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Mexican tropical cream cheese yield using low-fat milk induced by *trans*-10, *cis*-12 conjugated linoleic acid: effect of palmitic acid

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ABSTRACT

The *trans*-10, *cis*-12 isomer of conjugated linoleic acid (CLA) reduces milk fat in dairy cows, causing a lower cheese yield. This could be mitigated by adding palmitic acid (PA) to the diet, as it stimulates fat synthesis in mammary gland. The objective of this study was to evaluate the effect of adding PA to the diet of grazing dairy cows complemented with *trans*-10, *cis*-12 CLA on the yield and fatty acid (FA) profile of Mexican tropical cream cheese. The lowest cheese yield was the result of the treatment with CLA alone. Supplementing PA to the cows with low fat concentration in their milk, from the effect of the *trans*-10, *cis*-12 isomer, mitigates the decrease in cheese yield. The process of cheese making does not modify the profile of FAs, so the supplemented *cis*-9, *trans*-11 CLA isomer transfers to the cheese, giving added value to the Mexican tropical cream cheese.

Rendimiento de queso crema tropical Mexicano utilizando leche baja en grasa inducido por el *trans*-10, *cis*-12 ácido linoleico conjugado: efecto del ácido palmítico

RESUMEN

El isómero *trans*-10, *cis*-12 del ácido linoleico conjugado (ALC) en vacas lactantes reduce la grasa en leche, ocasionando menor rendimiento de queso, fenómeno que podría mitigarse adicionando ácido palmítico (AP) en la dieta, ya que éste estimula la síntesis de grasa en leche. El objetivo del estudio fue evaluar el efecto de adicionar AP a la dieta de vacas lactantes en pastoreo complementadas con *trans*-10, *cis*-12 ALC en el rendimiento y perfil graso de queso crema tropical Mexicano. El menor rendimiento de queso fue resultado del tratamiento con ALC solo. Complementar ácido palmítico a vacas con baja concentración de grasa en leche a consecuencia del isómero *trans*-10, *cis*-12 mitiga la reducción del rendimiento en queso. El proceso de elaboración del queso no modifica el perfil de ácidos grasos, por lo que el isómero *cis*-9, *trans*-11 ALC complementado se transfiere al queso, dándole valor agregado al queso crema tropical Mexicano.

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1. Introduction

Tropical cream cheese is a traditional Mexican cheese that belongs to the group of soft, fresh, pressed cheeses. It is original from the states of Chiapas and Tabasco, where its apparent annual consumption is 2.1 kg per citizen (Cervantes-Escoto & Villegas-De Gante, 2012). Said consumption together with its nutritional value, potentializes cheese as an adequate means to incorporate ingredients that add a functional food value. Such is the case of conjugated linoleic acid (CLA), considered functional food given its potential anticarcinogenic effect and its capacity to decrease fat in fatty and muscle tissues, attributed the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers (Yang et al., 2015). This can be achieved by adding ingredients that contain both CLA isomers to the diet of dairy cows (Bauman, Harvatiné, & Lock, 2011), thus increasing said isomers in the milk, and consequently in cheese. However, the *trans*-10, *cis*-12 isomer inhibits fat synthesis, and consequently decreases fat concentration in milk (Schiavon et al., 2016), negatively affecting cheese yield (Chen et al., 2009), since all

fats after casein are the most important component in the dry matter composition of cheese (Guinee, Mulholland, Kelly, & Callaghan, 2007). Additionally, this fat decrease in milk caused by the *trans*-10, *cis*-12 ALC isomer increases the protein-to-fat ratio (PFR) (Chen et al., 2009), which should be between 0.70 and 0.90. Values outside this range cause negative effects in cheese firmness and increase the rate of syneresis in cheese. To this regard, Guinee et al. (2007) reported that for each 0.1 point increase in PFR, cheese yield decreases by 4%. In face of this problem, it is necessary to propose alternatives that increase CLA concentration in milk, and consequently in cheese, without the latter's yield being affected. Recent studies suggest that palmitic acid (PA) can be a feasible option, since, according to Loftén et al. (2014), the supply of purified sources of this acid to dairy cows increases fat concentration in milk, which could decrease the loss of milk fat from the effect of the *trans*-10, *cis*-12 isomer, and consequently might stop the cheese yield from decreasing. The objective of this study was to evaluate the effect of adding PA to the diet of grazing dairy cows

supplemented with *trans*-10, *cis*-12 CLA isomer on the yield, chemical composition, and fatty acid (FA) profile of Mexican tropical cream cheese.

