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ORIGINAL ARTICLE

A comparison of milk fatty acid profile among three different dairy production systems in Los Ríos District, Chile#

Comparación del perfil de ácidos grasos de la leche entre tres sistemas distintos de producción lechera en la Región de Los Ríos, Chile

R Morales[a*], F Lanuza[a], I Subiabre[a], AM Carvajal[a], F Canto[a], EM Ungerfeld[b]

[a]Instituto de Investigaciones Agropecuarias, INIA Remehue, Osorno, Chile.

[b]Instituto de Investigaciones Agropecuarias, INIA Carillanca, Temuco, Chile.

RESUMEN

El objetivo de este estudio fue comparar tres sistemas distintos de producción lechera en la Región de Los Ríos, Chile, con énfasis en el perfil de ácidos grasos de la leche. Para ello se seleccionaron nueve predios representativos sobre la base de una encuesta y se clasificaron en tres sistemas de producción: pastoril (GS), mixto (MX) y ración totalmente mezclada (TMR). Muestras de leche de estanque se obtuvieron mensualmente durante 18 meses, para su posterior análisis de composición de leche y perfil de ácidos grasos. Los sistemas pastoril y mixto tuvieron la mayor concentración de ácidos grasos *n*-3 y ácido ruménico, lo que se asoció al alto consumo de pradera. Las muestras de leche de los sistemas pastoril y mixto tuvieron más del doble de ácido ruménico que la leche del sistema TMR al final del invierno y durante la primavera. Estos resultados concuerdan con estudios previos que mostraron altas concentraciones de ácidos grasos beneficiosos para la salud en leche de sistemas pastoriles.

Palabras clave: ácidos grasos, CLA, Holstein Friesian, calidad de leche.

SUMMARY

The objective of this study was to compare three different dairy production systems in Los Ríos District, Chile, with regards to milk fatty acids profile. Nine representative farms were selected from a survey and classified into three systems: grazing (GS), mixed (MX) and total mixed ration (TMR). Bulk milk tank samples were monthly obtained during an 18-months period, and milk components and fatty acid analyses were conducted. The grazing and mixed systems had higher concentrations of *n*-3 fatty acids and rumenic acid, which was associated to higher pasture intakes. Milk from grazing and mixed systems had twice as much rumenic acid than milk from TMR system in late winter and spring. These results are coincident with previous studies showing higher concentrations of fatty acids beneficial to human health in milk from grazing cows.

Key words: fatty acids, CLA, Holstein Friesian, milk quality.

INTRODUCTION

Milk fatty acid profile has an influence on human health (Lock and Bauman 2004). Rumenic acid (RA, Conjugated Linoleic Acid *cis*-9 *trans*-11-18:2) is a fatty acid present in ruminant milk fat and adipose tissues with positive attributes to human health. Rumenic acid in milk is produced during biohydrogenation of unsaturated fatty acids in the rumen and by endogenous synthesis in the mammary gland (Lock and Bauman 2004, Kalač and Samková 2010). Rumenic acid possess anticarcinogenic and immunostimulant properties among other effects (Whigham *et al* 2000, Albers and Wielen 2003, Haug *et al* 2007, Crumb 2011, Dilzer and Park 2012). Milk is also a source of n-3 fatty acids, which are claimed to prevent cardiovascular disease, type 2 diabetes, rheumatoid arthritis, asthma, and several cancers, amog others benefitial properties (Ruxton *et al* 2004, Hibbeln *et al* 2006, DeFilippis and Sperling 2006, Haug *et al* 2007).

It is well documented that the diet of the animal affects the fat content and fatty acid composition of milk (Bauman and Griinari 2003, Bauman *et al* 2008). Milk from animals fed on pasture contains more *n*-3 fatty acids and rumenic acid than milk from animals fed grain and concentrates (Kay *et al* 2005, Gómez-Cortés *et al* 2009), due to the high content of linolenic acid (*n*-3) in pasture fatty acids (Elgersma *et al* 2003, Vanhatalo *et al* 2007). Kelly *et al* (1998) and White *et al* (2001) reported that milk from grazing cows had double RA concentration in milk fat compared to milk from cows fed on corn-based mixed diet.

In recent years, consumers are demanding healthier food (Schnettler *et al* 2010, Realini *et al* 2014). A recent study has shown that consumers are willing to pay a premium price for products enriched with *n*-3 and/or CLA fatty acids (Realini *et al* 2014). In addition, Chilean consumers positively value animals fed on pasture and raised outdoors (Schnettler *et al* 2008, Morales *et al* 2013). The

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* rmorales@inia.cl

Chilean dairy sector has grown steadily in the last decades, with 77% of production taking place in the Los Ríos and Los Lagos regions (between 39° 37' and 44° 3' parallels south, ODEPA 2014). The majority of dairy production in the Los Ríos and Los Lagos regions relies on grazing of temperate pastures. Pasture production varies amply throughout the year, with low growth rates in winter and dry summers (Balocchi 1986). To face pasture deficits, conserved forage and grain are supplemented in winter, and forage crops such as fodder turnips (*Brassica rapa*) or raps (*Brassica napus*) are used in summer.

Milk produced in pasture-based Chilean systems may have a healthier fatty acids profile and potentially add value to Chilean dairy products. However, there is limited scientific information about the influence of different Chilean production systems on milk fatty acid profile. The objective of this study was to evaluate the fatty acids profile of milk produced in the Los Ríos region of Chile under three different dairy production systems.

MATERIAL AND METHODS

FARM SELECTION

Nine farms were selected out of a total of 90 farms surveyed. Farms were classified into three feeding systems, with three farms per system: grazing (GS), mixed (MX), and total mixed ration (TMR). Criteria to select the farms and main characteristics of each system are shown in the table 1.

