IJAS Iranian Journal of Applied Animal Science

Effects of Concentrate Source (Cottonseed *vs.* Barley) on Milk Performance and Fatty Acids Profile of Holstein-Friesian Dairy Cows

Research Article

A.I. Roca-Fernandez[1*], A. Gonzalez-Rodriguez[1], O.P. Vazquez-Yanez[2] and J.A. Fernandez-Casado[3]

[1] Centro de Investigaciones Agrarias de Mabegondo, INGACAL, Apdo, 10, 15080, A Coruña, Spain
[2] Fondo Gallego de Garantía Agraria, Apdo, 15781, Santiago de Compostela, Spain
[3] Laboratorio Agrario y Fitopatológico de Galicia, Apdo, 365, 15640, A Coruña, Spain

Received on: 19 Jan 2011
Revised on: 18 Mar 2011
Accepted on: 27 Mar 2011
Online Published on: Dec 2011

*Correspondence E-mail: anairf@ciam.es

Online version is available on: www.ijas.ir

ABSTRACT

Milk performance (protein and fat content) and milk fatty acids (FA) profile of dairy cows under supplementation with two sources of concentrate, oilseeds (C, cottonseed) at two levels, low (5kg DM/cow/day) and high (7 kg DM/cow/day), and cereal grains (B, barley) at high rate (7 kg DM/cow/day), were studied in three herds (n=36) of spring calving Holstein-Friesian dairy cows (200 days in milk). Animals were randomly assigned to one of three indoors (n=12) silage feeding regimes (C5, C7 and B7), using a TMR (total mixed ration) basal diet, containing 70-80% silage (grass: maize, 36: 64) and 30-20% concentrate. Daily milk yield (MY) was higher (P<0.001) at high level of supplementation (B7, 18.1 and C7, 17.9 kg/cow/day, respectively) compared to low level (C5, 15.7 kg/cow/day), and dairy cows at the highest level of concentrate showed the highest (P<0.05) body weight (B7, 605 and C7, 598, respectively). Milk protein content was lower (P<0.05) in the high level of cottonseed (C7, 30.7 g/kg DM) than in the barley treatment (B7, 32.7 g/kg DM). There were no differences among treatments in milk fat and milk urea content. Weekly milk FA profile of cow milk was determined using gas chromatography-mass spectrometry, during seventy days in autumn, and no differences were found among treatments in short, medium and long chain FA. Despite this, higher (P<0.05) contents of polyunsaturated fatty acids (PUFA) and linoleic acid were found in the C7 treatment compared to the C5 treatment (2.48 and 2.22 *vs.* 2.16 and 1.92 g/100 g of FA methyl esters, respectively). Including the high levels of cottonseed and barley as a concentrate source for feeding dairy cattle revealed similar MY and milk fat content. However, the high level of cottonseed in the diet of dairy cows showed decreased milk protein content and increased linoleic acid and PUFA levels.

KEY WORDS cottonseed, linoleic acid, lipid feed supplements, milk fat content, total mixed ration.

INTRODUCTION

When the goal of milk production is to enhance the conjugated linoleic acid (CLA) levels on milk fat, lipid feed supplements rich in C18:2n-6, as oilseeds, which provide the substrates for the production of *trans*-vaccenic acid (TVA) or rumenic acid (RA), has proven to be an effective way of decreasing saturated fatty acids (SFA) in milk fat of dairy cows (Lock and Garnsworthy, 2002), in particular C12:0, C14:0 and C16:0, while resulting in a small, if any, increases in linoleic acid content (Mele *et al.* 2006). This is not only due to the biohydrogenation of polyunsaturated fatty acids (PUFA) in the rumen (Lock and Garnsworthy, 2002), but also to the incorporation into the plasma of lipid fractions such as phospholipids and cholesterol esters, poorly used by the mammary gland in ruminants (Loor *et al.* 2005). In addition, a decrease in milk protein content has been frequently observed when lipid feed supplements are

used in dairy cow rations (DePeters and Cant, 1992; Shingfield *et al.* 2006). These reductions have been attributed to nutritional and endocrine factors such as reduced amino acid availability to the mammary gland and insulin resistance (DePeters and Cant, 1992), which appear to be linked. Although, decreases in milk protein content are relatively small, the effect of lipid supplementation on milk fat content can be more dramatic in the particular case of dairy cattle and it has consequently received greater attention by researchers during past (Bauman and Griinari, 2001; Roy *et al.* 2006; Shingfield and Griinari, 2007) in order to investigate the causes affecting milk fat depression (MFD).

