Irish Journal of Agricultural and Food Research **49:** 129–139, 2010

The effect of supplementary grass silage and standard concentrate on milk fat fatty acid composition and iodine value when cows are fed a whole rapeseed-based concentrate at pasture

E. Magowan[1]†, A.M. Fearon[2] and D.C. Patterson[1]
[1]*Agri-Food and Biosciences Institute, Agriculture Branch, Large Park, Hillsborough, Co. Down BT26 6DR, Northern Ireland*
[2]*Agri-Food and Biosciences Institute, Food Chemistry Branch, Newforge Lane, Belfast BT9 5PX, Northern Ireland*

The use of grass silage and concentrates to supplement fresh grass intake is commonly practised in dairy systems. However, the effects of such supplementation within a dietary regime designed to produce a spreadable butter are unknown. Sixteen Holstein Friesian cows were used in an incomplete changeover design to investigate the effect on milk fat of supplementation with grass silage (GS) or standard concentrate (SC) when offering a concentrate based on whole rapeseed at pasture (RC+G). A control diet of fresh grass and standard concentrate (SC+G) was also included. Diet had no effect ($P > 0.05$) on milk yield or on the lactose concentration of milk. The iodine value (IV; grams of iodine per 100 g milk fat) of milk fat with the RC+G diet was greater (43.9, $P < 0.05$) than with the SC+G diet (39.9). The iodine value of milk fat was reduced ($P < 0.05$) when RC+G+GS was offered (41.5 g/100g), but not when RC+G+SC was offered (43.1 g/100g), compared with when RC+G was offered. The proportion of unsaturated fatty acids in milk fat was higher ($P < 0.05$) when the RC+G diet was offered compared with either RC+G+GS or RC+G+SC. If supplementary feedstuffs are to be used in combination with a whole-rapeseed-based concentrate and pasture, then inclusion of standard concentrate would be preferred over grass silage because the negative impact on the iodine value of milk fat was less. However, further research is required to investigate the effect on IV of milk fat when a standard concentrate supplement is offered at levels that increase milk yield.

Keywords: concentrates; dairy cows; fatty acids; grass silage; iodine value; rapeseed

†Corresponding author: elizabeth.magowan@afbini.gov.uk; Tel.: +44 (0) 28 9268 2484; Fax: +44 (0) 28 9268 9594

Introduction

Milk fat produced by cows at pasture compared to indoors, when cows are commonly offered conserved forage, is generally softer and has a higher proportion of C18:1 *cis*-9 (oleic acid) and a lower proportion of C16:0 (palmitic acid) (Chilliard *et al*., 2007; Croissant *et al*., 2007). It is also known that manipulation of the diet of the dairy cow using concentrates containing high levels of unsaturated fatty acids (UFA) generally reduces the proportions of short- and medium-chain fatty acids (C4 to C16) and increases the proportion of one or more of the C18 fatty acids (C18:0 (stearic acid), C18:1 *cis*-9, C18:2 *n*-6 (linoleic acid; LA) or C18:3 *n*-3 (α-linolenic acid; ALA)) in milk fat (Ashes, Gulati and Scott, 1997; Demeyer and Doreau, 1999; Murphy, 2000; Chilliard, Ferlay and Doreau, 2001). Offering cows a highly unsaturated lipid concentrate at pasture has been found to result in milk fat containing high levels of UFA (Murphy, Connolly and McNeill, 1995b; Fearon *et al*., 2004; Glasser, Ferlay and Chilliard, 2008). However, little is known about the effect of additional feedstuffs, such as standard concentrate (SC) or conserved forages, on milk fat composition when dairy cows are offered, at pasture, concentrates with a high concentration of unsaturated lipid. These additional feedstuffs are commonly offered to dairy cows at pasture to maintain high milk yield whilst controlling body condition (Mayne and Laidlaw, 1999). The above technology (i.e., offering a concentrate with a high concentration of unsaturated lipid) is being used successfully in Northern Ireland to produce a highly spreadable butter that meets the definition of butter (EC Council Regulations 1898/87 and 2991/94), but with no adverse effects on the oxidative stability of milk fat, animal health or milk production (Fearon *et al*., 2004). The dietary regime used to produce the spreadable butter involves the inclusion of a concentrate containing whole rapeseed (400 g/kg) that has been damaged during the pelleting process, thus supplying a partially protected source of UFA to the rumen. A proportion of these UFA undergo biohydrogenation and desaturation before incorporation into milk fat, whereas others may be directly incorporated. The milk supplied to the factory for the production of spreadable butter must have a minimum iodine value (IV) of 43.0 g iodine per 100 g of milk fat to yield a perceptible improvement in butter spreadability at refrigerator temperature (as assessed by the manufacturer), yet still have sufficient solid fat to deliver good product body with little 'oiling off' at room temperature (Fearon *et al*., 2004). Measurement of IV is commonly used in the edible fats and oils industry to characterise the degree of unsaturation of a fat or oil and has been frequently applied to milk fat (Wright *et al*., 2001; Petursson, 2002).

