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Fatty Acid Profile in Milk of Jersey Cows Fed Green Forage or Hay

Research Article

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ABSTRACT

The objective of this study was to determine the concentration of fatty acids (FA) especially conjugated linoleic and trans fatty acids in milk from Jersey cows fed green alfalfa (GA) or alfalfa hay (AH). Forty Jersey cows that had from two to five calvings, 60 to 90 lactation days and were not pregnant were used in a confined production system. In one treatment (GA n=20) cows were fed chopped fresh green alfalfa and commercial concentrate. In the other one (AH n=20), they were fed alfalfa hay and the same commercial concentrate. A milk sample (10 mL) was collected from each cow. Total lipids, methyl-esterified fatty acids (FA), and conjugated linoleic acids (CLA) were determined by chromatography. The amount of saturated, monounsaturated and polyunsaturated FA were similar between treatments (P>0.05). The content of FA omega-3 and omega-6, as well as the proportion of omega-6:omega-3 were not affected by the type of forage (P>0.05). However, the concentrations of vaccenic acid-trans (P<0.01), rumenic acid (P<0.01), total FA trans (P<0.001), and total CLA (P<0.01) were higher in cows fed GA. We conclude that consumption of green chopped alfalfa in Jersey cows increased production of 18:1 n-7 trans-11 trans-vaccenic acid and CLA in milk.

KEY WORDS conjugated linoleic acids, dairy cows, fatty acids n-3, green alfalfa, vaccenic acid.

INTRODUCTION

The milk fat of ruminants contains 96-99% triglycerides (Barłowska and Litwińczuk, 2009), composed of 65-75% saturated fatty acids (SFA), and 30% of unsaturated fatty acids, mostly monounsaturated fatty acids (MUFA), with much less polyunsaturated fatty acids (PUFA). Fat percentage in milk and its FA content in ruminants depends on genetic, age, lactation state, environmental factors (Lock and Garnsworthy, 2003) and the animal's diet (Collomb *et al.* 2008), with particular emphasis on the type of forage consumed (Elgersma *et al.* 2006). Izumi *et al.* (2002) reported that the percentage of α- linolenic acid decreases to half, and simultaneously, the total fatty acids decrease to one-third, from fresh grass to hay. Therefore, the amount of α-linolenic acid in hay is reduced to approximately one-sixth of that in fresh grass. Oxidative loss of PUFA during field wilting represents a major loss in the food chain, with substantial losses of 18:3 n-3 during hay making and modest losses during wilting prior to ensiling. These losses are associated with the lipoxygenase system, a plant defence mechanism initiated in damaged tissues (Dewhurst *et al.* 2006). A diet based on fresh forage increases the amount of some FA that might have nutraceutical properties, particularly conjugated linoleic acids (CLA) and FA omega-3 (Nałęcz-Tarwacka *et al.* 2009; Falchero *et al.* 2010). The CLA represent a heterogeneous group of isomers of linoleic acids that are found mainly in ruminants' milk and meat

(Steinhart *et al*. 2003; Benjamin *et al*. 2005). When ruminants consume green forage, α-linolenic and linoleic acid from the feed are biohydrogenated by rumen microbes (Jenkins *et al*. 2008), which produce isomers such as CLA 18:2 n-7 cis-9 trans-11 (rumenic acid), 18:1 n-7 trans-11 or vaccenic trans acid (Palmquist, 1988). The CLA cis-9 trans-11 is especially important for human health because of its anti-cancer and anti-diabetes properties (Corl *et al*. 2003; Prieto-Manrique *et al*. 2018). These and other FA can escape further biohydrogenation and reach the small intestine, where they are absorbed and transported to the mammary gland that uses vaccenic *trans* as substrate for rumenic acid synthesis (Kay *et al*. 2008), which represents more than 82% of the CLA isomers in milk products (Chin *et al*. 1992). When cows graze, there are 500% more CLA in milk compared to cows that consume diets based on conserved forage or grain (Dhiman *et al*. 1999). Based on the previous information, the objective of this study was to determine the FA content especially conjugated linoleic and trans fatty acids in milk of Jersey cows fed alfalfa hay or green alfalfa.

