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Short communication. Effect of forage source (grazing *vs.* silage) on conjugated linoleic acid content in milk fat of Holstein-Friesian dairy cows from Galicia (NW Spain)

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Abstract

The aim of this study was to investigate the effect of different feeding proportions of forage —grazing *vs.* silage— on milk fatty acids (FA) profile and conjugated linoleic acid (CLA) content of autumn calving Holstein-Friesian cows (n = 61) at CIAM (Galicia, NW Spain). Three treatments (S, 100% silage; G/S, 50% grazing + 50% silage; G, 100% grazing) were set and milk FA profile of dairy cows was determined by gas chromatography-mass spectrometry. The G group showed a decrease in short ($p < 0.05$) and medium chain FA ($p < 0.001$), with an increase in long chain FA ($p < 0.001$) in comparison to the G/S and S groups, which showed the lowest levels ($p < 0.001$) of mono- and poly-unsaturated FA. The CLA content in milk fat increased ($p < 0.001$) linearly in relation to the increased proportion of fresh grass in the diet of dairy cows from 0.49 and 0.82 to 1.14 g/100 g FA for the treatments S, G/S and G, respectively. During spring and summer, the levels of CLA were three times higher ($p < 0.001$, +0.76 g/100 g FA) in milk from dairy cows at the G group than in cows at the S group and twice higher ($p < 0.001$, +0.40 g/100 g FA) than in cows at the G/S group. High proportion of grass in the diet of cows increased CLA content, with the highest levels of unsaturated FA and the lowest levels of saturated FA, increasing the added value of milk on grazing systems using available farm resources.

Additional key words: dairy cattle; forage proportion; grass feeding value; milk fatty acids.

Resumen

Comunicación corta. Efecto de la fuente de forraje (pasto *vs.* ensilado) sobre el contenido de ácido linoleico conjugado en la grasa láctea de vacas Holstein-Friesian en Galicia (NO España)

El objetivo de este estudio fue investigar el efecto de utilizar diferentes proporciones de forraje, pasto *vs.* silo, en el perfil de ácidos grasos (AG) de la leche y en el contenido de ácido linoleico conjugado (CLA) en vacas lecheras Holstein-Friesian (n = 61) de partos de otoño. Se establecieron tres tratamientos (S, 100% ensilaje; G/S, 50% pastoreo y 50% ensilaje; G, 100% pastoreo) y se determinó el perfil de AG de la leche por cromatografía de gases-espectrometría de masas. El grupo G mostró una disminución en los AG de cadena corta ($p < 0,05$) y media ($p < 0,001$), con un aumento en los AG de cadena larga ($p < 0,001$) en comparación con los grupos G/S y S, que mostraron unos contenidos menores ($p < 0,001$) de AG mono- y poli-insaturados. El contenido de CLA en la grasa láctea aumentó linealmente con el incremento en la proporción de pasto fresco en la dieta de las vacas lecheras desde 0,49 y 0,82 a 1,14 g/100 g de AG para los tratamientos S, G/S y G, respectivamente. Durante la primavera y el verano, los niveles de CLA fueron tres veces superiores ($p < 0,001$, +0,76 g/100 g de AG) en la leche de las vacas del grupo G respecto a la del grupo S y dos veces superiores ($p < 0,001$, +0,40 g/100 g de AG) a la del grupo G/S. Una alta proporción de pasto en la dieta incrementó el contenido de CLA, con niveles superiores de AG insaturados y menores de saturados, lo que aumenta el valor añadido de la leche producida en los sistemas de pastoreo que aprovechan los recursos existentes en las explotaciones.

Palabras clave adicionales: ácidos grasos leche; proporción forraje; vacuno; valor nutritivo pasto.