Since response patterns to lipid feed supplements are deeply influenced by the basal diet composition (Roy *et al.* 2006), different susceptibilities to variations in the ratio of starch to fibre (and associated to rumen pH) between ruminant species (Pulina *et al.* 2006) could partially contribute to explain the differences observed. Nevertheless, not so much research has been conducted in this area, trying to explain the response in milk performance (milk protein content and milk fat content) and milk FA profile of dairy cows to basal diets differing in the source of forages and/or concentrates and, moreover, in the proportion of forage to concentrate implemented when lipid feed supplements were used in the rations. An experiment carried out by Shingefield *et al.* (2005) compared the effect of forage type (maize silage *vs.* grass silage) and the proportion of concentrate (35:65 and 65:35) on milk FA profile of dairy cows reporting that the amount of starch in the diet could be an important determinant of the TVA content in milk fat. They also found that milk fat *trans-10* C18:1 concentration was positively associated with the intake of starch and the ratio of starch/neutral detergent fibre (NDF) in the diet (r^2=0.53 and 0.62, respectively) without focusing the investigation in milk protein content and fat content.

In this research, although starch was from different sources, its concentration was different across the diets; therefore, the effect of fermentability of starch on milk FA profile could not be evaluated. However, it is known that dairy cows consuming diets that contain maize silage as the only or major forage source appear to be more susceptible to MFD when unsaturated lipid feed supplements are used and in the present experiment we tried to determine the decrease on milk fat content produced using a TMR basal diet with high proportions of maize silage and a lipid feed supplement. Partial substitution of maize silage with other forage source such as alfalfa might alleviate this negative effect (Ruppert *et al.* 2003), however, we have preferred to use the same total mixed ration (TMR) basal diet differing the source of concentrate to evaluate the degree of unsaturation of the lipid feed supplement used as Harvatine and Allen (2006) did and/or the availability of the FA present as investigated, due to the chances of MFD occurring will increase.

The aim of our present work was to study the effect of changing the supplementation, under herd conditions indoors with the same grass-maize silage TMR, by using oilseeds (C, cottonseed) compared to cereal grains (B, barley) as concentrate source, on the animal performance with special reference to the milk FA profile of Holstein-Friesian cows in order to get a high added value milk produced directly on the farm.

MATERIALS AND METHODS

Animals, treatments and feeding regime

The study was conducted at the Centro de Investigaciones Agrarias de Mabegondo (CIAM), La Coruña, Spain (43°15'N; 81°18'W) using an indoors feeding regime experiment during 70 days in autumn (05/09-14/11), after 200 days in milk under grazing. When the experiment started, animals were in the last third of lactation with concentrate and silage (grass and maize) as forage in a TMR. Thirty-six primiparous and multiparous spring calving (in average 19th February 2007) Holstein-Friesian dairy cows, were balanced according to lactation number, pre-experimental milk yield (MY), milk protein and fat content, and set in three separate herds. The treatments implemented were: two herds supplemented at two levels of concentrate, low and high (C5, C7: 5 and 7 kg DM/cow/day) containing whole cottonseed (C), and one herd for contrast had a cereal grain concentrate, barley, at high level (B7, 7 kg DM cow/day).

Feed samples and chemical composition

Representative samples (0.5 kg) of all the ingredients from the three TMR diets used were collected, dried, milled, vacuum packed and stored at -20 °C until later chemical composition analysis using infrared reflectance spectroscopy by NIRS System 6500 (Foss Analytical, Hillerød, Denmark) done at the CIAM. Feed samples were analyzed for determination of dry matter (DM) content by TGA-601, LECO Corporation, St. Joseph, MI 49085-2396. Organic matter (OM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), water soluble carbohydrates (WSC) and digestibility *in vitro* of organic matter (IVODM) were calculated using the equations of calibration from Castro-García (1994).

The DM content of silage (grass and maize) and concentrate samples were determined weekly in order to adjust dietary allocation of silage and to maintain a consistent forage to concentrate ratio in the TMR basal diet, 80-70% of forage and 20-30% of concentrate for the two levels of supplementation used (5 and 7 kg DM/cow/day, respectively). The proportions of the ingredients in the two types

of concentrates used to feed the three herds of spring calving Holstein-Friesian dairy cows during the experimental period are presented in Table 1 and the chemical composition of the two types of silage (grass and maize) and the two types of concentrates consumed indoors during seventy days in autumn are presented in Table 2.

