FATTY ACIDS PROFILE OF FRESH CHEESE PRODUCED IN AN INTENSIVE SILVOPASTORAL SYSTEM IN THE TROPICS†

[PERFIL DE ÁCIDOS GRASOS DE QUESO FRESCO PRODUCIDO EN UN SISTEMA SILVOPASTORIL INTENSIVO EN EL TRÓPICO]

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SUMMARY

Intensive silvopastoral systems are being increasingly disseminated in the tropical areas of Mexico, Central and South America, so it is important to know the quality of the animal products generated in these systems. Fatty acid profile of fresh soft cheese (Domiati type) produced from milk of cows grazing *Leucaena leucocephala* associated with *Cynodon nlemfuensis* in an intensive silvopastoral system (ISS) was evaluated and compared with a cheese produced from cows grazing a traditional monoculture system (MS) of *C. nlemfuensis*. The legume *L. leucocephala* contains phenolic compounds that could affect the fatty acid biohydrogenation in the rumen and therefore the fatty acid profile of resultant milk and cheese. Milk samples from cows in both diets (systems) were processed monthly for cheese making and cheese samples were analyzed for fatty acid profile. The experimental period lasted for 10 months covering the rainy and dry season of a tropical area in Mexico. The results showed that the cheese produced in ISS had significantly lower content ($P<0.05$) of the saturated fatty acids lauric, myristic and palmitic and significantly higher content ($P<0.05$) of stearic, linoleic and γ-linolenic acids. Additionally, higher content ($P<0.05$) of the polyunsaturated fatty acids and omega-6 fatty acids were found in the cheese from ISS. The results suggest that fresh soft cheese produced from silvopastoral systems based on *L. leucocephala* has a better fatty acid profile than that produced from the traditional MS with grass only.

Keywords: Fatty acid profile; Fresh cheese; Intensive silvopastoral system; *Leucaena leucocephala*.

RESUMEN

Los sistemas silvopastorales intensivos se están difundiendo cada vez más en las áreas de México, Centro y Sudamérica, por lo que es importante conocer la calidad de los productos generados en estos sistemas. Se evaluó el perfil de ácidos grasos del queso fresco (tipo Domiati) producido a partir de leche de vacas en pastoreo de *Leucaena leucocephala* asociada con *Cynodon nlemfuensis* en un sistema silvopastoral intensivo (ISS) y se comparó con el de un queso producido a partir de vacas en pastoreo en monocultivo tradicional (MS) de *C. nlemfuensis*. La leguminosa L. leucocephala contiene compuestos fenólicos que podrían afectar la biohidrogenación de ácidos grasos en el rumen y, por lo tanto, el perfil de ácidos grasos de la leche y el queso resultantes. Muestras de leche de ambos sistemas fueron procesadas mensualmente para la fabricación de queso y muestras de queso fueron analizadas para determinar el perfil de ácidos grasos. El período experimental duró 10 meses, cubriendo la estación lluviosa y seca de un área tropical en México. Los resultados demostraron que el queso producido en ISS tuvo un contenido significativamente menor ($P <0.05$) de los ácidos grasos saturados láurico, mirístico y palmítico y un contenido significativamente mayor ($P <0.05$) de ácidos esteárico, linoleico y γ-linolénico. Además, se encontró un mayor contenido ($P <0.05$) de ácidos grasos poliinsaturados y ácidos grasos omega-6 en el queso de ISS. Los resultados sugieren que el queso fresco producido a partir de sistemas silvopastoriles con *L. leucocephala* posee un mejor perfil que el queso producido a partir del sistema tradicional de pastoreo en monocultivo de gramíneas.

Palabras clave: Perfil de ácidos grasos; queso fresco; sistema silvopastoril intensive; *Leucaena leucocephala*.