SAMPLING AND RECORDS

Milk production of each farm was recorded daily during the study period (18 months). Each farm was visited monthly between April 2012 and September 2013, and bulk-tank milk samples (250 ml) of milk produced in the morning and evening were collected. A 50-ml sub-sample was stored at 4 °C with a preservative (2-bromo-2-nitro-1,3-propanediol, Aldrich Chemistry, USA) for milk components analysis and somatic cells count. The remaining 200 ml were stored at –20°C for fatty acids analysis. Milk production data were obtained by interview questionnaire and farm records.

Pastures in GS and MX systems were both sown and improved natural pastures, the average botanical composition being: *Lolium perenne*, 70-75%, *Trifolium repens*, 5-10%, *Bromus* spp., 3-5%, and 5-10% of others species. All farms in the GS and MX systems employed strip grazing.

Pasture DM intake was indirectly estimated from cows' milk production and live weight as follows (Baker 1982):

$$Pasture\ DM\ intake = \frac{MEm + MEml + MELw - ConcME}{Pasture\ ME}$$

where MEm, MEml and MELw are the estimated Metabolizable Energy (ME) requirements for maintenance, milk yield and live weight change, respectively (AFRC 1993). ConcME and Pasture ME correspond to ME supplied by the concentrate supplement and pasture ME is the estimated ME concentration of pasture samples of each sampling.

Table 1. Criteria for selecting the farms that participated in the study and characteristics of each production system.
Criterios de selección y características de producción de los predios participantes en el estudio.

Criteria for farm selection
– Farm located in the Los Ríos region
– Similar period of confinement during lactation (within mixed system)
– Similar period of grazing (within grazing and mixed systems)
– Breed (Holstein Friesian)
– Production records available
– Official milk production control
– Similar farm size within each system
– Producer interest in participating in the study

System characteristics		
Grazing	Mixed	Total Mixed Ration
Milking cows: 185 ±13	Milking cows: 382 ± 28	Milking cows: 437 ± 44
Grazing all year around Strip grazing	Grazing in autumn, spring and summer. Strip Grazing Confinement in winter	Confinement all year around. Fresh pasture offered cut
Concentrate offered in parlour	Concentrate offered in parlour and TMR in confinement during winter	Cows fed a total mixed ration (TMR)
Conserved forage supplemented in autumn and winter. Summer catch crops (turnips)	Conserved forage supplemented in autumn and winter. Pasture irrigation in summer	
All-year around calving and Seasonal calving	Seasonal calving	All-year around calving

Pasture availability was estimated through electronic plate meter (F200, Farmworks, Fielding, New Zealand). Feeds were classified as conserved forage or concentrate (Lanuza 2011). Amounts of concentrate and conserved forage (silage, hay, etc.) offered were calculated from production records at each farm.

Samples of mixed ration were obtained from TMR farms at each monthly visit, and from the MX farms during the winter months visits. In case of the GS farms, and the MX farms during the spring, summer and autumn, the composition of the diet offered to cows at each farm was estimated from the amounts of each individual feed offered to the animals. Samples of the individual feedstuffs offered to the animals at each farm were obtained at each monthly farm visit. Simulated grazed pasture samples were collected on pasture strips. Samples of estimated diets were then reconstructed by mixing samples of each individual ingredient according to its proportion in the estimated diet. These composite samples were used for chemical and fatty acids analysis. A total of 162 samples (nine samples per 18 months) were analysed.

CHEMICAL AND FATTY ACIDS ANALYSES

Chemical and fatty acids analyses were conducted at Instituto de Investigaciones Agropecuarias (INIA), Chile. Dry matter (DM), crude protein (CP), EE and ash were determined as by AOAC (2005). Digestible Organic matter was estimated from *in vitro* digestibility obtained according to Tilley and Terry (1963). Neutral detergent fibre (NDF) was determined according to Sadzawka *et al* (2007). Milk fat, protein and lactose were analysed by near infrared spectroscopy (MILKOSCAN™, Foss Electrics, Hillerød, Denmark). Somatic cells were counted with a FOSSOMATIC™ analyser.

Fat was extracted from milk according to Rico *et al* (2007) and from feed samples as per Bligh and Dyer (1959) modified by Lumley and Colwell (1991). Extracted milk and feed fatty acids were methylated as by Ichihara *et al* (1996) and Hartman and Lago (1973), respectively. Samples were analysed by gas chromatography equipped with an FID detector (Shimadzu model GC 2010 Plus). Separation was conducted using a capillary column (SPtm-2560, Sigma-Aldrich, USA, 100 m x 0.25 mm x 0.2 µm) with an initial oven temperature of 140 °C and a 4°C min^{-1} ramp until 240 °C. Injector and detector temperatures were set at 260 °C. Helium was used as carrier gas at a flow rate of 0.5 mL min^{-1}. Injector split was set at 100:1. A mixture of fatty acid methyl esters (Supelco 37 Component FAME Mix, Supelco Analytical, USA), CLA ethyl ester (9c,11tr-Octadecadienoic, Larodan Fine Chemicals, Sweden), *trans*-11-vaccenic methyl ester (Perkin Elmer, USA), PUFA-2 (Supelco Analytical, USA) were used as external standards, and 19:0 (NU-CheckPrep, INC, Elysian, USA) was added before extraction as internal standard.

STATISTICAL ANALYSIS

Data were analysed by repeated measures with JMP®, with feeding system and month (time), and their interaction, as fixed effects. Farm was included as a random effect nested within feeding system:

response = intercept + feeding system + month + interaction + farm(feeding system, random) + residual

Non-significant ($P > 0.10$) interactions were eliminated. When the effect of the feeding system was significant ($P < 0.05$), least-square means were separated by Tukey's HSD test.