To achieve a milk fat with IV of ≥ 43.0 g/100g, dairy producers supplying milk into the spreadable butter scheme offer cows at spring/summer pasture *ca*. 3 kg/day of a concentrate based on whole rapeseed. However, the variation in IV between farms on the scheme was high (s.d. 4.6 g/100g; Fearon *et al*., 2003) and this threatened the success of the scheme, which operated over the narrow summer grazing season when milk production is at its peak. Magowan (2004) found that many producers offered supplementary feedstuffs (e.g., concentrates or conserved forages) within the spreadable butter regime and it was suspected that this may have been a significant factor causing the variation observed between herds.

Therefore, the aim of this study was to investigate the effect on milk fat composition of offering dairy cows supplementary

grass silage or standard concentrate whilst also offering, at pasture, a concentrate with a high concentration of unsaturated lipid.

Materials and Methods

Animals and diets

Sixteen Holstein Friesian dairy cows were balanced into four groups on the basis of milk yield (average 18.1, s.d. 4.4, L/day), days in milk (average 178, s.d. 89.5), parity, live weight and body condition score. All animals grazed together after morning and evening milking and were, therefore, offered the same pasture (perennial ryegrass) allowance each day. Fresh pasture was allocated every 24 h and each cow had a daily grass (G) allowance of approximately 16 kg dry matter (DM). Each group of cows was randomly allocated initially to one of four experimental diets: SC+G = a negative control diet involving a daily supplement of 3 kg (fresh weight,1.5 kg offered in the parlour post morning and evening milking) of a standard concentrate, RC+G = a positive control diet involving a daily supplement of 3 kg/day (fresh weight; 1.5 kg offered post morning and evening milking) of a concentrate based on whole rapeseed, RC+G+GS = the positive control diet supplemented with grass silage (GS) offered *ad libitum* for 1.5 h per day (45 min post morning and pre evening milking), and RC+G+SC = the positive control diet supplemented with 4.6 kg/day (fresh weight; 2.3 kg offered post morning and pre evening milking) of the standard concentrate. In the case of the RC+G+SC treatment, the 4.6 kg of SC was offered to balance the intake of DM from GS expected with the RC+G+GS treatment. The negative control diet was offered to the same four cows over the entire study period (6 weeks from mid August to late September) to provide a continuous contemporary baseline reference of underlying grass and milk fat composition and to reflect milk fat resulting from normal commercial practice. The RC+G, RC+G+GS and RC+G+SC diets were offered to the remaining 12 cows in an incompletely-balanced changeover design for two periods of 3 weeks (Table 1). The positive control treatment represented the recommended regime to achieve a milk fat with an IV of 43.0 g/100g. The RC+G+GS and RC+G+SC treatments were used to investigate the effect of including supplementary feedstuffs, GS or SC, with the recommended regime.