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The majority (up to 95%) of milk conjugated linoleic acid (CLA) content is derived by *de novo* synthesis and it is particularly rich in milk from grazing animals or from those fed with high fat diets (Khanal & Olson, 2004). In humid areas as Galicia (NW Spain) where sustainable milk production systems using available farm resources mainly fresh grass is a common practice by dairy farmers, it is expected that the highest CLA content in milk fat of Holstein-Friesian cows will be basically found on long periods of pasture feeding. Elgersma *et al.* (2003a) reported that zero-grazed cows were lower than grazed grass cows in milk rumenic acid (RA, C18:2 *cis-9, trans-11*) production. Mohammed *et al.* (2009) highlighted that it is now worth to investigate the possible mechanisms underpinning the differences in milk RA content among cows grazing, consuming silage as unique forage source only or a mixture of both feeding systems (grazing + silage). Analysis of fresh perennial ryegrass showed that 98% of extracted fat is esterified, with 2% in the free FA form, but when ensiled, esterified fat drops to 51% with the remaining 49% being comprised of free FA (Elgersma *et al.*, 2003a). The levels of α-linolenic acid also vary with environmental factors. The largest seasonal variation on milk FA profile was mainly attributed to the diet, significantly greater amounts of CLA were found in summer months when cows fed outdoors fresh grass than in winter when dried or ensiled forage was fed indoors by dairy cows (Thorsdottir *et al.,* 2004). It is therefore now well established that CLA and n-3 polyunsaturated fatty acids (PUFA) contents are increased under pasture-based milk production systems when compared with total mixed rations (TMR) based systems. The objective of our study was however to investigate the effect of feeding different forage proportions (grazing *vs.* silage) on milk FA profile of dairy cows comparing three forage diets.

The experiment was conducted at Centro de Investigaciones Agrarias de Mabegondo (CIAM) situated in Galicia, Spain (43°12’24’’N; 8°18’36’’W), from April 3 to August 20 in 2008. Mean daily temperature during the experimental period was 15.8°C, similar to the last 10-years average (16.1°C). Total rainfall during the trial was 334 mm, 67 mm higher than in the 10-years average. High photosynthetic active radiation from June to August was 10.0 MJ m^{-2} day^{-1} in 2008, as the last 10-years average (9.7 MJ m^{-2} day^{-1}). The soil type was a free draining, acid brown earth with a silt-loam texture and acid pH (5.5). The swards were initially sown with a mixture of 22 kg ha^{-1} of perennial ryegrass (*Lolium perenne* cv. Brigantia) and 4 kg ha^{-1} of white clover (*Trifolium repens* cv. Huia). At the time of the trial started, the swards were six years old and contained 80% of sown species. Basic fertilizer of P_2O_5 and K_2O, 84 kg ha^{-1}, was applied in February and Nitrogen, 135 kg ha^{-1}, was split in three occasions. Maize silage had 600 kg ha^{-1} of 8-15-15 (N, P_2O_5 and K_2O) at establishment.

The effect of offering three diets with different proportions of forage —grazing *vs.* silage— on milk FA profile seasonal production in dairy cows was investigated. Three groups of autumn calving Holstein-Friesian dairy cows were randomly assigned to one of three forage diets: S (100% silage, n = 11), G/S (50% grazing + 50% silage, n = 27) and G (100% grazing, n = 23), all supplemented with concentrate (6.3 kg DM cow^{-1} day^{-1}) composed of corn flour (31.0%), soybean hulls (34.0%), soybean meal (20.0%), cottonseed (12.0%), amender (1.0%), calcium carbonate (1.0%) and dicalcium phosphate (1.0%). A total mixed grass:maize (50:50) silage and concentrate ration (TMR) was fed indoors during all lactation in the S treatment and alternating with grazing in the G/S treatment. The G/S group received after the first milking a limited rate of silage, 20 kg cow^{-1} day^{-1}, around half of the estimated daily needs for dairy cows (7 kg DM cow^{-1} day^{-1}). The dry matter (DM) content of grass and silage was determined weekly in order to adjust dietary allocation of grass and silage for maintaining consistent forage to concentrate ratio (70:30) throughout the experimental period for the three forage diets. The nutrient composition of forage and concentrate is showed in Table 1. The experimental grazing area was 5.5 ha for the G/S treatment and 4.1 ha for the G treatment, with an average stocking rate (SR) of 4.91 and 5.61 cows ha^{-1}, respectively. The two groups of grazing