MATERIALS AND METHODS

The work was performed in La Paz, Baja California Sur (BCS), México located at 26 º 24' 16" N and 109 °54' 49" W with Jersey cows (n=40) of 450 ± 25 kg live weight and body condition of 3.5 ± 0.2 in a free stall housing production system. Cows had from two to five births, 60-90 lactation days and were not pregnant. Average production up to sampling was 15.0 ± 4.0 kg milk per day. Cows were randomly assigned to one of the two treatments. In treatment GA (n=20) cows were fed with chopped green alfalfa plus commercial concentrate. In treatment AH (n=20) they were fed with alfalfa hay (10-15 percent humidity) plus the same commercial concentrate for at least 60 days. In both groups, forage was offered throughout lactation considering daily consumption of dry matter per animal equivalent to 3% of live weight. The concentrate, based on cereals (14.5 percent of CP, maximum humidity 12%, minimum fat 2.20%, maximum fiber 9.5%, maximum ash 5.80%, nitrogen-free extract 56% and 1.5 Mcal de NE_L/kg) was offered individually during the two milking daily (2 kg per animal). By manual milking, 10 mL of milk of a single quarter were collected from each cow when it was between 60 and 90 days in lactation, because there is no effect on fatty acids in relation to the time of lactation (Kelsey *et al*. 2003). Milk samples were conserved in sterile test tubes and frozen at -80 ˚C until their chemical analysis was performed. Each milk sample was analyzed in triplicate, considering the mean of the three analysis as a repetition in the respective treatment.

Lipid extraction was performed using 2 mL of milk, to which 18 ml of chloroform methanol (1:2) were added in a vial containing butylated hydroxytoluene (BHT) as antioxidant and tricosanoic acid (23:0) as internal standard. Lipids were extracted according to the methodology proposed by (Bligh and Dyer, 1959). The extracted lipids were conserved in a nitrogen-saturated atmosphere until their analysis. Total lipid quantification was performed by the gravimetric method (Folch *et al*. 1956; Toyes-Vargas *et al*. 2016).

Milk samples were transesterified as follows Chand *et al*. (2001); 1 mL of BF3-methanol (10 percent) was added to the milk sample; then, 200 µL of KOH at 1.5 percent were added at 30 ˚C for 30 min.

Separation of CLA and fatty acid methyl esters (FAME) was performed using a DB-23 column of melted silica (50%-cyanopropyl)-methylpolysiloxane) of 60 m × 0.25 mm ID × 0.25 mm film (J&W Scientific, Inc.) from 60 to 220 ˚C temperature range using a flame ionization detector (FID) adapted in a gas chromatography (GC) 6890N Agilent Technologies.

FAME identification and quantification were made based on external and internal standards, respectively (Sigma; Bellefonte, PA, USA). CLA (18:2 n-7 cis-9 trans-11 and 18:2 n-6 trans-10 cis-12) were identified using external standards (Nu-Chek Prep, Inc, Elysian, MN, USA), and 18:1 n-7 trans-11 of Sigma (Bellefonte, PA, USA) and its identity were verified with a GC connected to a mass spectrophotometer. The treatment effect on the amount of FA in milk (g/100 g) was determined by a *t*-test procedure using SAS (SAS, 2001).

RESULTS AND DISCUSSION

Total lipids

Total lipid concentration in milk of cows fed with AH (5.5±1.1 g/100 g) or GA (1.7±0.4 g/100 g) was significantly different (P<0.05).

Saturated fatty acids

The content of SFA in milk is shown in Table 1. The total SFA was similar (P>0.05) between both AH and GA groups. Almost 55 to 58 % of the FA in both treatments was SFA.

Monounsaturated fatty acids

Total MUFA was similar between AH and GA, and individual concentration of the majority (Table 2) did not differ between treatments (P>0.05). The content of 16:1 n-9, 20:1 n-9, 20:1 n-11, and 20:1 n-7 was higher in cows fed GA (P<0.01; <0.05; <0.001; <0.001, respectively); of the total FA quantified, 32 and 34% resulted MUFA in GA and AH, respectively.