The standard concentrate contained (g/kg; fresh weight basis): barley 120, maize 210, molassed sugar beet pulp 350,

Table 1. The randomisation of cows within the experimental design

Cow number	Treatment[a]	
	Weeks 1 to 3	Weeks 4 to 6
1	SC+G	SC+G
2	SC+G	SC+G
3	SC+G	SC+G
4	SC+G	SC+G
5	RC+G	RC+G+SC
6	RC+G	RC+G+GS
7	RC+G	RC+G+GS
8	RC+G	RC+G+SC
9	RC+G+GS	RC+G
10	RC+G+GS	RC+G+SC
11	RC+G+GS	RC+G+SC
12	RC+G+GS	RC+G+SC
13	RC+G+SC	RC+G+GS
14	RC+G+SC	RC+G
15	RC+G+SC	RC+G
16	RC+G+SC	RC+G+GS

[a] SC+G = grazed grass supplemented with standard concentrate (3 kg/day); RC+G = grazed grass supplemented with rapeseed-based concentrate (3 kg/day); RC+G+GS = RC+G supplemented with grass silage (*ad libitum* access for 45 min post AM and pre PM milking); RC+G+SC = RC+G supplemented with standard concentrate (4.6 kg/day).

soya bean meal 250, minerals and vitamins 25, a molasses product (Molaferm) 25 and water 20. The composition of the rapeseed concentrate was confidential, but it can be reported that it contained 400 g whole rapeseed (fresh weight) per kilogram and that the fatty acid profile of the extracted oil included (g/kg total fatty acids): C18:1 *cis*-9, 529; C18:2 *n*-6, 260; C18:3 *n*-3, 104. The grass silage was made from the first re-growth of a perennial ryegrass sward, harvested in mid June and wilted for 48 h before ensiling. All cows received 0.5 kg/day of a standard high-magnesium concentrate containing (g/kg, fresh weight basis): barley 250, maize 300, soya bean meal 300, calcined magnesite 100, a molasses product (Molaferm) 30, water 20.

Sampling and analysis

The intake of GS by individual animals was recorded electronically daily (calibrated Calan-gate system) and residual concentrate was also weighed daily to permit individual cow intake to be calculated. Two representative samples of G, GS and each concentrate were collected weekly for compositional analysis (dry matter, ash, crude protein (CP), ether extract, water soluble carbohydrate (WSC), acid detergent fibre (ADF) and neutral detergent fibre (NDF)); the pH and gross energy of the GS were also determined. Compositional analysis was as outlined by Cushnahan and Gordon (1995) and Ferris *et al*. (2006). The *in vivo* digestibility of organic matter (D value) of the GS was determined using four castrated male sheep confined in digestibility crates. The digestibility study was conducted over 26 days, which included a 14-day pre-experimental feed-in period, a 6-day experimental feed-in period, and a 6-day collection period. Sheep were group-fed the experimental silage in a pen until day 7 after which they were transferred to digestibility crates and offered the experimental silage at maintenance. During the collection period feed intake was measured and faeces and urine output were collected daily, weighed, and stored at 4 °C. At the end of the collection period the faeces and urine were pooled per animal, homogenised and subsamples were retained for analysis.

Cows were milked twice daily throughout the study and yield was recorded. A composite preserved milk sample per cow, from six consecutive milkings, was collected each week and analysed for fat, protein, lactose and somatic cell count (United Dairies, Belfast). A separate milk sample (2 L) was also collected twice weekly from each cow, 24 h after the G, GS and concentrate samples were collected, to represent the output from that specific dietary intake. The cream was separated from the serum by centrifugation and churned into butter. Following clarification of the butter (British Standards Method 769: BSI 1961), the milk fat was analysed for IV and fatty acid composition. Wijs' method (AOCS, 1991) was used to determine the IV of milk fat. Fatty acid composition was determined, following preparation of the fatty acids as methyl esters (FAME; Amer, Kupranycez and Baker, 1985), by gas chromatography using a WCOT fused-silica capillary column (100 m long by 0.32 mm internal diameter) coated (0.25 mm thickness) with CP Sil 88 (Chrompack, London). The capillary column was placed in a Varian CP 3800 gas chromatograph (Varian Associates Ltd., Walton-on-Thames, UK) equipped with a temperature programmable injector operated in split mode and a flame ionisation detector. The carrier gas was He and the oven temperature was ramped from 50 to 225 °C to improve separation. Fatty acids were identified by their

retention time with reference to those of commercially available fatty acid standards (37 Supelco FAME mix, Sigma Aldrich Co. Ltd., Gillingham, UK) and were quantified using C13:0 and C21:0 as internal standards (Sigma Aldrich Co. Ltd., Gillingham, UK).