Abbreviations used: ADF (acid detergent fibre); BCS (body condition score); BW (body weight); CLA (conjugated linoleic acid); CP (crude protein); DHA (daily herbage allowance); DM (dry matter); FA (fatty acids); HM (herbage mass); IVODM (digestibility *in vitro* of organic matter); LCFA (long chain fatty acids); MCFA (medium chain fatty acids); MUFA (monounsaturated fatty acids); MY (milk yield); NDF (neutral detergent fibre); OM (organic matter); PDMI (pasture dry matter intake); PUFA (polyunsaturated fatty acids); RA (rumenic acid); SCFA (short chain fatty acids); SFA (saturated fatty acids); SR (stocking rate); UFA (unsaturated fatty acids).

Table 1. Chemical composition of feeds used in the diets (grazing, silage and concentrate)

Item	Fresh grass	Grass silage	Maize silage	Concentrate
Dry matter (%)	17.95	28.09	36.80	90.40
Organic matter (g kg^{-1} DM)	915	897	972	570
Crude protein (g kg–1 DM)	126	100	65	168
Acid detergent fibre (g kg^{-1} DM)	287	364	205	307
Neutral detergent fibre (g kg^{-1} DM)	532	529	419	418
Digestibility *in vitro* (g kg^{-1} DM)	779	623	717	795
Net energy lactation (Mcal kg^{-1})	1.34	1.00	1.46	1.59

Holstein-Friesian dairy cows, G/S and G, were offered a similar daily herbage allowance (DHA) (20-25 kg DM cow^{-1} day^{-1}) with a flexible grazing management system entering into paddocks when herbage reached at 15-20 cm of sward height and left at a residual height of 4 cm, assessed by a rising plate meter (Frame, 1981) from spring to summer months. On 20 August, grass supply was restricted by a strong summer drought.

Sixty-one autumn calving (November 29 ± 53.9 days) Holstein-Friesian cows from the experimental herd at CIAM, primiparous (n = 21) and multiparous (n = 40), lactation number (2.3 ± 1.2), pre-experimental milk yield (MY) (28.0 ± 6.43 kg cow^{-1} day^{-1}), milk protein (3.22 ± 0.25 g kg^{-1}), milk fat (3.68 ± 0.50 g kg^{-1}), body weight (BW) (565 ± 69.7 kg) and body condition score (BCS) (2.61 ± 0.69) were balanced and blocked into three groups of cows (S, G/S and G) fed with the described three forage diets. When the trial commenced cows were on average 119 days in milk.

Five random sward samples (0.33 m × 0.33 m) per paddock were taken before and after grazing, cutting to 4 cm of fresh grass above ground level with battery-operated shears. Sward heights were determined in the sampling area using a rising plate meter (Frame, 1981). Grass height (> 4.0 cm) was used to allocate each group of dairy cows after the morning milking using temporary pre- and back fencing, with no access to the previous grazed area. Pastures were not topped at all during the experimental period. After weighed, the sward samples were dried at 70°C during 24 h for herbage mass (HM) determination. Mixed sward samples, around 0.5 kg per paddock, were milled, then vacuum packed and stored at –20°C for chemical composition analysis made at CIAM using infrared reflectance spectroscopy by NIRS System 6500 (Foss Analytical, Hillerød, Denmark). Organic matter (OM), crude protein (CP), acid (ADF) and neutral detergent fibre (NDF), water soluble carbohydrates (WSC) and digestibility *in vitro* of organic matter (IVODM) were determined using the equations of calibration reported by Castro-García (1994). Silage DM intakes were estimated daily assuming losses estimated in other studies, around 20% for grass silage and 12% for maize silage (Phipps & Wilkinson, 1985). Pre- and post-grazing samples were used to estimate the following sward variables (Campbell, 1966; Hodgson, 1979):

— Herbage mass (HM) as kg DM ha^{-1}: $(A_i) + n_i*[(A_i - D_{i-1})*r_i^{-1}]$

— Daily herbage allowance (DHA) as kg DM cow^{-1} day^{-1}: HM*(cow*day)$^{-1}$

— Pasture dry matter intake (PDMI) as kg DM cow^{-1} day^{-1}: $[(A_i - D_i) + n_i*[(A_i - D_{i-1}*r_{i-1}^{-1})]*(cow*day)^{-1}$

— Herbage utilization as %: (PDMI*DHA^{-1})*100, where A_i = kg DM ha^{-1} before grazing; D_i = kg DM ha^{-1} after grazing; D_{i-1} = kg DM ha^{-1} after the previous grazing; n_i = number of grazing days (standing time) and r_i = number of days between D_{i-1} and A_i.

