.0%), so the MUFA content in the milk of this experiment is within the desirable ranges.

It has been reported that some SFA such as lauric, myristic and palmitic are associated with increased serum cholesterol in humans (Parodi, 2009). In this study, lauric and myristic acids decreased in CB60, the treatment where the presence of *C. bipinnatus* was higher. Also, the atherogenicity index for all treatments was under 4.0, indicative of low cardiovascular disease risk (Nantapo et al., 2014).

## CONCLUSIONS

It is concluded that silages with a high proportion of *Cosmos bipinnatus* have benefits on the fatty acid profile in milk as it increases the polyunsaturated content (positively associated with human health) and decreases the content of some saturated fatty acids (lauric and myristic acids), which are negatively associated with human health, compared to maize silage, which is the usual silage in these production systems.

### Author contribution

Conceptualization: C.M.A-J. Methodology: A.G-M., F.L-G. Validation: C.M.A-J. Analysis Laboratory: A.G-M., J.I.V-G., J.J.C-D. Investigation: A.G-M., J.I.V-G., J.J.C-D. Resources: C.M.A-J. Data curation: A.G-M., F.L-G. Writing original draft: A.G-M., J.I.V-G. Writing-review & editing: C.M.A-J., F.L-G. Translation: C.M.A-J. Visualization: C.M.A-J. Supervision: C.M.A-J. Project administration: C.M.A-J. Funding acquisition: C.M.A-J. All co-authors reviewed the final version and approved the manuscript before submission.

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