Table 1 Concentration (Mean±SE) of saturated fatty acids (g/100 g of FA) in milk of Jersey cows fed green alfalfa plus (GA) or alfalfa hay (AH) both plus commercial concentrate

Fatty acid	Treatment	
	GA	AH
14:0	10.07±1.08	12.63±3.80
15:0	1.49±0.11	1.11±0.71
16:0	31.18±2.26	32.30±6.46
17:0	0.83±0.12	0.78±0.23
18:0	11.03±0.88	11.00±1.72
20:0	0.10±0.03	0.19±0.06
22:0	0.38±0.06[a]	0.19±0.04[b]
Total	55.08±4.54	58.20±13.02

[a, b]: The means within the same row with different letter, are significantly different (P<0.001).

Table 2 Concentration (Media±EE) monounsaturated fatty acids (MUFA) (g/100 g de FA) in milk of Jersey cows fed with green alfalfa (GA) or alfalfa hay (AH) both plus commercial concentrate

Fatty acids	Treatment	
	GA	AH
14:1 n-8	1.15±0.19	0.88±0.44
15:1 n-8	0.51±0.10	0.74±0.84
16:1 n-9	0.33±0.14[c]	0.12±0.04[d]
16:1 n-7	1.64±0.25	1.65±0.46
16:1 n-5	0.65±0.08	0.58±0.25
17:1 n-8	0.35±0.09	0.33±0.26
18:1 n-9	20.64±2.96	21.81±6.73
18:1 n-7	0.96± 0.07	1.11±0.27
18:1 n-5	0.39±0.09	0.50±0.21
20:1 n-11	0.46±0.09[e]	0.15±0.10[f]
20:1 n-9	0.20±0.01[a]	0.10±0.01[b]
20:1 n-7	1.10±0.01[e]	0.41±0.25[f]
22:1 n-11	0.13±0.16	0.18±0.14
24:1 n-9	1.13±0.10	0.96±0.44
Total	34.21±5.64	31.97±10.79

[a, b]: The means within the same row with different letter, are significantly different (P<0.05).
[c, d]: The means within the same row with different letter, are significantly different (P<0.01).
[e, f]: The means within the same row with different letter, are significantly different (P<0.001).

Polyunsaturated fatty acids

Total PUFA did not differ between treatments (P>0.05). Table 3 shows that 20:3 n-6 was more abundant than that of group AH (P<0.01), and the concentration of 20:5 n-3 was higher in cows fed GA (P<0.01).

The rest of the PUFA analyzed resulted in a similar concentration between treatments (P>0.05). The total of FA omega-3, omega-6, and the relationship omega-6:omega-3 did not differ between treatments (P>0.05).

Table 4 shows that concentrations of 18:1 n-7 trans-11 and 18:2 n-7 cis-9 trans-11 were greater than in milk of cows in group GA (P<0.01), which contributed to making total CLA (P<0.01) and total concentration of trans FA (P<0.001) greater in milk of cows in this group.

Cows fed with green alfalfa recorded less fat percentage in milk probably as a result of less fiber content in the diet, as has been previously reported (Zebeli *et al.* 2006). Total fat in milk of the alfalfa hay group was slightly higher to the average value reported in Jersey cows (Anderson *et al.* 2007).

A higher proportion of SFA in alfalfa hay has been documented in other works (MacGibbon and Taylor, 2006; Lindmark, 2008).

In our study, the only SFA with a significantly different concentration between treatments was 22:0, which was higher in green alfalfa fed cows (P<0.001). The concentration of SFA in milk of cows fed with alfalfa hay was within the reported range in milking cows. However, in animals fed green alfalfa, the value was found lower than the minimum pointed out by Lindmark (2008), who mentioned SFA content was lower in summer when the cows are grazing fresh forage compared to winter, in which animal are stalled and consume dry feed.

MUFA values were higher than the average (26%) reported for milking cattle in grazing (Lindmark, 2008). Although the concentration of the majority of MUFA were similar between the milk from cows fed green or hay alfalfa in our study, another report has pointed out a lower concentration of MUFA in cows grazing green forage than when consuming silage (Frelich *et al.* 2009).