Statistical analysis

Statistical analysis was carried out using Genstat 6.1 (Lawes Agricultural Trust, Genstat, 2002). Analysis of variance (ANOVA) was carried out according to the changeover design. Due to the design of the study, the comparison between the negative control (SC+G) and the other treatments was based on the between-cow variation, while the comparison among the remaining treatments was based on the within-cow variation. The effect of week on milk yield and the concentrations of milk fat, protein and lactose, and on the IV of milk fat and fatty acid proportions in the milk fat was analysed using REML repeated measures ANOVA for the data from cows on the SC+G treatment. To analyse the effects of dietary regime, data from weeks 3 and 6 of the study (i.e., at the end of each changeover period) were combined for each dietary regime and analysed as outlined above using 'week' as a blocking factor. The incompletely balanced changeover design yielded effective replication of 8 cows per treatment (Table 1).

Results

Diet composition and intake

The chemical composition of the dietary components is presented in Table 2. There was no marked variation, from the mean composition presented in Table 2, over the 6 weeks of the study in the chemical or fatty acid composition of G, GS or the concentrates (as indicated by the s.d. values). The DM of the grass silage and its D-value indicated that it was good quality. The ether extract concentration of GS and G were similar (40.2 and 44.5 g/kg DM, respectively) but GS contained less ALA and more LA and C18:1 *cis*-9 than G (Table 2). The DM, CP, fibre and ash concentrations of SC and RC were similar and, as expected, the ether extract concentration of RC was considerably greater than that of SC. The intake of DM as SC by animals on the RC+G+CS treatment was lower than the intake of DM as GS by animals on the RC+G+GS treatment (Table 3).

Milk yield and composition

Milk yield and the concentration of lactose in milk were not affected by dietary treatment (Table 3). The inclusion of SC with RC+G reduced milk fat concentration and increased milk protein concentration compared to the RC+G or RC+G+GS diets. Data from cows on the SC+G treatment (negative control) showed that over the study, milk yield, milk composition, IV of milk fat, and fatty acid proportions for the most abundant fatty acids (C4:0 to C14:0, C16:0, C18:0), the long chain UFA (C18:1 *cis*-9, LA, ALA), and total conjugated linoleic acid isomers (CLA), did not vary significantly from the average values reported in Tables 3 and 4 (data for individual weeks not shown).

Effect of rapeseed concentrate compared with standard concentrate

When the RC+G diet was offered the proportions of *cis* UFA and saturated fatty acids (SFA) in milk fat increased and decreased, respectively ($P < 0.05$; Table 4), with a resultant increase ($P < 0.05$) in the IV of milk fat for cows on the SC+G treatment.

Table 2. Average composition of the feedstuffs used

Component[a]	Feedstuff				
	Grass	Grass silage	Standard concentrate	Rapeseed concentrate	High Mg concentrate
Dry matter (DM) (g/kg)	140 (5.2)[b]	311 (35.3)	873 (6.1)	898 (7.3)	866 (5.2)
pH	–[c]	4.01 (0.08)	–	–	–
D-value (g/kg DM)	–	749	–	–	–
Crude protein (g/kg DM)	201 (6.3)	112 (5.4)	196 (3.3)	182 (8.6)	215 (4.1)
NH_3-N (g/kg total N)	–	67.0 (2.1)	–	–	–
Ether extract (g/kg DM)	44.5 (4.2)	40.2 (4.4)	20.1 (2.1)	154 (13.4)	19.6 (1.3)
WSC (g/kg DM)	–	34.5 (6.3)	–	–	–
ADF (g/kg DM)	257 (13.1)	339 (10.8)	138 (4.8)	155 (5.3)	55.3 (1.6)
NDF (g/kg DM)	575 (15.2)	582 (12.3)	327 (8.2)	332 (9.1)	212 (6.7)
Ash (g/kg DM)	101 (3.4)	79 (4.3)	84 (1.9)	99 (3.1)	139 (2.2)
Gross energy (MJ/kg DM)	–	20.1 (4.10)	–	–	–
Fatty acids in DM (g/kg)					
C12:0	0.10 (0.04)	0.14 (0.09)	0	1.21 (0.53)	0
C14:0	0.24 (0.08)	0.18 (0.05)	0	0.66 (0.49)	0
C16:0	5.1 (0.37)	4.9 (0.41)	3.2 (0.23)	16.9 (5.0)	2.9 (0.16)
C16:1 *cis*-9	0.78 (0.13)	0.52 (0.14)	0	0.37 (0.12)	0
C18:0	0.52 (0.13)	0.39 (0.06)	0.43 (0.06)	5.3 (0.23)	0.40 (0.04)
C18:1 *cis*-9	0.70 (0.28)	0.92 (0.26)	3.3 (0.21)	75.9 (3.78)	3.1 (0.11)
C18:2 *n-6*	5.4 (0.59)	6.4 (0.35)	10.5 (0.34)	37.4 (2.06)	10.8 (0.25)
C18:3 *n-3*	31.2 (1.10)	26.1 (0.94)	2.1 (0.15)	13.9 (1.13)	1.7 (0.12)