The second term in HM and PDMI estimations $n_{i*}[(A_i - D_{i-1}*r_i^{-1})]$ was a correction factor for grass growth during grazing days.

Individual MY (kg cow^{-1} day^{-1}) was recorded daily at 08.00 h and 18.00 h milkings by Alprow System. Milk protein, fat and urea content were determined at each cow from two weekly milk samples collected in two successive evening and morning milkings. Milk samples were then pooled together and stored at –20°C until later analysis by the *Laboratorio Interprofesional Gallego de Análisis de Leche* (LIGAL) using infrared spectroscopy by MilkoScan FT6000 (Foss Electric, Hillerød, Denmark). Individual variables of each cow as BW and BCS were also recorded twice a month during the experiment. The BCS was scored by one experienced observer on a 1 to 5 scale (1 = severe undercondition and 5 = severe overcondition) with 0.25 increments (Wildman *et al.*, 1982).

A milk sample of 250 mL was collected weekly from each randomized five dairy cows per treatment and stored at –20°C for later analysis by the *Laboratorio Agrario y Fitopatológico de Galicia* (LAFIGA) using a gas chromatography–mass spectrometry, Agilent Technologies Model 6890N Network GC System, following the modifications of the extraction method proposed by Chouinard *et al.* (1999) and taking into account the considerations reported by Feng *et al.* (2004). The total saturated fatty acids (SFA) in milk fat were determined as the sum of C4:0 to C18:0. Short (SCFA, C4:0 to C10:0), medium (MCFA, C12:0 to C16:0) and long chain fatty acids (LCFA, C18:0 to C18:3) and the ratio between saturated (SFA, C4:0 to C18:0) and unsaturated fatty acids (UFA, C18:1 to C18:3) were calculated. The proportions of monounsaturated (MUFA, C18:1) and polyunsaturated fatty acids (PUFA, C18:2 to C18:3) were also determined.

Data were analyzed by analysis of variance for a completely randomized design using the General Linear Model (GLM) procedure (SAS Institute, 2005). The model used was as follows: $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where Y_{ij} was the observation of forage diet $_i$ and animal $_j$, for any of the dependent variables considered; μ was the overall mean; α_i was the fixed effect of the forage diet (i = 1, 100% silage; 2, 50% grazing + 50% silage; 3, 100% grazing) and ε_{ij} was the residual random error associated with the observation. When differences between forage diets appeared ($p < 0.01$) a Tukey's multiple comparison test was performed. Least square means (LSM) and standard error of the means (SEM) for the three forage diets used to feed the three groups of dairy cows were calculated for each dependent variable. Mean differences were declared significant at $p < 0.05$ for the three forage diets.