RESULTS

FEED COMPOSITION

Accumulated precipitation and low and high temperatures recorded during the study are shown in table 2. In general, accumulated precipitation was lower than historical for this area (1.330 mm). Low temperature in the autumn and winter of Year 2 were lower than in Year 1 (table 2). Pre and post-grazing sward heights of GS and MX systems are presented in the table 2.

Estimated intake of each dietary component is shown in table 3. The GS and MX systems were highest in fresh forage, and the TMR system had the highest amount of conserved forages and concentrate offered. Seasonal variation of fresh forage (kg Dry Matter d^{-1}) by production system is shown in figure 1. The TMR system had the lowest fresh forage: concentrate ratio (F:C), whereas GS had the highest. The GS system included fodder turnips in the summer (month 10: 5 kg DM d^{-1} and month 11: 2.8 kg DM d^{-1}). The total dry matter (DM) intake was higher in TMR than GS and MX.

The TMR diet had the highest estimated DM and the lowest NDF content (table 3). The grazing system had the lowest dietary *c*-9 *c*-12-18:2. In contrast, GS and MX had higher *c*-9 *c*-12 *c*-15-18:3 (α-linolenic) concentration than TMR in the diet (table 3). The diet of GS had higher 20:0 concentration than TMR.

MILK YIELD AND COMPOSITION

The TMR system had higher milk yield than GS, with MX being intermediate (table 4). Fat percentage was highest in GS and lowest in MX. Milk protein was not affected by system. Lactose content was lowest in GS and highest in TMR. Urea content was higher in GS than TMR, and MX was intermediate. Somatic cell count (SCC) was higher in TMR than in the other two systems.

MILK FATTY ACID COMPOSITION

Milk from GS had lower *c*-9 *c*-12-18:2, *n*-6:*n*-3 ratio and higher α-linolenic than TMR whereas MX was intermediate

Table 2. Accumulated precipitation, low and high temperature recorded at Cardal meteorology station (40º 22’ 31.4” S 72º 53’ 59.7” W) and pre and post-grazing height of Grazing and mixed systems recorded during 18 months of study.

Precipitación acumulada, temperatura mínima y máxima de la estación meteorológica del cardal (40º 22’ 31.4” S 72º 53’ 59.7” W) y altura pre y pospastoreo de los sistemas pastoreo y mixto registrados durante los 18 meses del estudio.

	Months																	
	Autumn			Winter			Spring			Summer			Autumn			Winter		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Accumulated precipitation (mm)	29.8	190	210	110	118	61.7	25.4	32.3	191	9.7	49	47.2	146	165	117	83.3	102	102
Low temperature (ºC)	19.3	19	16.2	15	15.5	23.3	19.3	25.2	22	34.1	29	26.5	24	20.5	15.4	15.6	16.2	21.6
High temperature (ºC)	0.9	–3.0	–4.4	–4.1	–3.2	–1.1	0.1	0.0	5.4	5.7	3.0	0.3	–2.0	–1.0	–2.5	–4.6	–5.1	–2.3
Pre-grazing height (cm)																		
Grazing system	10.3	8.4	7.9	4.4	5.2	8.5	8.4	10.0	9.0	7.4	6.8	7.1	5.7	5.5	4.5	5.1	5.8	5.4
Mixed system	8.6	8.8	7.4	6.1	5.5	9.8	11.1	10.0	9.1	8.8	6.3	8.2	7.6	6.1	5.1	5.8	4.7	8.6
Post-grazing height (cm)																		
Grazing system	5.5	4.6	5.0	3.8	3.8	6.0	6.7	7.9	6.4	6.3	6.1	5.5	4.3	4.5	4.3	4.5	4.1	4.6
Mixed system	5.3	5.7	4.5	4.5	4.4	5.8	7.7	7.4	6.0	7.0	4.8	4.8	4.6	4.2	4.0	4.4	4.2	4.9