2. Materials and methods

2.1. Experimental procedure and animals

The study was done in a private dairy farm located in the municipality of Huimanguillo, Tabasco, a Mexican tropical area (17°38'38.2" N; 93°23'36.9" W; 20 masl). The milk used to make the cheese was obtained from six lactating grazing dairy cows (31 ± 7 days in milk and 2.3 ± 0.8 calvings; mean ± standard deviation), ¾ American brown Swiss × Zebu, distributed into three groups of two cows each. The treatments were randomly assigned to each of the three groups in a crossover design. The cows had a 14-day adaptation period and three 21-day experimental periods. The treatments were base diet (control) (Table 1), base diet + 50 g CLA (CLA), and base diet + 50 g CLA + 412 g PA (CLA + PA). The base diet was composed of grazed forage (*Cynodon plectostachyus* 18% + *Brachiaria decumbens* 12% + *Brachiaria brizantha* 78%), 4 kg day⁻¹ of sorghum silage, and 4 kg day⁻¹ concentrate. The CLA was a mixture of microencapsulated FA that supplied 6 g *cis*-9, *trans*-11 and 6 g *trans*-10, *cis*-12 CLA (LutrellPure[®], BASF, Germany). The PA was a mixture of microencapsulated FA > 85% PA (JefoDairyFat 99%[®], JEFO, Canada). During each of the last 4 days of every experimental period, 10 L milk was obtained from each cow in every treatment to make the cheese, for a total of 8 cheeses per treatment, 24 cheeses per experimental period, and a total of 72 cheeses, thus making the total of cheese samples used in this study.

Table 1. Chemical composition and fatty acid profile of the diet components.

Tabla 1. Composición química y perfil de ácidos grasos de los componentes de la dieta.

Chemical composition %	Forage	Silage	Concentrate	CLA	PA
Dry matter	22.55	32.12	92.50	–	–
Organic matter	83.10	89.62	95.11	–	–
Raw protein	10.12	6.88	17.3	–	–
Neutral detergent fiber	70.42	63.14	22.63	–	–
Acid detergent fiber	44.18	39.14	6.90	–	–
Net energy for lactation ^a	1.54	1.11	1.80	2.46	3.07
	Fatty acids (g 100 g ⁻¹ FA)				
≤C11:0	–	–	–	3.11	–
C12:0	–	–	9.13	–	0.47
C14:0	0.79	1.35	3.79	0.45	2.28
C15:0	0.36	0.72	–	–	–
C16:0	24.36	23.68	15.47	11.60	86.53
C16:1n7	1.13	1.30	0.45	–	–
C17:0	0.87	1.08	–	–	–
C18:0	2.20	3.38	2.32	46.42	3.57
C18:1n9	1.97	12.57	26.45	12.08	6.17
C18:2n6	16.24	28.93	40.86	1.01	0.98
C18:3n3	50.95	24.56	–	–	–
<i>Cis</i> -9, <i>trans</i> -11 CLA	–	–	–	12.03	–
<i>Trans</i> -10, <i>cis</i> -12 CLA	–	–	–	12.26	–
C20:0	0.26	1.22	0.32	0.56	–
C22:0	0.87	1.21	1.21	0.48	–

CLA: conjugated linoleic acid; PA: palmitic acid.

^aCalculated according to the formulas of the National Research Council (2001) for dairy cows.

ALC: ácido linoleico conjugado; AP: Ácido palmítico.

^aCalculado de acuerdo a las fórmulas del National Research Council (2001) para vacas lecheras.