[a] D-value = digestible organic matter, WSC = water-soluble carbohydrate, NDF = neutral detergent fibre, ADF = acid detergent fibre.
[b] s.d. in parentheses.
[c] Not determined.

Effect of grass silage or standard concentrate as supplements to the positive control diet

Although the IV of milk fat was significantly lower with the RC+G+GS diet than with RC+G diet the inclusion of SC had no significant effect (Table 4). When the RC+G diet was supplemented with either GS or SC, the total proportion of *cis* UFA in the milk fat, especially C18:1 *cis*-9, declined ($P < 0.05$) and the total proportions of SFA and short- and medium-chain fatty acids increased ($P < 0.05$), compared with levels found with the RC+G diet. In particular, supplementary feeding of GS increased ($P < 0.05$) the proportion of C16:0 in milk fat. The proportion of LA in milk fat was not significantly affected by supplementing the RC+G diet with either GS or SC. The total CLA proportion in milk fat declined significantly ($P < 0.05$) when RC+G was supplemented with SC but not when the supplement was GS.

Discussion

The on-farm feeding protocol required for cows to produce milk with an IV of 43.0 g/100g, and therefore suitable to be churned into butter with improved spreadability, involves offering cows at pasture 3 kg/day of a concentrate formulated to contain *ca*. 400 g whole rapeseed per kilogram (Fearon *et al*., 2004). The high IV is achieved by utilising this highly unsaturated dietary lipid component, which

Table 3. Effect of dietary treatment on animal performance, feed intake and milk composition

Variable	Dietary treatment				s.e.d.[a]	s.e.d.[b]	Significance of effect
	SC+G	RC+G	RC+G+GS	RC+G+SC			
Dry matter intake (kg/day)							
Silage	0	0	6.2	0			
Standard concentrate	2.6	0	0	3.9			
Rapeseed-based concentrate	0	2.6	2.6	2.6			
High Mg concentrate	0.43	0.43	0.43	0.43			
Milk yield (kg/day)	17.0	18.7	20.1	16.8	2.00	1.96	
Milk composition (g/kg)							
Fat	40.7	40.8	42.4	36.4	2.34	1.60	*
Protein	36.3	35.6	35.5	37.1	0.73	0.57	*
Lactose	46.0	45.8	46.4	46.7	0.47	0.33	

[a] For comparisons between SC+G and the other 3 treatments.
[b] For comparisons among RC+G, RC+G+GS and RC+G+SC.

Table 4. Effect of dietary treatment on milk fat composition (g/kg total fatty acids) and iodine value (grams of iodine per 100 g of milk fat)