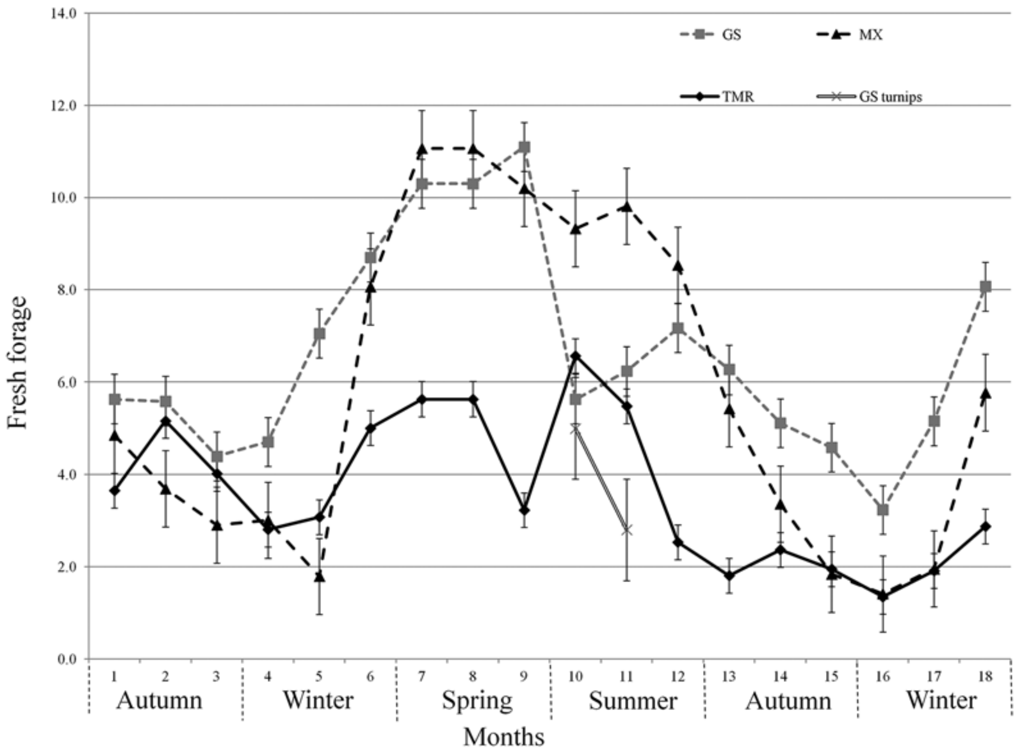

Figure 1. Seasonal variation of fresh forage production (kg Dry Matter d^{-1}) by system. GS= Grazing system; MX= Mixed system; TMR= Total Mixed Ration system. GS turnips= kg dry matter of turnips in Grazing systems.

Variación de producción de forraje fresco en la temporada (kg materia seca d^{-1}) por sistema de producción. GS= Sistema pastoril; MX= Sistema mixto; TMR= Ración totalmente mezclada. GS nabo= kg materia seca de nabo en sistema pastoril.

(table 5). Milk from GS and MX systems had higher RA and *c*-6 *c*-9 *c*-12-18:3 than milk from TMR.

The time by system interaction was significant for total saturated fatty acids (SFA, figure 2), 8:0, 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 18:0, total unsaturated fatty acids (UFA), *c*-9-18:1, RA (figure 3) and *n*-6 fatty acids (figure 4).

In months 1 and 3, GS had higher total saturated fatty acids than TMR, with MX being intermediate whereas in the month 12 MX was higher than TMR and GS was intermediate (figure 2).

In late winter and spring there was an increase in RA in GS and MX (figure 3) whereas differences in *n-6* fatty acids were observed during the two autumns of the study (figure 4), with GS being lower than TMR in the autumn of the first year and the mid and late autumn of the second year.

DISCUSSION

The milk fat content of GS system was similar to what was reported by Calvache (2009) for the same Chilean area and it is higher to what was reported by Palladino *et al* 2010 and White *et al* (2001) for Holstein Friesian breed (3.3-3.5 %). The three systems were higher to values reported by Pinto *et al* (1998) from milk of 16 dairy plants of regions of south of Chile (3.53 %).

The grazing systems had higher milk urea N concentration. Urea is the primary form of N excretion in mammals, and high concentration of milk urea N has long been known to reflect inefficient utilization of dietary N by ruminants (Broderick and Clayton 1997). When ammonia release rate in the rumen exceeds its rate of incorporation into microbial amino acids, excess ammonia is absorbed through the rumen wall and converted to urea in the liver, most of which is eliminated in urine (Wallace *et al* 1997). Even though the numerical differences in estimated dietary CP content among systems were not significant, protein in local pastures has been shown to be highly degradable (Valderrama and Anrique 2011), which could make N utilisation in the rumen inefficient in the GS system. Regarding SCC, housing increases the risk of environmental contamination associated with subclinical mastitis, which might explain the higher SCC in TMR[1] (Golbert *et al* 1992).

[1] Olivo CJ, LI Beck, A Mossat Gabbi, P Santini Charão, MF Sobczak, LF Gomes Uberty, JW Dürr, R Araújo Filho. 2005.Composition and somatic cell count of milk in conventional and agro-ecological farms: a comparative study in Depressão Central, Rio Grande do Sul state, Brazil. Livest Res Rural Dev 17 Article #72. Retrieved April 28, 2014, http://www.lrrd.org/lrrd17/6/oliv17072.htm