2.2. Elaboration of the cheese

The cheese was made with unpasteurized milk, simulating the artisanal process, using 10 L milk to which was added 1 mL microbial rennet (strength 1:10 000; Fromase 750, Mexico). The milk was set to rest for 12–18 h (pH 4.9–5.3), curdled, and drained for 12 h at 4°C. NaCl was added to the curds at a proportion of 4% of the milk. The cheese was then pressed for 4 h and packaged in polyethylene paper and cooled to 4°C.

2.3. Cheese yield

The cheese yield considering the wet fraction of the cheese is the real yield and was calculated according to the equation by Hu et al. (2013). Yield excluding said fraction is the adjusted yield for dry matter, and it was obtained following the equation by Fenelon and Guinee (1999).

2.4. Laboratory analysis

To determine the chemical composition of the diet, samples were taken from herbage, sorghum silage, and concentrate. They were grounded using a Willey mill with a 1-mm mesh. These were determined for dry matter, total protein, ash (Association of Official Analytical Chemists, 2000), neutral detergent fiber and acid detergent fiber (Van Soest, Robertson, & Lewis, 1991).

The determinations of the concentrations of fat, protein, and lactose in the milk were done by using a LactiCheck[™] (Page & Pedersen Inc., Hopkinton, MA, USA). To determine casein content, this was precipitated in milk at a pH of 4.6. Later, the serum was filtered and in the precipitate was determined the nitrogen content by the Kjeldahl method (Association of Official Analytical Chemists, 2000).

The cheese samples were determined for humidity, total protein, fat, ash (Association of Official Analytical Chemists, 2000), and pH (Kuchroo & Fox, 1982).

2.5. FA profile in milk and cheese

To determine the FA profile in milk and cheese, the methylation technique proposed by Palmquist and Jenkins (2003) was used, where the FAs are presented in the form of methyl esters. The FA methyl esters (FAME) were determined in a Hewlett-Packard 6890 chromatograph with an automatic injector with a silicon capillary column (100 m × 0.25 mm × 0.20 μm width, Sp-2560, Supelco). The identification of the FAs was done by comparing the retention times of each peak obtained in the chromatogram, with a standard of 37 FAME components (Supelco 37 FAME Components), and a specific standard for the C18:1 *trans* 11 (Sigma-Aldrich) and *cis*-9, *trans*-11 and *trans*-10, *cis*-12 (Nu-Check-Prep) isomers.

2.6. Statistical analysis

A crossover design was used and the results were analyzed using the MIXED procedure in Statistical Analysis Software [SAS] (2008). The means were compared with the adjusted Tukey's test. A statistical difference was considered when $p \leq 0.05$.

3. Results

3.1. Milk composition

The concentration of protein, lactose, and casein in milk did not differ ($p > 0.05$) among treatments. However, the milk fat of the cows under the CLA treatment decreased by 26.9% with respect to the treatment with CLA plus PA (CLA + PA), and 31.1% with respect to the control treatment (Table 2). This decrease in milk fat caused by CLA-alone treatment led to an increase ($p \leq 0.05$) in the PFR and casein-to-fat ratio (Table 2).

3.2. Cheese composition and yield

The moisture, pH, protein, and ash concentration in cheese did not differ ($p > 0.05$) among treatments, but the fat concentration did. The latter was lower in cheeses from the CLA treatment, which was 13.8% lower than the CLA + PA treatment and 14.8% lower than the control treatment. This also caused the cheeses from the CLA treatment to have the lowest yield ($p \leq 0.05$), both real and adjusted for dry matter (Table 2).

3.3. Profile of FAs in milk and cheese

The profile of FA in milk was similar to that in cheese (Table 3), where supplementing CLA to the cows decreased ($p \leq 0.05$) the FA <C16 and increased ($p \leq 0.05$) the FA >C16 with respect to the CLA + PA and control treatments. Moreover, there was an increase of 37.1% in the concentration of the *cis-9, trans-11* isomer in cheese, with respect to the control treatment (Table 4).

4. Discussion

4.1. Milk composition

The concentration of protein, lactose, and casein in milk was not different ($p > 0.05$) among treatments, although fat did decrease ($p \leq 0.01$) in milk samples as a result of

Table 2. Effect of the palmitic acid and conjugated linoleic acid on the composition of milk and tropical cream cheese yield.