Table 3 Concentration (Media±EE) of polyunsaturated and highly polyunsaturated fatty acids (g/100 g de FA) in milk of Jersey cows fed green alfalfa (GA) or alfalfa hay (AH) plus commercial concentrate

Fatty acids	Treatment	
	GA	AH
Polyunsaturated		
18:2 n-6	3.42±0.38	4.14±2.51
18:3 n-6	0.20±0.02	0.18±0.04
18:3 n-3	1.79±0.34	1.34±0.34
20:2 n-6	0.11±0.02	0.05±0.05
20:3 n-6	0.18±0.03[a]	0.50±0.15[b]
Total	9.05±1.76	8.30±4.26
Highly polyunsaturated		
20:4 *n*-6	0.22±0.14	0.14±0.09
20:5 *n*-3	0.22±0.03[a]	0.15±0.05[b]
22:6 *n*-3	0.44±0.09	0.29±0.18
Total	0.88±0.26	0.58±0.32
Omega-3	2.45±0.46	1.78±0.57
Omega-6	4.13±0.59	5.01±2.84
Relationship omega-6:omega-3	1.68	2.81

[a, b]: The means within the same row with different letter, are significantly different (P<0.01).

Table 4 Concentration (Media±EE) of conjugated linoleic and trans fatty acids (g/100 g of FA) in milk of Jersey cows fed green alfalfa (GA) or alfalfa hay (AH) both plus commercial concentrate

Fatty acids	Treatment	
	GA	AH
18:1 n-9 trans	0.91±0.19	0.66±0.13
18:1 n-7 trans-11	3.66±1.11[a]	1.79±0.22[b]
18:2 n-6 trans	0.85±0.17	0.68±0.58
18:2 n-7 cis-9 trans-11	1.44±0.46[a]	0.72±0.16[b]
18:2 n-6 trans-10 cis-12	0.18±0.08	0.11±0.11
Total trans	7.04[c]	3.96[d]
18:2 n-7 cis-9 trans-11 + 18:1 n-7 trans-11	5.10	2.51
Total CLA	1.62[a]	0.83[b]

[a, b]: The means within the same row with different letter, are significantly different (P<0.01).
[c, d]: The means within the same row with different letter, are significantly different (P<0.001).

We did obtain significantly higher levels of 16:1 n-9, 20:1 n-11, 20:1 n-9 and 20:1 n-7 in the milk of cows fed green alfalfa compared to hay alfalfa. Most of these MUFA can be readily synthesized in cows when necessary.

The PUFA proportion with respect to the total FA in our study resulted approximately 9% in both treatments, which is greater than that mentioned by Lindmark (2008). The transfer rate of C18:3 n-3 from feed to milk was highest for the two herbal hay diets (Ineichen *et al*. 2019). In another study, there was a trend to an increase of total PUFA in milk when cows were fed green alfalfa compared to those fed with hay. A similar result was observed in Chinampo (*Bos taurus*) cows grazing fresh forage in an open summer pasture (sarcocaulescent scrub) or consuming alfalfa hay (Ortega-Pérez *et al*. 2013). The milk fat of cows fed with high phenolic had higher proportions of polyunsaturated fatty acids compared to that of grass hay-based diets, low in nitrogen content (Ineichen *et al*. 2019). The only PUFA significantly affected by diet was 20:3 n-6, which was higher in the hay fed group.

There were very low levels of HUFA in milk, below one percent in both groups. Only the 20:5 n-3 was significantly different among the groups, with higher levels in the milk of cows fed green alfalfa, which indicates that there was not a lack of HUFA in milk in cows fed green alfalfa; In other study, the main n-3 FA in milk fat is linolenic acid (C18:3 cis-9,12,15), but small quantities of docosahexaenoic acid (22:6 n-3) and eicosapentaenoic acid (20:5 n-3) also occur (Ferlay *et al*. 2013), in spite of higher MUFA levels in this group that usually indicate lack of unsaturated fatty acids. The effect of nutritional supplementation with sunflower seed oil was evaluated in milk cows foraging on *Leucaena leucocephala* (green forage) in intensive silvopastoral systems and these effects contributed to the production of milk with greater quantities of UFA (Prieto-Manrique *et al*. 2018).

The total trans FA were significantly higher in the milk of cows fed green alfalfa, as expected. Of these FA, vaccenic acid (18:1 n-7 trans-11) was the most abundant both in milk of green alfalfa and hay fed cows, which is consistent with results reported by Lindmark (2008).