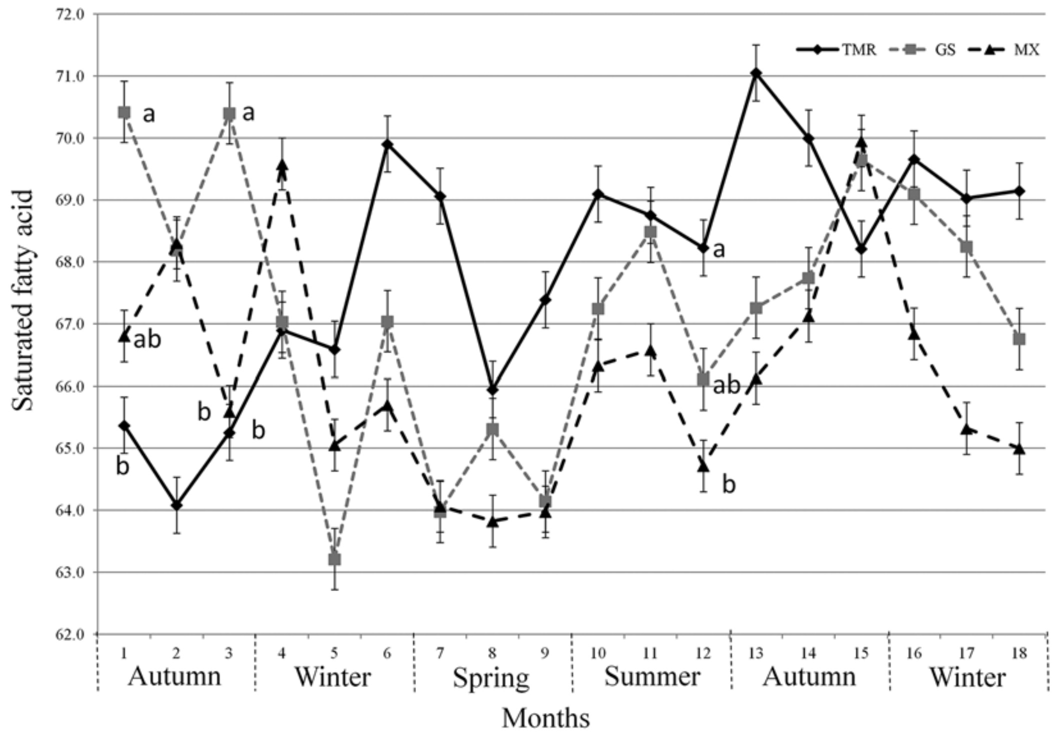

Figure 2. Seasonal variation of saturated fatty acids (g 100 g^{-1} of total fatty acids) by production system. GS= Grazing systems; MX= Mixed system; TMR= Total Mixed Ration system. [ab]Common letter within each month indicates no significant difference (P > 0.05).

Variación de ácidos grasos saturados en la temporada (g 100 g^{-1} de ácidos grasos totales) por sistema de producción. GS= Sistema pastoril; MX= Sistema mixto; TMR= Ración totalmente mezclada. Letras iguales dentro del mes no indican diferencia significativa (P > 0.05).

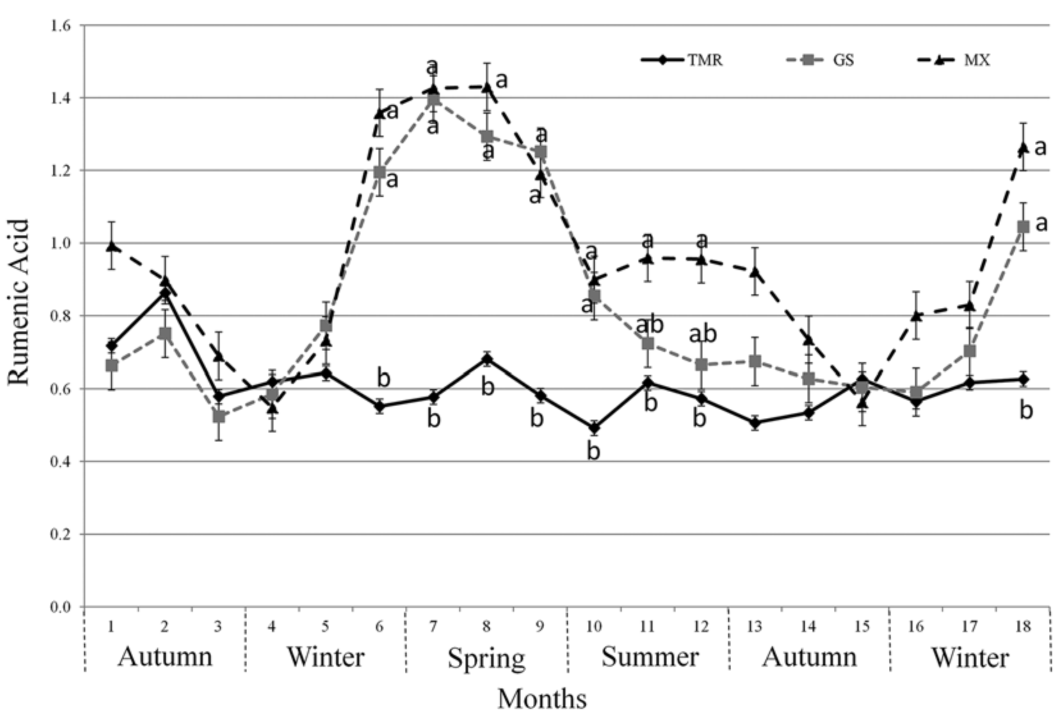

Figure 3. Seasonal variation of Rumenic acid (g 100 g^{-1} of total fatty acids) by production system. GS= Grazing systems; MX= Mixed system; TMR= Total Mixed Ration system. [ab]Common letter within each month indicates no significant difference (P > 0.05).

Variación de ácido ruménico en la temporada (g 100 g^{-1} de ácidos grasos totales) por sistema de producción. GS= Sistema pastoril; MX= Sistema mixto; TMR= Ración totalmente mezclada. Letras iguales dentro del mes no indican diferencia significativa (P > 0,05).

Table 3. Estimated amount of feeds offered and composition and fatty acids content of estimated diet in each feeding system.
Cantidad estimada de alimentos ofrecidos y perfil de ácidos grasos de la dieta para cada sistema.