Tabla 2. Efecto del ácido palmítico y ácido linoleico conjugado en la composición de leche y el rendimiento del queso crema tropical.

	Treatments			SEM
	Control	CLA	CLA + PA	
Milk composition (%)				
Protein	3.32	3.31	3.28	0.02
Lactose	4.80	4.79	4.75	0.04
Fat	3.86 ^a	2.83 ^c	3.64 ^b	0.06
Casein	2.66	2.66	2.59	0.03
Titration acidity (g/L)	1.52	1.52	1.47	0.06
Ratio				
Casein to protein	0.80	0.80	0.79	0.01
Protein to fat	0.86 ^b	1.17 ^a	0.90 ^b	0.06
Casein to fat	0.69 ^b	0.94 ^a	0.71 ^b	0.05
Cheese composition (%)				
Moisture	40.85	40.63	41.03	0.13
Protein	25.15	25.09	24.87	0.11
Fat	26.59 ^a	22.66 ^b	26.28 ^a	0.15
Ash	5.10	5.13	5.34	0.06
Cheese yield				
Real (kg cheese/100 kg milk)	14.13 ^a	12.81 ^b	14.04 ^a	0.30
Adjusted for dry matter (%)	8.39 ^a	7.62 ^b	8.29 ^a	0.13
pH	5.44	5.39	5.41	0.03

^{abc}Values with different letters in a row are different ($p \leq 0.05$).

SEM: standard mean error; CLA: conjugated linoleic acid; PA: palmitic acid.

^{abc}Valores con distinta literal en un renglón son diferentes ($p \leq 0.05$).

EEM: error estándar de la media; ALC: ácido linoleico conjugado; AP: Ácido palmítico.

Table 3. Effect of palmitic acid and conjugated linoleic acid on the *cis-9, trans-11* CLA isomer and the sum of saturated and unsaturated FA in milk and tropical cream cheese.

Tabla 3. Efecto del ácido palmítico y ácido linoleico conjugado en el isómero *cis-9, trans-11* ALC y la sumatoria de AG saturados e insaturados en leche y queso crema tropical.

Fatty acid (FA)	Control	CLA	CLA + PA	SEM
Milk				
C18:2 <i>cis-9, trans-11</i> CLA	1.12 ^c	1.68 ^a	1.54 ^b	0.03
Saturated FA	69.28 ^a	64.26 ^b	68.44 ^a	0.88
Mono-unsaturated FA	28.91 ^b	33.29 ^a	29.32 ^b	0.85
Poly-unsaturated FA	1.81 ^b	2.45 ^a	2.23 ^a	0.09
Cheese				
C18:2 <i>cis-9, trans-11</i> CLA	1.11 ^c	1.64 ^a	1.53 ^b	0.04
Saturated FA	69.25 ^a	64.19 ^b	68.17 ^a	0.74
Mono-unsaturated FA	27.77 ^b	32.00 ^a	28.19 ^a	0.75
Poly-unsaturated FA	2.98 ^b	3.83 ^a	3.64 ^a	0.17

^{abc}Values with different letters in a row are different ($p \leq 0.05$).

SEM: standard mean error; CLA: conjugated linoleic acid; PA: palmitic acid.

^{abc}Valores con distinta literal en un renglón son diferentes ($p \leq 0.05$).

EEM: error estándar de la media; ALC: ácido linoleico conjugado; AP: ácido palmítico.

Table 4. Effect of palmitic acid and conjugated linoleic acid on the fatty acid profile (g 100 g⁻¹ FA) of tropical cream cheese.

Tabla 4. Efecto del ácido palmítico y ácido linoleico conjugado sobre el perfil de ácidos grasos (g 100 g⁻¹ AG) del queso crema tropical.