Table 1 Proportions of the ingredients in the two types of concentrates (barley and cottonseed) used to feed indoors the three herds of Holstein-Friesian dairy cows during seventy days in autumn

	Concentrate	
	Barley	Cottonseed
Corn flour (%)	43.1	31.0
Soybean hulls (%)	28.5	34.0
Soybean meal (%)	23.5	20.0
Barley flour (%)	1.4	0
Whole cottonseed (%)	0	12.0
Amender (%)	1.0	1.0
Calcium carbonate (%)	1.5	1.0
Dicalcium phosphate (%)	1.0	1.0

Table 2 Chemical composition of the two types of silage (grass and maize) and concentrates (barley and cottonseed) consumed indoors by the three groups of spring calving Holstein-Friesian dairy cows during seventy days in autumn

	Silage		Concentrate	
	Grass	Maize	Barley	Cottonseed
Dry matter (%)	21.9	34.6	89.4	90.4
Crude protein (g/kg DM)	124	76	146	168
Neutral detergent fibre (g/kg DM)	530	423	249	418
Water soluble carbohydrates (g/kg DM)	216	412	343	342
Crude fat (g/kg DM)	ND	ND	31	49
Net energy lactation (Mcal/kg)	1.36	1.48	1.84	1.59

[1] ND: not determined.

The three herds of dairy cows were fed indoors with a total mixed grass: maize silage ration with a grass to maize proportion of 36:64.

The forage and concentrate intakes were estimated daily as the difference between the amounts offered and rejected by the animals located in each individual pen. Total intakes of silage and concentrate were of 16.0 and 4.6 kg DM/cow/day in the C5 treatment, 15.0 and 7.0 kg DM/cow/day in the C7 treatment and 15.0 and 6.6 kg DM/cow/day in the B7 treatment, respectively.

Animal measurements, sampling and milk analysis

A: Body weight and body condition score

Individual body weight (BW) and body condition score (BCS) of each animal were recorded twice a month from the start of the experiment. BCS was scored by one experienced observer on a 1 to 5 scale (1= severe undercondition and 5= severe overcondition) with 0.25 increments following the method described by Wildman *et al.* (1982).

B: Milk yield and composition

Milking took place at 08.00 h and 18.00 h daily. Individual milk yields (MY) (kg/cow/day) were recorded at each milking by Alprow System (Alfa DeLaval, France). Milk protein, fat and urea content were determined from two milk samples collected at each cow from two successive evening (tuesday) and morning (wednesday) weekly milkings. Samples, preserved with potassium dichromate, were pooled 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).

C: Milk fatty acids profile

Weekly 500 ml of raw bovine milk were collected from the same five dairy cows per treatment (a total of fifteen milk samples were managed per week) for determination of milk FA profile and stored at –20 °C until later analysis by the Laboratorio Agrario y Fitopatológico de Galicia (LAFIGA) using gas chromatography–mass spectrometry Agilent Technologies Model 6890N Network GC System following a modification of the extraction method proposed by Bligh and Dyer (1959) and taking into account the considerations made by Fagan *et al.* (2004).

Statistical analysis

All statistical analyses were carried out by SAS (SAS Institute, 2005). Production and chemical composition data were analyzed using a mixed model with treatment diets as fixed effects and dairy cows as random effects. Least square means (LSM) and standard error of the means (SEM) for the source and level of concentrate used to feed dairy cows were calculated for each dependent variable. Mean differences were compared using a Tukey's multiple comparison test and statistical significance were declared at $P<0.05$ for the main effects.

RESULTS AND DISCUSSION

Animal measurements and milk performance

The treatment effects on animal measurements of spring calving Holstein-Friesian cows, MY and milk composition (milk protein, fat and urea content) are presented in Table 3.

Table 3 Effect of feeding cottonseed or barley, at two levels of supplementation, on body weight, body condition score, milk yield and milk composition (milk protein, fat and urea content) of spring calving Holstein-Friesian dairy cows consuming indoors a TMR basal diet during seventy days in autumn

	Cottonseed		Barley		P-values[3]		
Treatments[1]	C5	C7	B7	SEM[2]	C5 *vs.* C7	C5 *vs.* B7	C7 *vs.* B7
BW (kg)	567	598	605	9.8	*	*	NS
BCS (1-5)	2.80	2.78	2.85	0.06	NS	NS	NS
MY (kg/cow/day)	15.7	17.9	18.1	0.3	***	***	NS
Milk protein (g/kg DM)	31.7	30.7	32.7	0.4	NS	NS	**
Milk fat (g/kg DM)	40.8	40.1	42.1	0.8	NS	NS	NS
Milk urea content (mg/kg)	130	128	142	15.7	NS	NS	NS

[1] Treatments: Cottonseed (C5, 5 and C7, 7 kg DM cow/day) and Barley (B7, 7 kg DM/cow/day).
[2] SEM: Standard error of the mean.
[3] P-values: NS: non significant differences; * P<0.05; ** P<0.01; *** P<0.001.

Body weight (BW) was higher (P<0.05) at the high level of supplementation in both B7 and C7 treatments compared to the C5 treatment due to higher intake of concentrate in the diet of dairy cows feeding 7 kg DM/cow/day of concentrate. However, no significant differences were found between treatments as for BCS. MY was higher (P<0.001) at the high level of supplementation in both types of concentrates, barley (+2.4 kg/cow/day) and cottonseed (+2.2 kg/cow/day) for B7 (18.1 kg/cow/day) and C7 (17.9 kg/cow/day) respectively, compared to the low level of supplementation in the cottonseed treatment (C5, 15.7 kg/cow/day). However, there were no significant differences between high levels of concentrate sources, cottonseed *vs.* barley, on MY per cow (Figure 1a).