Component[1]	Dietary treatment[2]				s.e.d.[3]	s.e.d.[4]	Significance of effect
	SC+G	RC+G	RCG+GS	RC+G+SC			
C4:0	21.6	21.9	22.0	22.0	1.68	1.05	
C6:0	15.4^{ab}	13.7^{a}	15.2^{ab}	16.3^{b}	1.58	0.82	*
C8:0	9.6^{ab}	7.9^{a}	9.5^{b}	10.6^{b}	1.11	0.61	***
C10:0	20.5^{ab}	15.8^{a}	20.4^{b}	23.7^{b}	2.49	1.50	***
C12:0	24.4^{ab}	19.6^{a}	25.5^{b}	28.7^{b}	2.73	1.78	***
C14:0	97.3^{ab}	84.4^{a}	99.1^{b}	101.6^{b}	7.36	5.51	**
C14:1 *cis*-9	11.7	10.8	13.7	12.0	1.36	1.07	
C15:0	11.6	11.2	12.2	11.8	0.60	0.46	
C16:0	265.9^{ab}	251.0^{a}	276.9^{b}	257.4^{a}	16.53	7.33	**
C16:1 *trans*	11.9	11.7	11.0	11.2	0.60	0.44	
C16:1 *cis*-9	17.6^{ab}	16.2^{ab}	17.8^{b}	15.0^{a}	1.43	1.07	*
C17:0	6.2	5.9	5.7	5.9	0.36	0.23	
C18:0	125.9^{ab}	126.1^{b}	111.6^{a}	114.1^{ab}	9.69	6.36	*
C18:1 *trans*[†]	33.8	37.5	34.7	37.5	3.76	2.00	
C18:1 *cis*-9	287.3^{ab}	324.1^{b}	284.7^{a}	289.4^{a}	21.17	9.89	***
C18:2 *trans, trans-9,12*	2.8	2.7	2.8	3.3	0.27	0.24	
C18:2 *n-6* (LA)	12.4^{a}	15.6^{ab}	13.5^{a}	18.0^{b}	1.94	1.94	*
C18:3 *n-3* (ALA)	8.6	6.8	7.6	8.2	0.76	0.58	
CLA	15.7^{ab}	17.1^{b}	15.9^{ab}	13.9^{a}	2.06	1.39	*
Sum of C4:0 to C14:0	188.7^{ab}	163.4^{a}	191.5^{b}	202.9^{b}	20.13	14.01	**
Total *trans* acids	48.5	52.0	48.4	52.1	3.96	1.93	
Total SFA	598.3^{ab}	557.9^{a}	598.8^{b}	590.7^{b}	20.53	10.86	***
Total *cis* UFA+CLA	353.2^{ab}	390.2^{b}	352.9^{a}	357.3^{a}	19.64	10.36	**
Iodine value	39.9^{a}	43.9^{b}	41.5^{a}	43.1^{b}	1.52	0.54	***

[1] LA = linoleic acid, ALA = α-linolenic acid, CLA = conjugated linoleic acid, SFA = saturated fatty acid, UFA = unsaturated fatty acids.
[2] See Table 1.
[3] For comparisons between SC+G and the other 3 treatments.
[4] For comparisons among RC+G, RC+G+GS and RC+G+SC.
[†] Total C18:1 *trans*, predominately vaccenic acid (C18:1 *trans*-11).
[a,b,c] Means without a superscript in common are significantly different.

provides the animal with partially protected, unsaturated fatty acids since the whole rapeseed has its seed coat partially damaged during pelleting. Following ruminal biohydrogenation, these fatty acids have the potential to supply the mammary gland with a high proportion of C18:0 for desaturation by the Δ-9 desaturase (stearoyl-CoA desaturase) enzyme system in the mammary gland. In addition, some dietary C18:1 *cis*-9 and polyunsaturated fatty acids (PUFA) may escape microbial hydrolysis and biohydrogenation in the rumen (Ashes *et al*., 1997). Intake of the whole-rapeseed-based concentrate is complemented with fresh grazed grass, which also contains a high proportion of C18 PUFA, especially ALA, in the lipid fraction (Gilliland *et al*., 2002). Although grass-derived ALA is not protected from microbial hydrolysis and biohydrogenation in the rumen, it can contribute to the unsaturated fraction of milk fat, mainly as C18:1 *cis*-9, following ruminal biohydrogenation and subsequent desaturation in the mammary gland (Murphy, 2000). In the current study, the effect of offering the RC+G diet resulted in milk fat compositional changes described above and found by previous workers (Murphy *et al*., 1995b). Indeed, when the RC+G diet was offered the IV of milk fat increased by 10% compared with the negative control regime.