Fatty acid	Treatments			SEM*
	Control	CLA	CLA + PA	
C4:0	1.52 ^a	1.29 ^b	1.07 ^c	0.08
C6:0	2.15 ^a	1.68 ^b	1.40 ^b	0.12
C8:0	1.35 ^a	0.98 ^b	0.78 ^b	0.09
C10:0	2.72 ^a	2.13 ^b	1.50 ^c	0.20
C11:0	0.40 ^a	0.32 ^{ab}	0.21 ^b	0.04
C12:0	3.35 ^a	2.90 ^a	2.05 ^b	0.19
C13:0	0.17 ^a	0.15 ^{ab}	0.12 ^b	0.01
C14:0	11.59 ^a	10.83 ^a	8.93 ^b	0.56
C14:1	1.34 ^a	0.99 ^b	1.10 ^b	0.08
C15:0	1.17 ^b	1.28 ^a	1.01 ^c	0.03
C16:0	31.38 ^b	27.39 ^c	39.96 ^a	0.70
C16:1 <i>cis-9</i>	2.03 ^b	1.86 ^b	2.70 ^a	0.13
C17:0	0.81 ^a	0.86 ^a	0.65 ^b	0.04
C17:1	0.28	0.32	0.31	0.04
C18:0	10.16 ^b	11.71 ^a	8.03 ^c	0.47
C18:1 <i>cis-9</i>	22.79 ^c	28.48 ^a	25.08 ^b	0.83
C18:1 <i>trans-11</i>	0.22	0.27	0.22	0.03
C18:2 <i>cis-9, cis-12</i>	3.49 ^a	3.43 ^a	2.32 ^b	0.38
C18:2 <i>cis-9, trans-11</i>	1.02 ^b	1.64 ^a	1.53 ^a	0.05
C18:3 <i>cis-9, cis-12, cis-15</i>	0.31	0.33	0.27	0.03
C20:0	0.11	0.13	0.11	0.01

^{abc}Values with different letters in a row are different ($p \leq 0.05$).

SEM: standard mean error; CLA: conjugated linoleic acid; PA: palmitic acid.

^{abc}Valores con distinta literal en un renglón son diferentes ($p \leq 0.05$).

EEM: error estándar de la media; ALC: ácido linoleico conjugado; AP: ácido palmítico.

supplementing with CLA and CLA + PA to cows due to the effect of the *trans-10, cis-12* CLA isomer. This isomer inhibits the expression of enzymes involved in the *de novo* FA synthesis and the intracellular transportation and absorption in the mammary gland of preformed FA coming from the blood (Schiavon et al., 2016). Consequently, the fat concentration in milk was reduced in 26.7% under CLA-alone treatment, and only 5.7% under CLA + PA treatment, when compared to the control treatment. This result is undoubtedly due to the inclusion of PA (C16:0), increasing its availability in the mammary gland, stimulating the synthesis of triglycerides (Hansen & Knudsen, 1987), and consequently the concentration of fat in the milk increases (Piantoni, Lock, & Allen, 2013). This indicates that the *trans-10, cis-12* CLA isomer does not affect the incorporation of dietary C16:0 into milk fat, as said incorporation is through the capture

and transportation of C16:0 as a FA preformed in the mammary gland (Loften et al., 2014), and the *trans*-10, *cis*-12 CLA isomer mainly inhibits *de novo* synthesis of FA, not that from capture of preformed FA (Bauman et al., 2011).

The PFR in the milk from cows under the CLA treatment was higher ($p \leq 0.01$) due to the loss of milk fat from the effect of the *trans*-10, *cis*-12 isomer, which caused a decrease in the yield of the cheese from this treatment (Guinee et al., 2007).