This is in contrast to data reported by DePeters and Cant (1992) which showed an increase on MY of Holstein-Friesian dairy cows by feeding lipid supplements. Nevertheless, Shingfield *et al.* (2005) found similar responses on MY when the diet of dairy cows was supplemented with lipid feed supplements.

Supplementing dairy cow diets with high amounts of plant oils often cause a drop in feed intake and, therefore, MY (Chilliard *et al.* 2001) might be reduced possibly as a result of their negative effects on feed digestibility and rumen fermentation.

This does not agree with the present results because the total intake in the two herds supplemented with the high levels of concentrate were similar (B7, 21.6 and C7, 22.0 kg DM/cow/day) when barley and cottonseed were used and no significant differences were found between both concentrate sources on MY. Milk protein content was lower (P<0.01, 2.0 g/kg DM) at the high level of concentrate in the cottonseed treatment (C7, 30.7 g/kg DM) compared to the barley treatment (B7, 32.7 g/kg DM) (Figure 1b).

This result agrees with other reports (DePeters and Cant, 1992; Shingfield *et al.* 2006) because feeding dairy cows with lipid feed supplements usually decreases milk true protein.

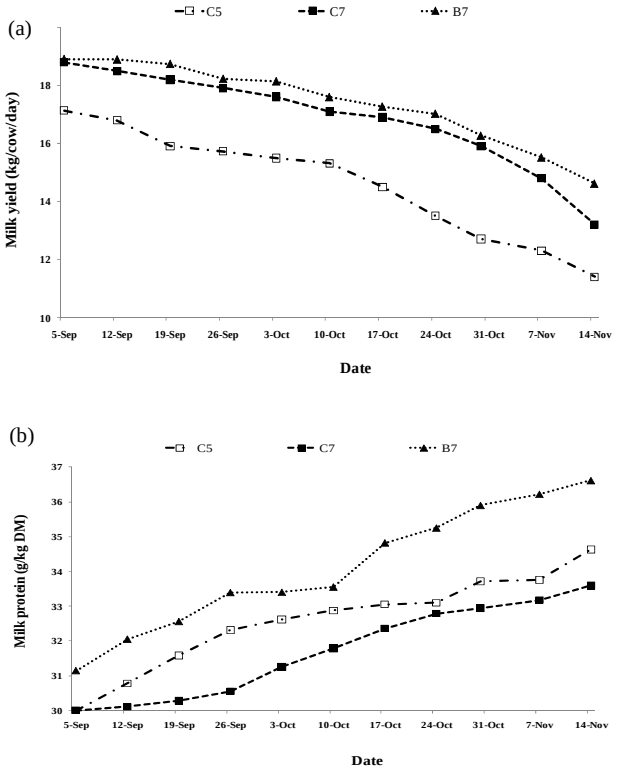

Figure 1 (a) Milk yield (kg/cow/day) and (b) milk protein content (g/kg DM) in three herds of spring calving confined Holstein-Friesian dairy cows consuming two sources of concentrate, at two levels of supplementation, during autumn. Treatments: see Table 3

The decrease in milk protein because of high rate of fat intake might be mediated through a decrease in the amount of blood flow per unit milk volume, which then results in a change in the delivery of amino acids, glucose, acetate and long chain fatty acids (LCFA) to the mammary gland (Cant *et al.* 1993a,b). Recent studies also demonstrated a decisive

role of endocrine signals (especially from the lactogenic hormones hydrocortisone, insulin and prolactin) integrating information on nutrient availability and enhancing the modulating protein translation (Burgos *et al.* 2010).

Milk fat, milk urea content and MFD did not show any differences between treatments in this study when a TMR basal diet containing 70-80% silage (64% maize and 36% grass) and 30-20% concentrate (barley *vs.* cottonseed) was used to feed indoors Holstein-Friesian cows. This agrees with the results of Bu *et al.* (2007) and AbuGhazaleh and Holmes (2007) which reported no effect of dietary lipid feed supplements on milk fat content. Shingfield *et al.* (2006), however, reported a marked reduction in milk fat content when dietary lipid supplements were used in the ration of ruminants. Bauman and Griinari (2003) suggested that MFD is related to the direct action on the mammary gland of specific FA isomers derived from the ruminal metabolism of dietary unsaturated fatty acids (UFA). Baumgard *et al.* (2002) established that post-ruminal infusions of *cis-9, trans-12* CLA inhibited *de novo* milk FA synthesis in dairy cows and dietary induced MFD has been related to increased formations of these isomers in the rumen (Bauman and Griinari, 2003). *Trans-10* C18:1 has been also reported to be associated with MFD (Shingfield *et al.* 2006) and concentrations of it usually increases linearly with lipid feed supplements where MFD was reported, with average values of 2.84 and 7.72 g/100 g of the total FA reported by Loor *et al.* (2004) and Shingfield *et al.* (2006), respectively.