INTRODUCTION

Consumers have become more informed about the fat content of dairy products since the majority of fatty acids (FAs) in milk are saturated (Kalač and Samková, 2010). More attention has been given to the medium and long-chain fatty acids than short-chain fatty acids since their confirmed correlation with the coronary

† *Submitted August 16, 2018 – Accepted July 5, 2019.*
ISSN: 1870-0462

heart disease (CHD) (Bhupathiraju and Tucker, 2011). It has been established that the risk of CHD is more associated to the fatty acids lauric C12:0, myristic C14:0 and palmitic C16:0 (Shingfield *et al.*, 2008). On the other hand, unsaturated fatty acids like omega-3, omega-6 and conjugated linoleic acid (CLA) may provide important health benefits for humans such as protection against cardiovascular diseases (Williams, 2000) or be involved in anti-carcinogenic, immunomodulatory, and anti-diabetic activities (Sokoła-Wysoczańska *et al*., 2018; Corazzin *et al*., 2019).

In the tropics, several countries have implemented the intensive silvopastoral systems for feeding cattle, a modality of agroforestry based on associations of tropical grasses with trees and shrubs, which promotes plantations of legume trees such as *Leucaena leucocephala* at high densities (more than 10,000 plants/ha), intercropped with pastures and directly grazed by livestock (Cuartas-Cardona *et al.,* 2014). This legume fodder shrub has a number of attributes that make it well-adapted to that system, resulting in improved forage quality (Cuartas-Cardona *et al.*, 2014) and higher milk and meat production (Murgueitio *et al*., 2015).

It is well known that diet composition and the type of forage eaten by the cow play a major role in modulating the fatty acid composition of milk by influencing their precursors produced in the rumen and those available in the blood (Vasta *et al.*, 2008; Kalač and Samková, 2010). However, there are few, if any, studies on the FAs profile of cheese produced from milk of cows grazing in intensive silvopastoral systems based on *L. leucocephala*. This legume contains phenolic compounds (Estrada-Lievano *et al.*, 2009) that could affect ruminal fatty acid biohydrogenation and increase bypass of polyunsaturated fatty acids (PUFA) (Jayanegara *et al.*, 2011). This could affect positively the nutritional value of milk and dairy products (Vasta *et al.*, 2008). The objective of this study was to evaluate the fatty acid profile and the nutritional value of fresh soft cheese produced from milk of cows grazing *L. leucocephala* in an intensive silvopastoral system.

MATERIALS AND METHODS

Location and duration of the study

The study was carried out at the Faculty of Veterinary Medicine and Animal Science, University of Yucatan Mexico (89.37ºW, 20.52ºN). The region has a sub-humid tropical climate with a rainy season in summer (García, 1988). The study lasted 10 months, covering the rainy season (July-October 2013) and the dry season (November 2013-April 2014), where monthly milk samples were processed for cheese manufacture from cows under two different diets.

Management of cows

Milk to be processed (cheese making) was obtained from twenty four crossbred cows (*Bos indicus x Bos taurus*) >3 calvings which were divided in two homogenous groups (treatments) according to their body weight and milk yield records (Mohammed Mohammed *et al*., 2016). The first group of cows (n=12) was under a monoculture grass system (MS), and grazed Stargrass (*C. nlemfuensis*) with a stocking rate of 2 AU/hectare (1 animal unit (AU) = 450 kg live weight). Body weight of cows in this system was 532 kg ± 82.6 kg with an average milk yield of 15.3 ±1.5 kg/day. The second group of cows (n=12) was under an intensive silvopastoral system (ISS), and grazed mixed paddocks of *L. leucocephala* (36,000 plants/ha) and *C. nlemfuensis* with a stocking rate of 2.4 AU/ha. Body weight of cows in this system was 522 kg ± 75.6 kg, with an average milk yield of 14.3 ±5.9 kg/day. Both groups of cows grazed from 17:00 to 05:00 h and from 08:00 to 13:00h and were supplemented at milking times (06:00 and 15:00h) to satisfy their energy and protein requirements for milk production (AFRC, 1993). Because of the low content of protein and metabolisable energy of *C. nlemfuensis*, cows in MS were supplemented with a commercial concentrate, composed of corn grain 60%, soybean meal 30% and soybean husks 10%. Cows in ISS were supplemented only with sorghum grain as an energy source. Average daily dry matter intake and metabolizable energy intake per cow were 15.2 kg and 179.5 MJ EM for ISS and 11.5 kg and 117.4 MJ EM for MS. Detailed feed chemical composition and dry matter intakes were previously reported by Mohammed Mohammed *et al*., (2016).