On average, the G/S and G groups completed 4-5 grazing rotations with a total of 139 grazing days. The G group had less grazing days per rotation ($p < 0.001$, –6.9 days) than the G/S group (34.8 days), with lower herbage utilization (40.1%) in the group G/S than in the G group (89.2%) due to lower SR and higher substitution rate. The pre- and post-grazing sward heights were lower in the G group (16.4 and 4.7 cm, respectively) than in the G/S group (17.3 and 7.2 cm, respectively). Cows in the G/S group were allocated to greater HM (kg DM ha^{-1}) ($p < 0.05$, +278) and DHA (kg DM cow^{-1} day^{-1}) ($p < 0.05$, +4.60) than animals in the G group, with an average HM of 3,069 kg DM ha^{-1} and a DHA of 19.34 kg DM cow^{-1} day^{-1}, respectively. Sward quality was higher ($p < 0.01$) in the G group compared to the G/S group, with higher CP (G, 144 *vs.* G/S, 118 g kg^{-1} DM), WSC (G, 182 *vs.* G/S, 148 g kg^{-1} DM) and IVODM (G, 785 *vs.* G/S, 756 g kg^{-1} DM), and lower ADF (G, 271 *vs.* G/S, 289 g kg^{-1} DM) and NDF (G, 525 *vs.* G/S, 573 g kg^{-1} DM). Total DM intake (kg DM cow^{-1} day^{-1}) was lower in the S group compared to the G/S ($p < 0.05$, –1.6) and G ($p < 0.01$, –2.4) groups, with no significant differences between them (Table 2). Pasture DM intake (kg DM cow^{-1} day^{-1}) was higher in the G group than in the G/S ($p < 0.001$, +7.7) and S ($p < 0.001$, +17.3) groups, according to the grazing proportion of fresh grass in the three forage diets. Silage DM intake (kg DM cow^{-1} day^{-1}) was higher in the S group than in the G/S ($p < 0.001$, +8.0) and G groups ($p < 0.001$, +14.9) due to the silage proportion

Table 2. Pasture and animal production parameters from the three groups of dairy cows

	Diets[1]			SEM[2]	*p*-values[3]		
	S	G/S	G		S *vs.* G/S	S *vs.* G	G/S *vs.* G
Feeding regime (kg DM cow^{-1} day^{-1})							
Pasture DM intake	0^a	9.6^b	17.3^c	0.12	***	***	***
Silage DM intake	14.9^a	6.9^b	0^c	0.12	***	***	***
Concentrate DM intake	6.3^a	6.3^a	6.3^a	0.14	NS	NS	NS
Total DM intake	21.2^a	22.8^b	23.6^b	0.34	*	**	NS
Animal performance							
Milk yield (kg cow^{-1} day^{-1})	23.3^a	21.5^b	22.6^c	0.11	***	**	***
Milk protein (g kg^{-1})	30.3^a	30.6^a	31.6^b	0.20	NS	***	**
Mil fat (g kg^{-1})	39.6^a	38.3^{ab}	37.4^b	0.30	NS	*	NS

[1] Diets: (S) 100% silage, (G/S) 50% grazing + 50% silage, (G) 100% grazing. [2] Standard error of the mean (SEM). [3] NS: not significant ($p \geq 0.05$); Significance: ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$. [a-c] Means within a row with different superscripts differ ($p < 0.05$).

in the ration. In spite of all groups received the same level of concentrate DM intake (6.3 kg DM cow^{-1} day^{-1}) in the diet, the G and G/S groups produced less MY than the S group (23.3 kg cow^{-1} day^{-1}) due probably to the high differences on the quality of grass forage all along the experimental period. The highest milk protein content (g kg^{-1}) was found in the G group compared to the S ($p < 0.001$, +1.30) and G/S ($p < 0.01$, +1.00) groups, with no differences among them. Milk fat content (g kg^{-1}) was higher ($p < 0.05$, +2.20) in the S group than in the G group.

The milk FA profile for the three groups of cows is showed in Table 3. There was a significant higher value in the concentrations of SFA in the S ($p < 0.001$, +3.90 g/100 g of FA) and G/S ($p < 0.001$, +3.39 g/100 g of FA) groups than in the G group (61.95 g/100 g of FA), due mainly to the concentrations of C18:0 for the G/S group and C16:0 and C14:0 for the S group, and to a minor extent by the differences in the concentrations of other SFA as C6:0, C8:0, C10:0 and C12:0. The lowest content of SCFA was found in the G group, due to lower levels of C6:0, C8:0 and C10:0, compared to the other two groups of cows (S and G/S). The lowest content of MCFA, with the lowest levels of C12:0, C14:0 and C16:0, was also found in the G group. However, the highest content of LCFA and the highest levels of UFA (MUFA and PUFA) were found in the G group, due to higher levels of C18:0, C18:1, C18:2 and C18:3, compared to the S and G/S groups. The predominant CLA isomer determined, accounting around 75-85% of the total, was the RA (C18:2 cis-9, trans-11) being three times higher ($p < 0.001$, +0.76 g/100 g of FA) during spring (April-May) in the G group than in the S group and twice higher ($p < 0.001$, +0.40 g/100 g of FA) than in the G/S group. The linolenic acid content was also different among the three forage diets, with higher amount in the G group compared to the G/S and S groups. In our study, substantial variation in milk FA profile of dairy cows especially in CLA content across the main grazing season (April-August) was observed. At that time a better milk FA profile, with less SFA, more UFA and CLA content, was found from cows in the G group than in the S group. This was more related to the variation on diet composition than anything else, due mainly to the forage proportion and the