However, producers on the spreadable butter scheme, described above, often offered supplementary feedstuffs such as conserved forage or concentrate (Magowan, 2004) in order to meet the metabolic requirements of cows with high milk yield (Mayne and Laidlaw, 1999). In the current study, milk yield was similar for all dietary treatments offered. Although supplementing RC+G with GS had no effect on milk composition, inclusion of SC increased the concentration of protein and reduced that of fat in milk, in line with effects of concentrate supplementation at grass reported by other workers (see review by Bargo *et al*., 2003).

Offering supplements with the RC+G diet had an adverse effect on the required milk fat composition. Supplementing with GS reduced the IV of milk fat below that required for the production of spreadable butter. When SC was used as the supplement the IV of milk fat was not significantly reduced, even though the total proportions of short- and medium-chain fatty acids, SFA and *cis* UFA+CLA (in particular C18:1 *cis*-9), in the milk fat were similar with the RC+G+SC and RC+G+GS treatments. Overall, although the effect of SC inclusion on IV was not significant, it is likely that such changes in fatty acids would affect the properties of the butter (Wright *et al*., 2001). The reasons for the difference in effects on the IV of milk fat are unclear and it appears that the relationships between IV of milk fat, fatty acid profile and spreadability of the resultant butter warrants further investigation. It was noted that the intake of GS dry matter was significantly higher than the intake of SC dry matter in this study. Therefore, if a higher allowance of SC was offered (similar to actual intake of dry matter as GS or to an allowance that increased milk yield) the effects on IV of milk fat may be significant, with consequences for the spreadability of butter.

In consecutive studies, Murphy *et al.* (1995a,b) investigated the impact on milk fat composition of including oilseeds in the diet of dairy cows when either G or GS was offered as the basal forage. They found that when GS replaced fresh grass as the basal forage, the proportion of C18:1 *cis*-9 in the milk fat was reduced (from 290 to 239 g/kg total fatty acids) while that of C16:0 was increased (from 237 to 307 g/kg total fatty acids). There appears to

be a lack of information in the literature regarding the effects of offering GS in combination with G but, similar to the effects found by Murphy *et al.* (1995a,b), offering GS with the RC+G diet in the current study increased the proportion of the C16:0 in milk fat while the proportion of C18:1 *cis*-9 was markedly reduced. Similar effects on the proportions of C16:0 and C18:1 *cis*-9 were seen in the current study when SC was offered as the supplementary feedstuff with the RC+G diet. In the current study, the supplementary feedstuffs had the potential to increase rumen fill, which would depress appetite and reduce the subsequent consumption of fresh grass. Unfortunately, grass intake was not measured in this study but it has been reported (Mayne and Wright, 1988; Morrison and Patterson, 2007) that supplementation of dairy cows with conserved forage during summer grazing reduced pasture DM intake more than when the diet was supplemented with a concentrate. It has also been reported that increasing the amount of concentrate offered to dairy cows at pasture reduced grazing time and hence grass intake (Bargo *et al*., 2003). The GS and SC used in the current study had a lower proportion of PUFA than the fresh grass and the negative effects of GS may have been exaggerated since the cows consumed a greater amount of GS than SC on a dry matter basis. Supplementation with GS or SC in the present study may therefore have lowered the IV of milk fat by reducing the intake of G, and thereby reducing the proportion of PUFA entering the rumen and being incorporated, directly or indirectly, as 18:1 *cis*-9 into milk fat. In support of this, when GS and SC were offered there was also a decline in the proportions of C18:0 and total CLA, which are products of ruminal biohydrogenation, in milk fat. The reduction in these products of ruminal biohydrogenation was possibly due to a reduction in the supply of dietary PUFA to the rumen microorganisms responsible for these processes.