4.2. Cheese composition and yield

Given the high PFR value (1.17) in the cheeses as a result of the CLA-alone treatment, with respect to the other treatments (CLA + PA = 0.90; control = 0.86), they had the lowest yields, both real and adjusted for dry matter. This is because the increase in PFR causes a higher syneresis rate (Fox et al., 2016), negatively affecting the firmness and yield of the cheese (Guinee et al., 2007). Also, there is an inverse correlation ($r = -0.88$) between the PFR and cheese yield (Guinee et al., 2007), which explains the lower yield in cheeses as a result of the CLA-alone treatment. The fact that milk fat decreased as a consequence of the *trans*-10, *cis*-12 isomer in the CLA-alone treatment represented that the cheese derived from this milk had the lowest yield, as casein and milk fat make up more than 85% of the dry matter in cheese (Fox et al., 2016). To this regard, Fekadu et al. (2005) mention that there is a positive correlation ($r = 0.79$) between fat concentration and cheese yield. This means that the higher the fat concentration, the greater the cheese yield, or vice versa. For example, Schiavon et al. (2016), who supplemented dairy cows with 5.5 g day⁻¹ *trans*-10, *cis*-12, observed a decrease in fat milk concentration (23.2%), causing the cheese yield to decrease by 10.2%. In the present study, supplementing the cows with 6 g day⁻¹ *trans*-10, *cis*-12 in the CLA-alone treatment caused a decrease in milk fat of 26.7%, while the cheese yield decreased by 9.4% with respect to the control. However, Sinclair, Lock, Early, and Bauman (2007) observed results contrary to these, when supplementing the diet of lactating sheep with a supplement that contained the *trans*-10, *cis*-12 CLA isomer. They observed a decrease in the concentration of milk fat, but not in cheese yield. This difference in results might be related to the fact that the fat reduction reported by Sinclair et al. (2007) caused an increase of 0.15 points in the PFR, while in our study, the increase was 0.31 points when the milk fat decreased from the addition of CLA, and therefore, there was a decrease in cheese yield.

The cheese yields found under the control and CLA + PA treatments were not different ($p < 0.05$) between them. This result shows that the inclusion of C16:0 mitigated the milk fat decline from the effect of the *trans*-10, *cis*-12 isomer, causing the PFR of the control and CLA + PA treatments (0.86, control; 0.90, CLA + PA) not to be modified ($p > 0.05$). Consequently, the cheese yield in both treatments did not differ either.

4.3. Profile of FAs in milk and cheese

The *cis*-9, *trans*-11 CLA isomer increased in the milk fat of the cows supplemented with CLA (Table 3). The efficiency of transference of the mentioned isomer to the milk fat due to the CLA-alone treatment was 9.3%, while that one due to the CLA+PA treatment it was 7.0%. These values are within the range (4–10%) of transference of *cis*-9, *trans*-11 CLA,

when it is supplied to lactating cows in a protected form (Giesy, McGuire, Shafil, & Hanson, 2002).

The FA profile of the cheese was similar to that of the milk (Table 3), which means that the cheese making process was not a determining factor in the composition of the FA profile. Luna, Juárez, and De La Fuente (2007) indicate that the standard cheese making processes does not change the profile of the FA, including the CLA isomers. Additionally, modifications to the carbonated structure of the FA have only been observed from the effect of heat. Nevertheless, for this to happen, the cheese making process must happen at temperatures above 63°C and exposition times longer than 30 min (Herzallah, Humeid, & Al-Ismael, 2005). In the present study, the cheeses were made with unpasteurized milk, so the heat factor was not determining in any stage of the cheese making. Therefore, the FAs in the cheese were not modified.

The short and medium chain FA decreased and the sum of unsaturated FA increased in the cheeses under the CLA treatment. This result is due to the *trans*-10, *cis*-12 CLA isomer, which causes the same effect in the FA profile in milk (Schiavon et al., 2016). This, added to the fact that the cheese making process did not modify the FA profile from milk to cheese, caused the *cis*-9, *trans*-11 CLA isomer to increase in the cheeses in the CLA and CLA + PA treatments. This allows these cheeses to have a FA profile with functional food potential (Yang et al., 2015).

5. Conclusion

Supplementing PA to grazing dairy cows with low fat concentration in their milk, from the effect of the *trans*-10, *cis*-12 isomer, mitigates the decrease in cheese yield. The cheese making process does not modify the FA profile, so the supplemented *cis*-9, *trans*-11 CLA isomer in the diet transfers to the cheese, giving added value to the Mexican tropical cream cheese.

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Compliance with ethical standards

Care of the animals in this study followed the guidelines of Mexican federal law of animal health (DOF 25-07-2007).

Declaration

All of the listed authors have participated in the development of this research and wrote this document.

Disclosure statement

No potential conflict of interest was reported by the authors.

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