Table 3. Milk fatty acids composition (g/100 g of total FA) of the three forage diets used to feed autumn calving Holstein-Friesian dairy cows during the experimental period in 2008

	Diets[1]			**SEM[2]**	***p*-values[3]**		
	S	**G/S**	**G**		**S *vs.* G/S**	**S *vs.* G**	**G/S *vs.* G**
$C_{4:0}$, Butyric acid	4.26^a	4.73^a	4.33^a	0.37	NS	NS	NS
$C_{6:0}$, Caproic acid	2.31^a	2.39^b	2.22^c	0.03	*	*	***
$C_{8:0}$, Caprylic acid	1.32^a	1.34^a	1.18^b	0.02	NS	***	***
$C_{10:0}$, Capric acid	2.93^a	2.86^a	2.42^b	0.07	NS	***	***
$C_{12:0}$, Lauric acid	3.26^a	3.12^a	2.65^b	0.09	NS	***	***
$C_{14:0}$, Myristic acid	10.98^a	10.66^a	10.18^b	0.17	NS	***	*
$C_{16:0}$, Palmitic acid	30.44^a	30.20^a	27.92^b	0.47	NS	***	***
$C_{18:0}$, Stearic acid	10.35^{ab}	10.04^a	11.05^b	0.32	NS	NS	*
$C_{18:1}$, Oleic acid	21.81^a	21.49^a	23.70^b	0.35	NS	***	***
$C_{18:2}$, Linoleic acid	2.86^a	2.76^a	3.13^b	0.06	NS	**	***
$C_{18:2\ cis\text{-}9,\ trans\text{-}11}$, CLA	0.49^a	0.82^b	1.14^c	0.04	***	***	***
$C_{18:3}$, Linolenic acid	0.40^a	0.50^b	0.62^c	0.02	***	***	***
SCFA[4]	10.82^{ab}	11.31^a	10.15^b	0.33	NS	NS	*
MCFA[5]	44.68^a	43.99^a	40.75^b	0.63	NS	***	***
LCFA[6]	35.41^a	34.78^a	38.50^b	0.66	NS	**	***
SFA[7]	65.85^a	65.34^a	61.95^b	0.46	NS	***	***
UFA[8]	25.06^a	24.75^a	27.45^b	0.41	NS	***	***
MUFA[9]	21.81^a	21.49^a	23.70^b	0.35	NS	***	***
PUFA[10]	3.26^a	3.25^a	3.75^b	0.70	NS	***	***

[1, 2, 3] See Table 2. [4] Short chain fatty acids (C4:0 to C10:0). [5] Medium chain fatty acids (C12:0 to C16:0). [6] Long chain fatty acids (C18:0 to C18:3). [7] Saturated fatty acids (C4:0 to C18:0). [8] Unsaturated fatty acids (C18:1 to C18:3). [9] Monounsaturated fatty acids (C18:1). [10] Polyunsaturated fatty acids (C18:2 to C18:3). [a-c] Means within a row with different superscripts differ ($p < 0.05$).

level of intake of UFA, especially by feeding fresh grass with higher content of linoleic acid (Precht & Molkentin, 2000).