Feed (DM)	Production system			S.E.M	*P*-value		
	Grazing	Mixed	Total Mixed Ration		Time (T)	System (S)	T × S
Fresh forage kg d^{-1}	6.62^{a}	5.78^{a}	3.69^{b}	0.43	0.058	0.007	0.63
Conserved forage kg d^{-1}	3.66^{b}	4.17^{b}	7.24^{a}	0.40	0.004	0.002	0.021
Concentrate kg d^{-1}	4.84^{b}	6.76^{b}	10.00^{a}	0.64	0.002	0.004	< 0.001
Fresh forage: concentrate ratio	1.39^{a}	0.90^{b}	0.38^{c}	0.096	0.17	0.001	0.91
Turnips kg d^{-1}	0.44	---	---	---	---	---	---
Total DMI kg d^{-1}	15.6^{b}	16.7^{b}	20.8^{a}	4.108	0.51	0.001	0.47
Diet composition							
Dry Matter %	24.7^{b}	29.5^{ab}	34.2^{a}	1.36	0.005	0.006	0.94
Crude Protein % DM	20.0	19.6	18.6	1.09	0.91	0.63	0.94
Digestible Organic Matter % DM	75.0	74.6	76.3	0.50	0.005	0.18	0.30
Neutral Detergent Fibre % DM	40.5^{a}	37.8^{a}	31.5^{b}	1.52	0.71	0.007	0.23
Ether extract % DM	3.24	3.14	3.22	0.214	0.013	0.93	0.75
Fatty acids content g 100 g^{-1} of total fatty acids							
14:0	1.46	1.19	1.19	0.12	0.001	0.28	0.021
16:0	31.1	30.7	26.2	3.32	0.89	0.29	0.53
c-9-16:1	0.85^{a}	0.74^{ab}	0.48^{b}	0.075	0.93	0.038	0.68
18:0	10.0^{a}	9.34^{a}	5.21^{a}	1.91	0.001	0.20	0.078
c-9-18:1	18.6	16.3	23.3	2.40	0.016	0.15	0.021
c-9 *c*-12-18:2	19.4^{b}	27.1^{ab}	33.1^{a}	2.90	0.001	0.022	0.47
c-9 *c*-12 *c*-15-18:3	17.0^{a}	14.3^{ab}	9.36^{b}	8.576	0.21	0.019	0.079
20:0	0.89^{a}	0.62^{ab}	0.59^{b}	0.061	0.21	0.025	0.18
c-9-20:1	0.19	0.085	0.10	0.091	0.67	0.67	0.81

[ab]Common letter within a row indicates no significant difference among production systems (P > 0.05).
Fresh forage: grazing and/or cut *Lolium multiflorum* pasture (Total Mixed Ration system).
Conserved forage: pasture and corn silage, pasture and alfalfa.
SEM: Standard error of the mean;
[ab]Letras iguales dentro de la fila indica ausencia de diferencias significativas entre sistemas de producción (P > 0,05).
Forraje fresco: pastoreo o *soiling* de *Lolium multiflorum* (Sistema ración totalmente mezclada).
Forraje conservado: pradera y ensilaje de maíz, pradera y alfalfa.
SEM: Error estándar.

Total SFA were similar to values reported by Pinto *et al* (2002) from milk fat from dairy plants in the south of Chile. Several studies indicated that concentrate supplementation increased SFA and reduced UFA content (Rego *et al* 2004, Bargo *et al* 2006) in the present study, this relation was no clear (figure 2).

The low proportion of *c*-9 *c*-12-18:2 and higher proportion of α-linolenic in milk fat from GS can be explained by the dietary concentration of *c*-9 *c*-12-18:2 and α-linolenic fatty acids of GS (table 3). Dietary concentration of fatty acids is related to fresh forage intake of each system, where fresh grass contains a high proportion (50 % -75 %) of total FA content as α-linolenic, whereas grains have higher concentrations of *c*-9 *c*-12-18:2 (Elgersma *et al* 2006). Some authors have reported similar results in cows fed cut fresh forage (Ferlay *et al* 2006), grazing cows (Bargo *et al* 2006), and TMR complemented with 12 h grazing (Morales-Almaráz *et al* 2010). White *et al* (2001) and Gómez-Cortés *et al* (2009) reported that higher concentrate in the diet resulted in higher concentration in milk fat of *n*-6 fatty acids than grazing. Other reports indicate that grazing increased α-linolenic content in milk fat (Wyss *et al* 2010, Morales-Almaráz *et al* 2010, Rutkowska *et al* 2012). In this respect, increments in α-linolenic would represent a benefit of diets based on pasture from the point of view of milk health attributes (Gómez-Cortés *et al* 2009).

Milk fat from GS and MX had approximately 50% more RA than milk fat from TMR (table 5). Rumenic acid is produced as an intermediate of the biohydrogenation process occurring in the rumen, where dietary unsaturated fatty acids (mainly *c*-9 *c*-12-18:2 and α-linolenic) undergo

Table 4. Milk yield and components by production system.

Cantidad y componentes de leche por sistema de producción.

Response	Production Systems			S.E.M	*P*-value		
	Grazing	Mixed	Total Mixed Ration		Time (T)	System (S)	T × S
Milk per cow kg d^{-1}	23.0^{b}	25.3^{ab}	28.7^{a}	7.128	0.000	0.016	0.025
Fat %	4.14^{a}	3.68^{b}	3.84^{ab}	0.184	0.000	0.043	0.004
Protein %	3.47	3.43	3.39	0.0353	0.000	0.064	0.052
Lactose %	4.74^{c}	4.85^{b}	4.92^{a}	0.0188	0.011	< 0.001	0.122
Urea g ml^{-1}	0.308^{a}	0.298^{ab}	0.284^{b}	0.00651	0.000	0.006	0.069
Somatic cell count $\times 10^{3}$ cel ml^{-1}	172.7^{b}	163.9^{b}	210.7^{a}	16.8	0.001	0.013	0.001

[ab]Common letter within a row indicates no significant difference among production systems (P > 0.05).
SEM: Standard error of the mean.
[ab]Letras iguales dentro de la fila no indican diferencia significativa entre sistemas de producción (P > 0,05).
SEM: Error estándar.