Cheese making

Cheese making (Domiati type) was performed monthly from pooled milk samples, obtained weekly at the morning and afternoon milkings. Detailed cheese making, as well as milk and cheese composition (fat, protein, ash) were reported in Mohammed Mohammed *et al*., (2016).

Fatty acid profile of cheese

After cheese making, a sample of 100 g was lyophilized and stored at −20 ºC until analysis for fatty acids. Fat was extracted from cheese samples using Folch method according to Iverson *et al.* (2001). Fatty acid methyl ester (FAME) was prepared according to Joseph and Ackman (1992) using boron trifluoride (BF_3). One µl of each FAME sample was injected into a TR-FAME capillary column (120m×0.25mm i.d., 0.25µm film thickness Thermo P/N: 260M166L) connected to GC system Agilent Technologies 7820A fitted with flame ionization detector. The oven temperature was programmed as follows: the initial temperature held at 150ºC for 5 min, increased to 180ºC at 10ºC/min, increased to 220ºC at 1.5ºC/min and kept there for 10 min. The injector and detector temperature were 250ºC and 280ºC, respectively. Helium was used as the carrier and make-up gas at 1.3 ml/min and 25 ml/min, respectively. The split ratio was 1:100. Fatty acid methyl esters were identified by comparison of retention times with standards: 37 Component FAME Mix (Sigma-Aldrich 47885); α-linolenic acid (Fluka 62160), Eicosapentaenoic acid methyl ester (Fluka 17266) and Docosahexaenoic acid methyl ester (Fluka 05832); and quantified in relation to the internal standard Heptadecanoic acid (C17:0), which prepared with a concentration of 10 mg/ml in Hexane (200 µl of C17:0 added to 50 µl extracted lipid before FAME preparation).

Statistical analysis

Data of FAs were analyzed using the GLM procedure of IBM SPSS Statistics 22 (SPSS for windows, 2013). A completely randomized design with a factorial arrangement 2×2 was used. The statistical model included effects of diet (MS and ISS), season (rainy and dry) and the interactions. The interaction effect was removed from the final model when it was not significant ($P>0.05$).

RESULTS AND DISCUSSION

The concentration of fatty acids in dairy products such as fresh cheese, are largely dependent on the fatty acid content of the raw milk (Nudda *et al*., 2005). In the current study, cheese fat from ISS had lower content of lauric C12:0 ($P<0.05$), myristic C14:0 ($P<0.05$) and palmitic C16:0 ($P<0.05$) acids compared with cheese from MS (Table 1). The grouped content of these saturated fatty acids was significantly lower ($P<0.05$) in ISS (Table 2). Lauric and mysristic are medium-chain fatty acids which are synthesized exclusively *de novo* in the mammary gland. Palmitic acid is the main saturated fatty acid present in the milk of the majority of mammals (Markiewicz-Kęszycka *et al*., 2013) and can be derived either *de novo* or from the uptake of circulating lipids (Neville and Picciano, 1997). The lower content of saturated fatty acids in cheese fat from ISS would be associated with a higher supply of long-chain fatty acids to the mammary gland of the cows in that system. Vanhatalo *et al*. (2007) reported increased supply of long-chain fatty acids to the mammary gland in cows fed legume compared with cows fed silage based diets. These authors suggested that long-chain fatty acids inhibit *de novo* synthesis of milk fatty acids in the udder. In the same way, Kadegowda *et al.* (2008) and Shingfield *et al.* (2009) reported that several trans isomers from C18:1 may decrease lipogenesis in the mammary gland. The results in the current study agree with Praga-Ayala (2015) who reported significantly lower concentration of low-chain fatty acids in fresh cheeses from a silvopastoral system based on *L. leucocephala*. Lauric, myristic and palmitic acids are the only three fatty acids in cow´s milk associated with increasing risk of cardiovascular diseases in humans (Mensink *et al*., 2013). On the other hand, stearic acid (C18:0) was significantly higher ($P<0.05$) in cheese fat from ISS. A significant interaction ($P<0.05$) between diet and season was found for that saturated fatty acid (Fig. 1), which was higher in ISS on the rainy and dry seasons. This result would be related with a higher estimated daily intake of lipids (g/cow/day) for cows in ISS (326.1 in the rainy season and 379.0 in the dry season) compared with cows in MS (300.8 in the rainy season and 253.8 in the dry season). It is reported that *L. leucocephala* have a higher content of stearic acid (9.6 % FAME, Jayanegara *et al.* (2011)) than Stargrass (6.5 % FAME, Gomes *et al.* (2015)). Prieto-Manrique *et al*. (2016) found higher stearic acid in the digesta in an *in vitro* studio, when *L. leucocephala* was included at 14% of forage-based diets. Stearic acid is reported to have a neutral effect on plasma LDL cholesterol for humans (Fernandez and West, 2005). However, Bonanome and Grundy (1988) reported that consumption of diets high in C18:0 resulted in a decline in plasma total cholesterol.