The key point of our study was to assess the effect of forage source, grazing or silage, on milk FA profile of dairy cows during the main grazing season, 20 weeks, from April to August in the humid conditions of Galicia, using different proportions of grazing *vs.* silage, providing a good opportunity to evaluate the cumulative treatment effects of three forage diets on sward quality, PDMI and milk performance and quality of dairy cows at pasture. The main result of our study pointed that high grazing proportion of fresh grass in the diet of dairy cows was the main responsible of the highest levels of CLA, linolenic acid and PUFA in milk fat and also it highlighted that appropriate grassland management practices are necessary to be implemented at farm leave for increasing sward quality.

Pasture DM intake in our trial was higher in the G group than in the G/S group, with a slightly higher grazing pressure and better sward quality (higher CP content, WSC and IVODM and lower ADF and NDF), as happened in other experiment carried out by Roca-Fernández *et al.* (2009) when two SR (high *vs.* low) were compared. After a review of the literature, the effect of varying DHA could not be well established on milk concentrations of either CLA or linolenic acid content (Dewhurst *et al.*, 2006). But with a high DHA of 20 kg DM cow^{-1} day^{-1} and a low HM of 1,600 kg DM ha^{-1}, Roca-Fernández *et al.* (2011) reported higher PDMI with lower stem and dead DM yield (> 4.0 cm) and higher sward quality. Lower HM had higher intake of total FA, mainly of linolenic and linoleic acids, which were related to the high herbage content of both FA in grasses (Palladino *et al.*, 2009).

Elgersma *et al.* (2003b) demonstrated that FA content in grasses declined, with lower concentrations of C18:3, after longer periods of regrowth. Regrowth periods affected the total milk FA concentration in our trial with significantly lower concentrations of C18:3 after a longer period of regrowth in the G/S compared to the G group. Elgersma *et al.* (2005) also hypothesized that the protein content in the herbage, the leaf-blade proportion of the canopy and regrowth period of the sward, might affect milk fat content and proportions of FA in the herbage. The milk CLA response was also related to the PDMI, CP and IVODM of swards increasing from the G/S group to the G group. Mohammed *et al.* (2009) found a strong correlation between RA yield (g day^{-1}) and total substrate intake from herbage snips. Differences in forage quality from the use of grass and maize silage in the G/S and S groups harvested the year before in May, after 6 weeks of growth, and the use of grazed grass in the G/S and G groups of our experiment with different growth stages and qualities, after 4-5 weeks of grazing rotations, affected CP and fibre levels and digestibility of forages, with different total DM intake in the three forage diets. The G group with higher IVODM and better sward quality produced more milk with better milk FA profile, higher content of MUFA and PUFA, than the G/S group. But both grazing cows (G and G/S groups) produced less MY than the S (grass and maize silage) group, with significantly lower milk protein and higher milk fat content, due probably to the differences in growth stage between the forages affecting also the efficiency of microbial protein synthesis in the rumen. Mohammed *et al.* (2009) when compared three forage sources all grazing, grazing and silage and zero-grazing found an increased efficiency of milk RA production per 100 g of substrate intake (grazing *vs.* silage) in the cows grazing compared to the other feeding groups as animals were able to produce more RA yield. When the proportion of fresh grass was increased in the diet of dairy cows the levels of PUFA in milk fat were higher, especially CLA and n-3 PUFA, with lower levels of SFA when compared to the TMR systems based on silage feeding rations (Dewhurst *et al.*, 2006), these results also agree with data of our trial due to the levels of CLA were three times higher in milk fat from grazing outdoors cows than in silage indoors cows.

In conclusion, the differences in substrate intake provided as forage source (100% silage, 50% grazing + 50% silage and 100% grazing) in the ration during the lactation curve of Holstein-Friesian cows, appeared to contribute to the differences in milk FA profile between the three forage diets compared. The lowest amount of SFA and the highest content of UFA, with an increased efficiency on milk CLA production, were found in cows grazing fresh grass compared to those feeding silage. It is now important to check the milk production system selected by Galician dairy farmers, taking into account the cows' feeding patterns and the response on milk quality to different forage sources and how sward characteristics will affect grass feeding value and milk quality in terms of FA profile.

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References

Campbell AG, 1966. Grazed pasture parameters. 1. Pasture dry-matter production and availability in a stocking rate and grazing management experiment with dairy cows. J Agric Sci Camb 67: 199-210.