In the present study, C18:1did not show any differences between treatments and no evidence of MFD were found in Holstein-Friesian dairy cows feeding cottonseed at high rate.

Milk fatty acids profile

The treatment effects on milk FA profile of Holstein-Friesian dairy cows feeding a TMR basal diet with two different sources of concentrate is presented in Table 4.

Table 4 Effect of feeding cottonseed or barley, at two levels of supplementation, on milk fatty acids profile (g/100 g of FAME) of spring calving Holstein-Friesian dairy cows consuming indoors a TMR basal diet during seventy days in autumn

	Cottonseed		Barley		P-values[3]		
Treatments[1]	C5	C7	B7	SEM[2]	C5 *vs.* C7	C5 *vs.* B7	C7 *vs.* B7
$C_{4:0}$, Butyric acid	14.09	12.24	11.53	1.98	NS	NS	NS
$C_{6:0}$, Caproic acid	2.05	2.18	2.14	0.09	NS	NS	NS
$C_{8:0}$, Caprylic acid	1.18	1.22	1.25	0.06	NS	NS	NS
$C_{10:0}$, Capric acid	2.61	2.64	2.80	0.13	NS	NS	NS
$C_{12:0}$, Lauric acid	3.02	3.03	3.35	0.16	NS	NS	NS
$C_{14:0}$, Myristic acid	10.19	10.48	10.36	0.29	NS	NS	NS
$C_{16:0}$, Palmitic acid	32.95	33.06	33.95	0.76	NS	NS	NS
$C_{18:0}$, Stearic acid	7.06	7.83	6.26	0.33	NS	NS	**
$C_{18:1}$, Oleic acid	16.24	16.97	16.26	0.56	NS	NS	NS
$C_{18:2}$, Linoleic acid	1.92	2.22	2.12	0.08	*	NS	NS
$C_{18:2\ cis\text{-}9,\ trans\text{-}11}$, CLA	0.35	0.39	0.37	0.02	NS	NS	NS
$C_{18:3}$, Linolenic acid	0.24	0.26	0.27	0.01	*	**	NS
SCFA[4]	19.93	18.28	17.72	1.73	NS	NS	NS
MCFA[5]	46.16	46.57	47.66	1.10	NS	NS	NS
LCFA[6]	25.46	27.28	24.91	0.81	NS	NS	NS
Ratio SFA/UFA[7]	3.98	3.74	3.84	0.18	NS	NS	NS
MUFA[8]	16.24	16.97	16.26	0.56	NS	NS	NS
PUFA[9]	2.16	2.48	2.39	0.09	*	NS	NS

[1, 2, 3] See Table 3.
[4] Short chain fatty acids (SCFA, C4:0 to C10:0).
[5] Medium chain fatty acids (MCFA, C12:0 to C16:0).
[6] Long chain fatty acids (LCFA, C18:0 to C18:3).
[7] Ratio saturated (SFA, C4:0 to C18:0)/unsaturated (UFA, C18:1 to C18:3) fatty acids.
[8] Monounsaturated fatty acids (MUFA, C18:1).
[9] Polyunsaturated fatty acids (PUFA, C18:2 to C18:3).

There were no significant differences between treatments on C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:1 and C18:2 *cis-9, trans-11* CLA. The C7 treatment showed an increased (P<0.01) amount of C18:0 (7.83 g/100 g of fatty acids methyl esters, FAME) compared to the B7 treatment (6.26 g/100 g of FAME). However, source of concentrate had no effect on C18:2 content. But as expected, linoleic acid (g/100 g of FAME) was higher (P<0.05) at the high level of cottonseed (C7, 2.22) compared to the low level (C5, 1.92). Differences between the C5 and B7 treatments were also found for linolenic acid, with higher (P<0.01) amount (g/100 g of FAME) in the barley treatment (B7, 0.27) than in the cottonseed treatment (C5, 0.24). Moreover, higher (P<0.05) levels of linolenic acid were found in the C7 treatment (0.26 g/100 g of FAME) than in the C5 treatment (0.24 g/100 g of FAME). In this study, C18:0 concentration increased (P<0.01) with cottonseed supplementation as reported by Bu *et al.* (2007) and Loor *et al.* (2005) when linseed was used as a concentrate source to feed cows supporting the C22:6 effect on TVA accumulation.