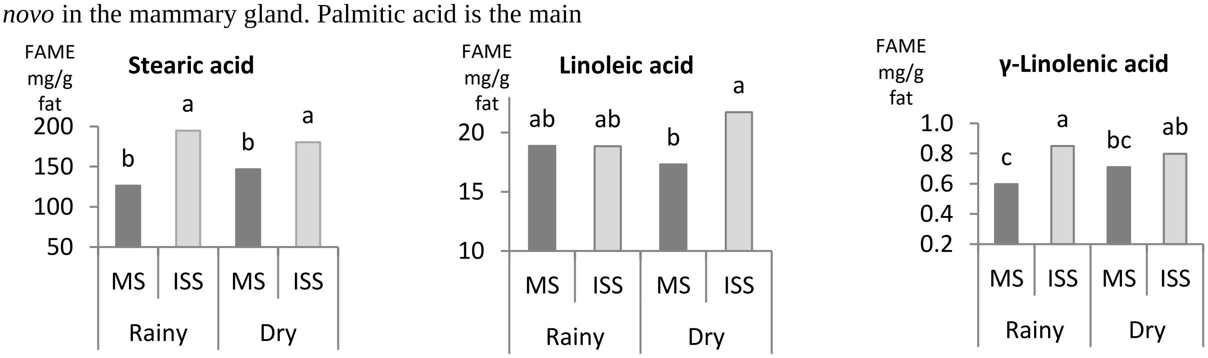

Fig. 1 Stearic, linoleic and y-linolenic fatty acids (FAME mg/g fat) in cheeses produced in monoculture (MS) and intensive silvopastoral system (ISS), in the rainy and the dry seasons. Different letters above histogram bars indicate statistical differences ($P<0.05$).

Concerning to trans fatty acids, elaidic acid (C18:1n9t) was significantly higher (P<0.05) in cheese from ISS, but the content of linolelaidic acid (C18:2n6t) did not differ between diets (Table 1). In ruminants, trans fatty acids are produced by bio-hydrogenation of linoleic and linolenic acids in the rumen and, in consequence, may appear in milk or dairy products (Sommerfeld, 1983). Prieto-Manrique *et al*. (2016) reported that linoleic and linolenic acids were both increased in the diet and digesta with a dietary inclusion of 14 % of *L. leucocephala*. Clinical studies have suggested that trans fatty acids, particularly C18:1, increase the risk of coronary heart disease (Precht and Molkentin, 2000); however, Meijer *et al.* (2001) reported that diets high in elaidic acid did not increase blood cholesterol levels in hamsters.