Castro-García P, 1994. Espectroscopía de reflectancia en el infrarrojo próximo (NIRS) y evaluación nutritiva de pastos. Tesis Doctorales en microfichas Nº 408. Servicio de Publicaciones de la Universidad de Santiago, Galicia, España. pp: 121. [In Spanish].

Chouinard PY, Corneau L, Barbano DM, Metzger LE, Bauman DE, 1999. Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. J Nutr 129: 1579-1584.

Dewhurst RJ, Shingfield KJ, Lee MRF, Scollan ND, 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Anim Feed Sci Technol 131: 168-206.

Elgersma A, Tamminga S, Ellen G, 2003a. Comparison of the effects of grazing versus stall-feeding of cut grass on milk fatty acid composition of dairy cows. Grassl Sci Eur 8: 271-274.

Elgersma A, Ellen G, Van Der Horst H, Muuse BG, Boer H, Tamminga S, 2003b. Influence of cultivar and cutting date on the fatty acid composition of perennial ryegrass. Grass Forage Sci 58: 323-331 (also Erratum Grass Forage Sci 59: 104).

Elgersma A, Maudet P, Witkowska IM, Wever AC, 2005. Effects of N fertilization and regrowth period on fatty acid concentrations in perennial ryegrass (*Lolium perenne* L.). Ann Appl Biol 147: 145-152.

Feng S, Lock AL, Garnsworthy PC, 2004. Technical note: A rapid lipid separation method for determining fatty acid composition of milk. J Dairy Sci 87: 3785-3788.

Frame J, 1981. Herbage mass. In: Sward measurement handbook (Hodgson J, Baker RD, Davies A, Laidlaw AS, Leaver JD, eds.). British Grassland Soc, Grassland Res Inst, Hurley, UK. pp: 39-69.

Hodgson J, 1979. Nomenclature and definitions in grazing studies. Grass Forage Sci 34: 11-18.

Khanal RC, Olson KC, 2004. Factors affecting conjugated linoleic acid (CLA) content in milk, meat, and egg: a review. Pak J Nutr 3(2): 82-98.

Mohammed R, Stanton CS, Kennelly JJ, Kramer JKG, Mee JF, Glimm DR, O'Donovan M, Murphy JJ, 2009. Grazing cows are more efficient than zero grazed and grass silage-fed cows in milk rumenic acid production. J Dairy Sci 92: 3874-3893.

Palladino RA, O'Donovan M, Murphy JJ, McEvoy M, Callan J, Boland TM, Kenny DA, 2009. Fatty acid intake and milk fatty acid composition of Holstein cows under different grazing strategies: herbage mass and daily herbage allowance. J Dairy Sci 92: 5212-5223.

Phipps RH, Wilkinson JM, 1985. Maize silage. Chalcombe Publ, USA. 48 pp.

Precht D, Molkentin D, 2000. Frequency distributions of conjugated linoleic acid and trans fatty acid contents in European bovine milk fats. Milchwissenschaft 55(12): 687-691.

Roca-Fernández AI, González-Rodríguez A, Vázquez-Yáñez OP, 2009. Response to grazing pressure and supplementation on milk production by dairy cows. Grassl Sci Eur 14: 436-439.

Roca-Fernández AI, O'Donovan M, Curran J, González-Rodríguez A, 2011. Effect of pre-grazing herbage mass and daily herbage allowance on perennial ryegrass swards structure, pasture dry matter intake and milk performance of Holstein-Friesian dairy cows. Span J Agric Res 9(1): 86-99.

SAS Institute, 2005. User's guide: statistics. SAS Inst. Inc., Cary, NC, USA. 49 pp.

Thorsdottir I, Hill J, Ramel A, 2004. Seasonal variation in cis-9, trans-11 conjugated linoleic acid content in milk fat from Nordic countries. J Dairy Sci 87(9): 2800-2803.

Wildman EE, Jones GM, Wagner PE, Boman RL, Troutt HF Jr, Lesch TN, 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J Dairy Sci 65: 495-501.