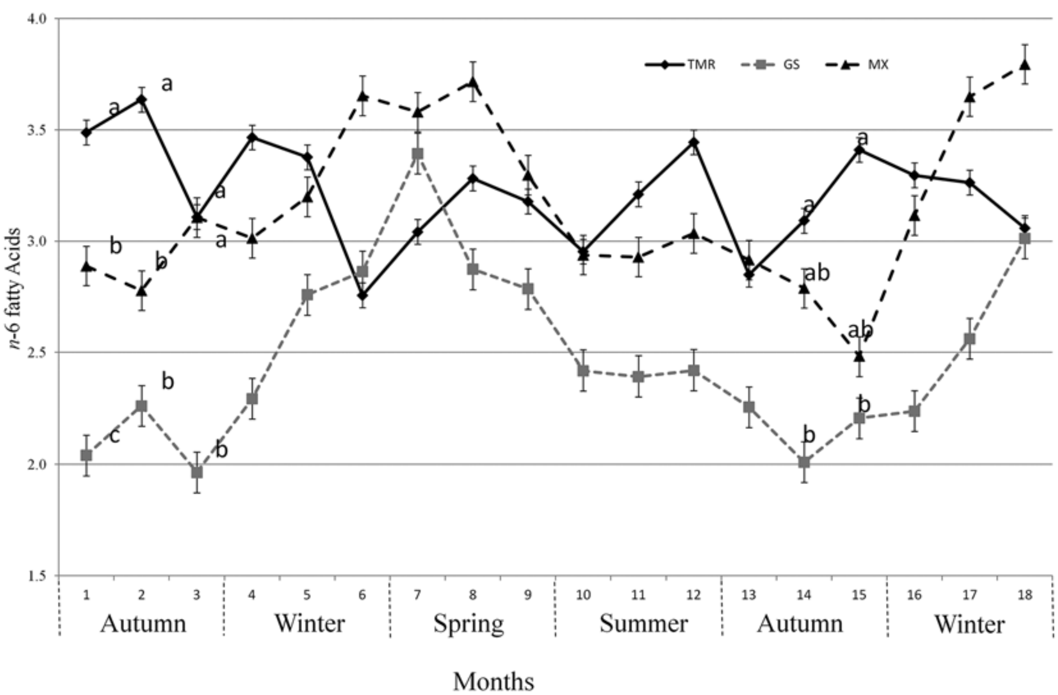

Figure 4. Seasonal variation of n-6 fatty acids (g 100 g^{-1} of total fatty acids) by production system. GS= Grazing systems; MX= Mixed system; TMR= Total Mixed Ration system. [ab]Common letter within each month indicates no significant difference (P > 0.05).

Variación de ácidos grasos n-6 en la temporada (g 100 g^{-1} de ácidos grasos totales) por sistema de producción. GS= Sistema pastoril; MX= Sistema mixto; TMR= Ración totalmente mezclada. Letras igual dentro del mes no indican diferencia significativa (P > 0,05).

successive steps of isomerisation and reduction (Lock and Bauman, 2004, Kalač and Samková, 2010). Most RA in milk fat is actually synthesised by endogenous conversion of *t*-11-18:1 (*trans*-vaccenic acid) by the enzyme Δ-9-desaturase in mammary gland (Bauman and Griinari, 2003), *t*-11-18:1 also being an intermediate of ruminal biohydrogenation. The positive effect of pasture intake on RA in milk fat has been previously reported (Kelly *et al* 1998, Ferlay *et al* 2006, Gómez-Cortés *et al* 2009). On the other hand, RA content is negatively affected by the level of concentrate in the diet (Shingfield *et al* 2010, Aldai *et al* 2013). Rumenic acid content in milk fat in GS and MX treatments in the present study are somewhat higher than those reported by Aviléz *et al* (2012, 2013), who also worked with grazing systems under the conditions of Southern Chile.

Table 5. Fatty acid composition (g 100 g^{-1} of total fatty acids by production system.
Perfil de ácidos grasos (g 100 g^{-1} de ácidos grasos totales) por sistema de producción.

Fatty acid	Productive Systems			S.E.M	*P*-value		
	Grazing	Mixed	Total Mixed Ration		Time (T)	System (S)	T × S
Total SFA	67.19	66.17	67.98	0.0753	0.018	0.30	0.0006
4:0	2.03	1.92	1.91	0.052	0.61	0.26	0.20
6:0	1.37	1.29	1.32	0.033	0.11	0.34	0.080
8:0	0.98	0.94	0.95	0.043	0.0003	0.84	0.015
10:0	2.45	2.40	2.53	0.181	0.0001	0.89	0.0005
11:0	0.29	0.29	0.30	0.029	0.0001	0.92	0.001
12:0	3.07	3.02	3.26	0.276	0.0002	0.81	0.0002
13:0	0.16	0.17	0.19	0.025	0.27	0.62	0.009
14:0	10.77	10.63	11.03	0.483	0.0001	0.84	0.002
15:0	1.08	1.12	1.19	0.082	0.017	0.70	0.0008
16:0	30.54	31.04	32.34	0.822	0.027	0.35	0.033
17:0	0.54	0.61	0.61	0.049	0.0001	0.53	0.29
18:0	13.75	12.58	12.19	1.085	0.0001	0.60	0.0005
20:0	0.17	0.15	0.15	0.010	0.064	0.64	0.26
UFA	32.31	33.34	31.58	0.756	0.031	0.32	0.0006
c-9-14:1	0.91	0.90	0.93	0.086	0.018	0.97	0.48
c-9-16:1	1.28	1.24	1.27	0.071	0.0007	0.94	0.15
c-9-17:1	0.22	0.25	0.22	0.017	0.99	0.35	0.57
t-11-18:1	0.88	1.34	0.70	0.208	0.50	0.60	0.28
c-9-18:1	25.50	25.71	24.61	0.594	0.12	0.43	0.003
c-7-18:1	0.05	0.12	0.29	0.144	0.37	0.52	0.22
t-9 *t*-12-18:2	0.27	0.31	0.26	0.025	0.71	0.33	0.52
c-9 *c*-12-18:2	1.30^{b}	1.81^{ab}	2.28^{a}	0.143	0.15	0.008	0.058
c-9 *c*-12 *c*-15-18:3	0.74^{a}	0.69^{ab}	0.53^{b}	0.049	0.12	0.043	0.35
c-6 *c*-9 *c*-12-18:3	0.10^{a}	0.08^{a}	0.073^{b}	0.0032	0.28	0.005	0.35
c-9 *t*-11-18:2	0.83^{a}	0.96^{a}	0.61^{b}	0.048	0.27	0.006	0.008
Unidentified	0.49	0.49	0.44	0.019	0.0001	0.13	0.58
n-3	0.74^{a}	0.70^{ab}	0.53^{b}	0.049	0.11	0.043	0.35
n-6	2.49	3.16	3.21	0.21	0.94	0.088	0.019
n-6:*n*-3	3.41^{b}	4.75^{ab}	6.64^{a}	0.59	0.042	0.023	0.27