On the other hand, there were no significantly differences between diets or seasons for the content of oleic acid (C18:1n9c) in cheeses, whose values were 250.7 and 252.1 mg/g in MS and ISS, respectively (Table 1). Oleic acid is the most abundant monounsaturated fatty acid in milk (Markiewicz-Kęszycka *et al*., 2013) and its concentration can be increased mainly by oil supplements to the diet (Palmquist, 2006) or by using fat-protected supplements. Mahecha *et al*. (2008) increased oleic acid concentrations in the milk of Lucerna cows under a silvopastoral system with *L. leucocephala* but using bypass fat supplements.

A significant interaction (P<0.05) between diet and season was found for the values of linoleic acid (C18:2n6c) and γ-linolenic acid (C18:3n6), which were higher in cheese fat from ISS during the dry and rainy season, respectively (Fig. 1). Gamma linolenic acid is a polyunsaturated long-chain fatty acid which is produced in the body from linoleic acid by the enzyme delta-6-desaturase (Kapoor and Huang, 2006). Those results could have been effect of a higher supply of linoleic and linolenic acids coming from the Leucaena fodder (Prieto-Manrique *et al*., 2016) and probably due to seasonal differences in consumption of this legume. These fatty acids contribute with the total content of omega-6 fatty acids which were significantly higher (P<0.05) in cheese from ISS (Table 2). Gamma linolenic acid seems to have an important role in modulating inflammatory response (Kapoor and Huang, 2006). On the other hand, cheese content of α-linolenic acid (C18:3n3) did not differ significantly between diets. Linoleic and α-linolenic acids are essential fatty acids for humans that must be obtained from the diet, and they can be converted to omega-6 and omega-3 families of C20 and C22 long-chain PUFA by a series of desaturation and elongation reactions (Parodi, 2004). Then, the higher C20:1 and C22:1n9 found in cheeses from ISS could be associated with the higher concentration of linoleic acid found in these cheeses.

The content of PUFA was significantly higher (P<0.05) in cheese fat from ISS (Table 2) which is particularly important for human health because of the general trend of replacing dietary saturated fatty acids (C12:0–C16:0) with PUFA in order to decrease blood LDL cholesterol and hence decrease the risk of CHD (Sokoła-Wysoczańska *et al*., 2018). This result could be additionally explained by the higher total phenol content, particularly condensed tannins, reported in *L. leucocephala*. Vasta *et al.* (2009) pointed out that tannins can reduce the PUFA biohydrogenation process through the inhibition of the activity of ruminal microorganisms, resulting in higher bypass of these FA from the rumen to the mammary gland.

Table 1 Fatty acid profile (FAME mg/g fat) of fresh soft cheese (Domiati type) as affected by diet and season.

Fatty acids	Diet		Season		SEM	P value		
	MS	ISS	Rainy	Dry		Diet	Season	Interaction
C12	1.52[a]	1.30[b]	1.43	1.40	0.04	**0.002**	0.62	0.09
C14	120.90[a]	110.83[b]	117.87	114.53	2.92	**0.04**	0.55	0.10
C14:1	11.48[a]	9.47[b]	18.23[a]	5.30[b]	1.52	**0.01**	**<0.001**	0.11
C15	13.07	13.00	13.82	12.52	0.42	0.57	0.06	**0.003**
C16	335.85[a]	302.61[b]	314.52	322.37	5.81	**0.001**	0.39	0.12
C16:1	17.30[a]	12.72[b]	14.86	15.11	0.59	**<0.001**	0.67	0.35
C17:1	3.94	3.53	3.43	3.94	0.23	0.54	0.30	0.30
C18	138.66[b]	186.18[a]	160.59	163.64	6.60	**<0.001**	0.67	**0.02**
C18:1n9t	47.25[b]	51.93[a]	49.70	49.51	1.21	**0.02**	0.93	0.09
C18:1n9c	250.67	252.09	242.10	257.57	7.29	0.84	0.34	0.58
C18:2n6t	10.54	11.22	11.44	10.50	0.75	0.81	0.56	0.36
C18:2n6c	17.94[b]	20.57[a]	18.86	19.52	0.59	**0.04**	0.51	**0.04**
C18:3n3	1.19	1.25	1.27	1.19	0.08	0.54	0.62	0.22
C18:3n6	0.67[b]	0.82[a]	0.72	0.75	0.03	**<0.001**	0.37	**0.03**
C20:1	3.59[b]	4.04[a]	3.70	3.89	0.10	**0.02**	0.30	0.68
C22:1n9	0.66[b]	0.75[a]	0.66	0.74	0.02	**0.03**	0.05	0.56