Supplementing dairy cow diets with linolenic acid oil source as linseeds usually increases milk *cis-9, trans-11* CLA content under confinement (Loor *et al.* 2005; Bu *et al.* 2007) and grazing (AbuGhazaleh and Holmes (2007)) feeding systems. However, in the present experiment there were no differences between treatments for *cis-9, trans-11* CLA and the levels found in the C7 treatment were in line with those reported by Roca-Fernández *et al.* (2010) in a herd feeding indoors a similar TMR basal diet, during spring-summer, to this used in the current experiment. Also, Precht and Molkentin (2000) highlighted that the average concentrations of RA in raw bovine milk in most countries are between 0.40 and 1.00 g/100 g of FAME and these values during pasture feeding are twice to three times higher than those during barn feeding due to lower proportion of grazed grass in the ration of dairy cows. The increase in milk linoleic acid content with cottonseed at high level (C7 treatment) was relatively smaller compared with their intake. Similar low increases in n-3 FA were reported by other authors (Loor *et al.* 2005; Bu *et al.* 2007) when n-3 lipid feed supplements were used. This low transfer of n-3 FA from feed to milk fat might be explained by extensive ruminal biohydrogenation of linoleic acid or by their partitioning toward other tissues within the body (Harfoot and Hazlewood, 1997).

Despite there were no significant differences between treatments for short (SCFA), medium (MCFA) and long chain (LCFA) fatty acids, higher values of SCFA and LCFA and lower values of MCFA were observed in both cottonseed treatments (C5 and C7) compared to the barley treatment (B7). The lowest ratio of SFA/UFA was observed in the C7 treatment (3.74), with the highest levels of UFA (19.45 ± 0.64 g/100g of FAME) and MUFA (16.97 ± 0.56 g/100g of FAME). The levels of PUFA (g/100 g of FAME) were higher (P<0.05) at the high level of cottonseed (C7, +0.36) compared to the low level of concentrate (C5, 2.16). The SCFA and MCFA have mainly a mammary gland origin in contrast to the LCFA, principally of a dietary origin. Furthermore, feeding oilseeds, rich in the LCFA, might have also exerted an inhibitory effect on the MCFA in the mammary gland. Clapperton and Banks (1985) attributed 80% of this inhibitory action of feed lipid supplements to the increase of the absorption of the LCFA while Hansen and Knudsen (1987) attributed this result, mainly to the increase of the C18:1 isomers concentrations. Both cases could have happened in the present experiment regarding the results obtained in the C7 treatment due to a more increased ruminal biohydrogenation activity of C18:2.

Significant main effects on milk FA profile related to the diet were found in our study with the highest linoleic acid and PUFA content in milk fat from Holstein-Friesian dairy cows supplemented with cottonseed at high level (C7 treatment). Diets with a high concentrate to forage ratio fed with unsaturated oils have shown a reduction in milk fat production to the largest extent (Bauman and Griinari, 2003). Nevertheless, in our experiment the proportion of concentrate fed in the diet of dairy cows was only of 22.33, 31.82 and 30.56% in the C5, C7 and B7 treatments, respectively. Biohydrogenation of PUFA in the rumen is usually reduced with high-concentrate diets causing accumulation of *trans-11* C18:1 isomers (Kalscheur *et al.* 1997). In dairy cows fed high-concentrate diets without (Piperova *et al.* 2002) or with (Piperova *et al.* 2000) vegetable oils rich in linoleic acid, *trans-10* C18:1 content in milk fat was as high or higher than *trans-11* C18:1. *Trans-7, cis*-9 C18:2 and *trans-10, cis*12 C18:2, along with cis-9, *trans-11* C18:2, also increased primarily when the high-concentrate diet was supplemented with oil high in linoleic acid (Piperova *et al.* 2000). Nevertheless, in our experiment despite higher content of PUFA and linoleic acid were found at high level of supplementation with cottonseed (C7 treatment), there was no significant evidence of accumulation of *trans-11* C18:1 in milk fat. As dairy cows progressed into lactation, the proportion of FA C14:0 or less, C16:0 and C18:2 increased. The proportion of FA C16:1 and C18:1 decreased correspondingly. This observation is consistent with the utilization of body fat for milk synthesis in early lactation because SCFA were expected to be relatively lower during fat mobilization.

Experiments assessing the effects of concentrate to forage ratio on milk fat composition and FA profile of cows have used maize silage alone, alfalfa hay alone, maize silage plus alfalfa or grass silage, or even maize silage plus

mixtures of small cereal grain silages (i.e., barley and triticale) as the forage source, with corn grain being the primary source of starch in the concentrate mixture (Kalscheur *et al.* 1997; Piperova *et al.* 2000). From these experiments, high linoleic acid oils (i.e., corn or soybean oil) were the preferred substrate for biohydrogenation. In our experiment, average starch content of maize silage was of 32.47% and the preferred source of concentrate for biohydrogenation was cottonseed instead of the fact that the barley concentrate showed a higher percentage of corn flour and soybean. According to Chilliard *et al.* (2001), the type of forage and supplemental UFA alter *trans-11* C18:1 and CLA isomers in milk fat to varying extents in dairy cows. Differences in starch source might account for some of the observed variation in the MFD response in dairy cows feeding high-concentrate diets (Bauman and Griinari, 2003).