^{ab}Common letter within a row indicates no significant difference (P > 0.05). SEM: Root mean squared error; SFA: saturated fatty acids; UFA: unsaturated fatty acids; *n*-6:*n*-3: fatty acid ratio.
Letras iguales dentro de la fila no indican diferencia significativa (P > 0,05). SEM: Error del cuadrado medio; SFA: ácidos grados saturados; UFA: ácidos grasos insaturados; *n*-6:*n*-3: relación ácidos grasos.

Considerable attention has been paid to the relative proportion of *n*-6 and *n*-3 fatty acids, as diets with *n*-6:*n*-3 ratios higher than 4.0 have been suggested to increase risk for certain cancers and coronary heart disease (British Department of Health, 1994, Hibbeln *et al* 2006). In this respect, only milk from the GS system had a *n*-6:*n*-3 ratios lower than 4.0 (table 5). Similar results were reported by Khan *et al* (2012), who speculated that the decrease they observed in α-linolenic and total *n*-3 content, and the increase in the *n*-6:*n*-3 ratio in milk fat, were the results of reduced α-linolenic intake.

In late winter and spring there was an increase in RA in GS and MX (figure 3). This increase coincided and is likely due to increase of pasture intake in those seasons (figure 1), with greatest pre and post-grazing pasture heights (table 2). Pasture production in southern Chile varies amply throughout the year, with low growth rates in winter and dry summers (Balocchi1986). Other studies revealing seasonal variation in CLA content of milk fat with the highest concentrations in warm seasons in grazing cows and the lowest concentrations in winter have been reported (Lock and Garnsworthy 2003, Elgersma *et al* 2006, Collomb *et al* 2008, Wyss *et al* 2010).

Jahreis *et al* (1997) compared conventional farming with high external inputs of fertiliser and concentrates, with or without grazing during summer, against low external input organic farming with summer grazing. They found that organically produced milk had the highest content of RA in milk fat, especially during May through September, which was the grazing period. In another study, milk from a grazing system and an organic, low external input system, had higher concentrations of nutritionally desirable fatty acids (RA and α-linolenic) compared to milk from a high-input system (Butler *et al* 2008). In agreement, Dhiman *et al* (1999) indicated that increasing the proportion of grazed grass from pasture in the diet of dairy cows linearly increased the RA content of milk. Similarly, White *et al* (2001) found that cows grazing warm-season crabgrass (*Digitaria sanguinalis*) supplemented with 5.5 kg of commercial concentrate/cow per day produced significantly higher RA concentrations in milk compared to confinement-fed cows.

During the summer months (February and March), RA was higher in MX than in TMR, whereas GS was intermediate. High RA content in milk from MX systems could be due to: (1) higher pasture intake and (2) pasture of better quality in MX compared to GS as a result of irrigation (Nissen and Robert 2009). Irrigation also increases pasture growth rate, and as a consequence pasture intake in MX (figure 1). Content of *c*-9 *c*-12 *c*-15-18:3 is affected by season and date of cutting, with the highest values in spring (Dewhurst *et al* 2001), and consequently concentrations of all CLA isomers in milk decrease as pastures mature (Aviléz *et al* 2012). In addition, during summer animals in GS were supplemented with higher proportion of fodder turnips *(Brassica rapa)* in the diet (figure 1). It is reported that inclusion of turnips decrease the RA concentration in the milk (Thomson *et al* 2000), and this might affect to RA content in GS.

Dietary content of linolenic acid is evidently central to explain differences among production systems in milk fat linolenic and rumenic acid. Nevertheless, differences in milk composition among the production systems studied can also be influenced by factors other than the diet. For example, the three production systems studied differed with regard to calving seasons (table 1), which caused variation among them with regard to lactation stage at different times of the year. Lactation stage is known to affect milk fat content (Phillips 2010). Regarding the fatty acids, several studies reported that there is an increase of 6:0 to 14:0 during the first 3 months of lactation and a decrease thereafter, whereas 18:0 fatty acids follow an inverted pattern (Kay *et al* 2005, Garnsworthy *et al* 2006, Stoop *et al* 2009, Bilal *et al* 2014). On the other hand, RA increased with lactation stage (Kay *et al* 2005, Stoop *et al* 2009). However, these authors indicate that changes in milk fat composition over lactation are not explained by changes in overall fat percentage.

The results of the present study should be interpreted with caution. Even though we selected farms as homogenous as possible within each system regarding aspects unrelated to feed management, they would evidently differ both in nutritional and non-nutritional aspects. Furthermore, as explained, estimation of intake of silage and supplements were done from each farm records and not thus not standardised among farms, and are therefore not strictly comparable.

Milk fat from grazing and mixed systems had higher *n*-3 and rumenic fatty acids concentrations, in particular during late winter and spring. Higher *n*-3 and RA content in grazing and mixed systems milk fat associated with high pasture intake in these systems. These results indicate that grazing contributes to improve milk nutritional quality.

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