Means with different letters in the same row within system or season differ significantly (P<0.05). *ISS* intensive silvopastoral system, *MS* monoculture system, *SEM* standard error of mean.

Table 2 Major fatty acids classes (FAME mg/g fat) and C14 desaturase index % of fresh soft cheese (Domiati type) as affected by diet and season.

Fatty acids	Diet		Season		SEM	P value		
	MS	ISS	Rainy	Dry		Diet	Season	Interaction
C12+C14+C16	458.27[a]	414.74[b]	433.82	438.29	8.14	**0.002**	0.73	0.08
MCFA	500.11[a]	449.94[b]	480.73	471.22	8.58	**0.001**	0.46	0.08
LCFA	475.10[b]	532.38[a]	492.47	511.25	12.00	**0.01**	0.38	0.20
MUFA	287.64	282.60	282.98	286.55	7.65	0.84	0.83	0.65
PUFA	19.79[b]	22.64[a]	20.86	21.46	0.58	**0.02**	0.53	0.06
n3-FA	1.19	1.25	1.27	1.19	0.08	0.54	0.62	0.22
n6-FA	18.60[b]	21.39[a]	19.59	20.27	0.59	**0.02**	0.48	**0.04**
C14 index*	8.25	7.87	13.50[a]	4.44[b]	1.08	0.67	**<0.001**	0.77

Means with different letters in the same row within system or season differ significantly (P<0.05).
ISS intensive silvopastoral system, *MS* monoculture system, *MCFA* medium-chain fatty acids (C12:0-C16:0), *LCFA* long-chain fatty acids (>C16), *MUFA* monounsaturated fatty acids (C14:1, C16:1, C17:1, C18:1n9c, C20:1, C22:1n9), *PUFA* polyunsaturated fatty acids (C18:2n6c, C18:3n3,C18:3n6), *n-3FA* omega-3 fatty acid (C18:3n3), *n-6FA* omega-6 fatty acids (C18:2n6c, C18:3n6), *SEM* standard error of mean
*C14-desaturase activity index = C14:1 ÷ (C14:1 + C14:0) ×100 (Heck *et al.*, 2009)

The effect of season was not significant (P>0.05) for all FAs, except for C14:1, which was higher in the rainy season. Accordingly, a significant increase (P<0.05) in C14-Δ9 desaturase index was found in the rainy season compared with the dry season (Table 2). This index is the best indicator of the activity of the Δ9 desaturase enzyme because all of C14:0 in milk fat is produced via *de novo* synthesis in the mammary gland (Lock and Garnsworthy, 2003). These results could be related to the early stage of the forage grazed in the rainy season, which could have resulted in increases of the desaturase activity of the mammary gland (Morand-Fehr *et al.*, 2007).

CONCLUSIONS

The cheese produced from milk of cows grazed in an intensive silvopastoral system with *L. leucocephala* associated with *C. nlemfuensis* has lower concentration of saturated fatty acids and higher content of omega-6 and PUFA than that produced from a traditional monoculture system with grass only.

ACKNOWLEDGMENTS

The authors are grateful with the financial support from the project “Cargas-Leche-SSPi, F.P.M., A.C.”. This work is a partial requirement for a Ph.D. degree funded to the first author by the Consejo Nacional de Ciencia y Tecnologia (CONACYT) (grant number 510528), Mexico.

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