CONCLUSION

Using a high level of cottonseed or barley, as a concentrate source for feeding dairy cattle, gave similar milk yield and milk fat response in an indoors silage feeding trial. Including lipid feed supplements (as cottonseed) in the diet of dairy cows can improve nutritional and added value of milk, by increasing the linoleic acid and polyunsaturated fatty acids levels in milk fat, despite some decrease in the milk protein content. Modifying the milk fatty acids profile of Holstein-Friesian dairy cows through the diet, by supplementation with concentrates containing cottonseeds, could be a good tool to be implemented by dairy farmers at farm level to improve profitability of milk production.

ACKNOWLEDGEMENT

The authors wish to thank Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) for their financial support under the project RTA2005-00204-00. Gratitude is also extended to all the farm staff from the Dairy Section Unit at the Animal Production Department in the CIAM for the care of the experimental animals and the assistance with all the measurements undertook and to the technical staff from the Plant Science Department for the analysis of forage and concentrate samples throughout the study. A mention is too due to all the technical staff at the Food Quality Department in the LAFIGA for the determination of milk fatty acids profile and to the LIGAL for the analysis of milk composition.

REFERENCES

AbuGhazaleh A.A. and Holmes L.D. (2007). Diet supplementation with fish oil and sunflower oil to increase conjugated linoleic acid levels in milk fat of partially grazing dairy cows. *J. Dairy Sci.* **90,** 2897-2904.

Bauman D.E. and Griinari J.M. (2001). Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. *Livest. Prod. Sci.* **70,** 15-29.

Bauman D.E. and Griinari J.M. (2003). Nutritional regulation of milk fat synthesis. *Annu. Rev. Nutr.* **23,** 203-227.

Baumgard L.H., Weber W.J., Kazmer G.W., Zinn S.A., Hansen L.B., Chester-Jones H. and Crooker B.A. (2002). *Trans*-10, *cis*-12 conjugated linoleic acids decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. *J. Dairy Sci.* **85,** 2155-2163.

Bligh E.G. and Dyer W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian J. Biochem. Physiol.* **37**, 911-917.

Bu D.P., Wang J.Q., Dhiman T.R. and Liu S.J. (2007). Effectiveness of oils rich in linoleic and linolenic acids to enhance conjugated linoleic acid in milk from dairy cows. *J. Dairy Sci.* **90,** 998-1007.

Burgos S., Dai M. and Cant J. (2010). Nutrient availability and lactogenic hormones regulate mammary protein synthesis through the mammalian target of rapamycin signaling pathway. *J. Dairy Sci.* **93,** 153-161.

Cant J.P., DePeters E.J. and Baldwin R.L. (1993a). Mammary uptake of energy metabolites in dairy cows fed fat and its relationship to milk protein depression. *J. Dairy Sci.* **76(8)**, 2254-2265.

Cant J.P., DePeters E.J. and Baldwin R.L. (1993b). Mammary amino acid utilization in dairy cows fed fat and its relationship to milk protein depression. *J. Dairy Sci.* **76(3)**, 762-774.

Castro-García P. (1994). Espectroscopía de reflectancia en el infrarrojo próximo (NIRS) y evaluación nutritiva de pastos. Tesis doctorales en microfichas 408. Servicio de Publicaciones de la Universidad de Santiago, Galicia, España. Pp. 121.

Chilliard Y., Ferlay A. and Doreau M. (2001). Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acids (CLA) and polyunsaturated fatty acids. *Livest. Prod. Sci.* **70,** 31-48.

Clapperton J.L. and Banks W. (1985). Factors affecting the yield of milk and its constituents, particularly fatty acids, when dairy cows consume diets containing added fat. *J. Sci. Food Agric.* **36**, 1205-1211.

DePeters E.J. and Cant J.P. (1992). Nutritional factors influencing the nitrogen composition of bovine milk: A review. *J. Dairy Sci.* **75(8)**, 2043-2070.

Fagan P., Wijesundera C. and Watkins P. (2004). Determination of mono- and di-acylglycerols in milk lipids. *J. Chromatogr. A* **1054(1,2)**, 251-259.

Hansen H.O. and Knudsen J. (1987). Effect of exogenous long chain fatty acids on individual fatty acid synthesis by dispersed ruminant mammary gland cells. *J. Dairy Sci.* **70**, 1350-1354.