The amount of 18:1 n-7 trans-11 in milk of cows in treatment green alfalfa was higher than that in alfalfa hay. Falchero *et al.* (2010) obtained a similar concentration to that of group AH in Red-Pie, Gray Alpine, and Tarantaise milking cows grazing green alfalfa (*Festuca nigrescens*). However, in another study with Jersey cows, the concentration of 18:1 n-7 trans-11 was similar when they were grazing green alfalfa than when a total mixed ration was offered (Palladino *et al.* 2010). A recent report mentions that when Finnish Ayrshire cows were fed fresh forage, the concentration of 18:1 n-7 trans-11 in the omasum was two times more abundant than in cows fed grass silage (Halmemies-Beauchet-Filleau *et al.* 2013).

In our study, the amount of 18:1 n-7 trans-11 in milk of cows fed green alfalfa was slightly less than the concentration obtained in grazing Flckvieh and Holstein cows (Frelich *et al.* 2009). The secretion of C18:1 t11 in relation to the amount of C18:2 n-6 + C18:3 n-3 ingested was highest for diet consisting of hays with high proportion of herbs with either low phenolic content (Ineichen *et al.* 2019).

In our work, concentration of 18:2 n-7 cis-9 trans-11 was greater in milk from the cows fed green alfalfa GA group, where an amount slightly less than 18:2 n-7 cis-9 trans-11 was observed, compared with another study (Frelich *et al.* 2009) where Italian Red-Pie, Gray Alpine, and Tarantaise milking cows grazing fresh forage (*Festuca nigrescens* and *Trifolium alpinum*) were used. In our study, we also observed that the sum of 18:2 n-6 trans-10 cis-12 and 18:2 n-7 cis-9 trans-11 contributed to a greater quantity of both trans FA and total CLA in milk of the cows fed green alfalfa.

The first step of the linoleic acid biohydrogenation pathway consists of enzymatic isomerization by cis-12, trans-11 isomerase, which turns the cis-12 bond into a trans-11. This isomerase has a specific requirement for free carboxylic groups, and particularly ones with the isolated diene characterized by a geometry cis-9, cis-12 (Buccioni *et al.* 2012).

From the 18:2 n-6 and 18:3 n-3 acids, isomers of biological value are produced such as 18:2 n-7 cis-9 trans-11 and 18:1 n-7 trans-11 (Jalc *et al.* 2007). Although a number of different forms of CLA are present in milk fat, including trans-10, cis-12 CLA, it is cis-9, trans-11 CLA that is of interest as a functional food component. CLA 18:2 n-7 cis-9 trans-11, represents 75 to 90% of the total CLA in milk fat, and its presence is related to the biohydrogenation of polyunsaturated fatty acids in the rumen (Bauman *et al.* 2006).

Although CLA 18:2 n-7 cis-9 trans-11 is an intermediate in the biohydrogenation of linoleic acid, the principal source of CLA 18:2 n-7 cis-9 trans-11 in milk fat is from endogenous synthesis in the mammary gland. Vaccenic acid, an intermediate in the rumen biohydrogenation of both linoleic and linolenic acid, is the substrate and its conversion to CLA 18:2 n-7 cis-9 trans-11 is catalyzed by Δ^9-desaturase, an enzyme present in the mammary gland and other tissues (Griinari and Bauman, 1999; Bauman *et al.* 2006; Bernard *et al.* 2009). The intake of fresh grass elevated also the concentration of CLA (Bargo *et al.* 2006); this explains why the CLA content in milk fat from cows fed green fodder (Frelich *et al.* 2009).

In general, milk from cows with daily rations of fresh grass could be successfully distinguished from milk from cows with no fresh grass in their diet (Capuano *et al.* 2014), as Jersey cows fed with chopped green alfalfa increased the amount of FA 18:1 n-7 trans-11 and of CLA, specifically, 18:2 n-7 cis-9 trans-11 in milk. This type of production system would also result in milk characterized by elevated proportions of desired fatty acids (Ineichen *et al.* 2019).

CONCLUSION

The results showed that consumption of green chopped alfalfa in Jersey cows increased production of 18:1 n-7 trans-11 trans-vaccenic acid and CLA in milk.

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