Harfoot C.G. and Hazlewood G.P. (1997). Lipid metabolism in the rumen. Pages 382–426 in The Rumen Microbial Ecosystem. P.N. Hobson and C.S. Stewart. (1997). Chapman & Hall, London, UK.

Harvatine K.J. and Allen M.S. (2006). Fat supplements affect fractional rates of ruminal fatty acid biohydrogenation and passage in dairy cows. *J. Nutr.* ***136,*** 677-685.

Kalscheur K.F., Teter B.B., Piperova L.S. and Erdman R.A. (1997). Effect of dietary forage concentration and buffer addition on duodenal flow of *trans*-C18:1 fatty acids and milk fat production in dairy cows. *J. Dairy Sci*. **80,** 2104-2114.

Lock A.L. and Garnsworthy P.C. (2002). Independent effects of dietary linoleic and linolenic fatty acids on the conjugated linoleic acid content of cows' milk. *Anim. Sci*. **74,** 163-176.

Loor J.J., Ueda K., Ferlay A., Chilliard Y. and Doreau M. (2004). Biohydrogenation, duodenal flow, and intestinal digestibility of trans fatty acids and conjugated linoleic acids in response to dietary forage:concentrate ratio and linseed oil in dairy cows. *J. Dairy Sci.* **87**, 2472-2485.

Loor J.J., Ferlay A., Ollier A., Doreau M. and Chilliard Y. (2005). Relationship among trans and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. *J. Dairy Sci.* **88,** 726-740.

Mele M., Buccioni A., Petacchi F., Serra A., Banni S., Antongiovanni M. and Sechiari P. (2006). Effect of forage/concentrate ratio and soybean oil supplementation on milk yield, and composition from Sarda ewes. *Anim. Res.* **55,** 273-285.

Piperova L.S., Teter B.B., Bruckental I., Sampugna J., Mills S.E., Yurawecz M.P., Fritsche J., Ku Y. and Erdman R.A. (2000). Mammary lipogenic enzyme activity, trans fatty acids and conjugated linoleic acids arc altered in lactating dairy cows fed a milk fat-depressing diet. *J. Nutr.* **130,** 2568-2574.

Piperova L.S., Sampugna J., Teter B.B., Kalscheur K.F., Yurawecz M.P., Ku Y., Morehouse K.M. and Erdman R.A. (2002). Duodenal and milk trans octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate that postabsorptive synthesis is the predominant source of cis-9- containing CLA in lactating dairy cows. *J. Nutr.* **132,** 1235-1241.

Precht D. and Molketin J. (2000). Frequency of conjugated linoleic acid and *trans* fatty acid contents in European bovine milk fats. *Milchwissenschaft* **55,** 687-691.

Pulina G., Nudda A., Battacone G. and Cannas A. (2006). Effects of nutrition on the contents of fat, Protein, somatic cells, aromatic compounds, and undesirable substances in sheep milk. *Anim. Feed Sci. Technol.* **131,** 255-291.

Roca-Fernández A.I., González-Rodríguez A., Vázquez-Yáñez O.P. and Fernández-Casado J.A. (2010). High reliance on grass for an improved milk fatty acids composition. *J. Dairy Sci.* **93 (E-Suppl. 1),** 757.

Roy A., Ferlay A., Shingfield K.J. and Chilliard Y. (2006). Examination of the persistency of milk fatty acid composition responses to plant oils in cows given different basal diets, with particular emphasis on *trans*-C18:1 fatty acids and isomers of conjugated linoleic acid. *Anim. Sci*. **82,** 479-492.

Ruppert L.D., Drackley J.K., Bremmer D.R. and Clark J.H. (2003). Effects of tallow in diets based on corn silage or alfalfa silage on digestion and nutrient use by lactating dairy cows. *J. Dairy Sci.* **86,** 593-609.

SAS Institute. (2005). SAS User's Guide: Statistics, SAS Institute Inc., Cary, NC, USA. 49 Pp.

Shingfield K.J., Reynolds C.K., Lupoli B., Toivonen V., Yurawecz M.P., Delmonte P., Griinari J.M., Grandison A.S. and Beever D.E. (2005). Effect of forage type and proportion of concentrate in the diet on milk fatty acid composition in cows given sunflower oil and fish oil. *Anim. Sci.* **80**, 225-238.

Shingfield K.J., Reynolds C.K., Hervás G., Griinari J.M., Grandison A.S. and Beever D.E. (2006). Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. *J. Dairy Sci.* **89,** 714-732.

Shingfield K.J. and Griinari J.M. (2007). Role of biohydrogenation intermediates in milk fat depression. *Eur. J. Lipid Sci. Tech.* **109,** 799-816.

Wildman E.E., Jones G.M., Wagner P.E., Boman R.L., Troutt H.F.Jr. and Lesch T.N. (1982). A dairy cow body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.* **65**, 495-501.