On the other hand, the negative effects on the IV of milk fat may have been mediated by the different fibre concentrations of the dietary components and the resulting impact on rumen function and microbial population. The ADF concentration of the GS used was 30% higher than that of the G. Higher fibre concentrations in the diet, for example when fresh grass is replaced by GS, have been reported to favour the *de novo* synthesis of C14:0 and C16:0, with lower levels of C18 fatty acids (mono-unsaturated fatty acids (MUFA) and PUFA, including CLA) being synthesised (Grummer, 1991; Chilliard *et al*., 2001). The review by Vlaeminck *et al*. (2006) highlighted the fact that changes in forage type (e.g., from G to GS) and the supply of neutral detergent fibre (e.g., supplementation with SC) can affect the production of odd- and branched-chain fatty acids in the rumen due to the changes in both the substrate and the rumen environment. It has been suggested that an increased fibre concentration reduces the toxic effects of long-chain (C ≥18) UFA on the bacteria involved in rumen fermentation and the production of precursors for lipogenesis in the mammary gland (Pantoja *et al*., 1996). An increase in *de novo* synthesis of C16:0 using short-chain fatty acids from fibre digestion would explain the increased proportion of C16:0 in milk fat when GS was used as the supplementary feedstuff. Unfortunately, detailed triacylglycerol analysis was not available, which could have identified the origin of the C16:0. If C16:0 had originated from dietary sources, esterification would have been expected predominately at the *sn*-2 position in the high molecular weight triacylglycerols, whereas if it had originated as a result of *de novo* synthesis,

esterification would mainly have been at the *sn*-1 position in the low molecular weight triacylglycerols (Dimick, McCarthy and Patton, 1966; Parodi, 1981).

A high forage:concentrate ratio has also been shown to reduce the rate of outflow from the rumen (Khorasani, Okine and Kennelly, 2001); thus inclusion of GS may have increased the exposure of dietary lipid to lipolysis and biohydrogenation in the rumen. This is supported by the fatty acid profiles presented in Table 3, where supplementation with GS resulted in a higher proportion of short- and medium-chain fatty acids (C4:0 to C14:0) in the milk fat compared with the RC+G treatment. However, animals receiving supplementary SC (RC+G+SC treatment) also had higher levels of short- and medium-chain fatty acids ($P < 0.05$) compared with RC+G, and the levels were similar to those for treatments RC+G+GS and SC+G. Murphy (2000) noted that, in general, offering a diet with a low forage:concentrate ratio reduces milk fat concentration due to a lower rumen pH and a lower acetate:propionate ratio, and hence reduced *de novo* synthesis. In the present study, milk fat concentration was significantly reduced by supplementation with SC but it is unknown whether the change in forage:concentrate ratio was dramatic enough to be the main factor responsible for the changes in the proportion of short- and medium-chain fatty acids.

Further research is required to investigate the effect on the IV of milk fat and on the fatty acid profile when a higher allowance of SC is offered which either matched that of GS or which would increase milk yield.

Acknowledgements

The authors wish to thank the staff of the dairy unit of the Agri-Food and Biosciences Institute Hillsborough, Dr. D.J. Kilpatrick (Biometrics Department) for assistance with statistical analysis and Mr. Alan Beattie for technical assistance.

References

Amer, M.A., Kupranycez, D.B. and Baker, B.E. 1985. Physical and chemical characteristics of butter fat fractions obtained by crystallization from the melt. *Journal of the American Oil Chemist's Society* **62:** 1551–1557.

AOCS (American Oil Chemist's Society). 1991. 'Sampling and analysis of commercial fats and oils'. *Iodine value of fats and oils Wij's method* (Official Method) Cd 1-25. American Oil Chemists Society, Chicago, USA, 25 pages.

Ashes, J.R., Gulati, S.K. and Scott, T.W. 1997. Potential to alter the content and composition of milk fat through nutrition. *Journal of Dairy Science* **80:** 2204–2212.

Bargo, F., Muller, L.D., Kolver, E.S. and Delahoy, J.E. 2003. Production and digestion of supplemented dairy cows on pasture. *Journal of Dairy Science* **86:** 1–42.

Chilliard, Y., Ferlay, A. and Doreau, M. 2001. Effect of different types of forage, animal fat or marine oils in cow's diet on milk fat secretion and composition especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livestock Production Science* **70:** 31–48.

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J. and Doreau, M. 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *European Journal of Lipid Science Technology* **109:** 828–855.

Croissant, A.E., Washburn, S.P., Dean, L.L. and Drake, M.A. 2007. Chemical properties and consumer perception of fluid milk from conventional and pasture-based production systems. *Journal of Dairy Science* **90:** 4942–4953.

Cushnahan, A. and Gordon, F.J. 1995. The effects of grass preservation on intake, apparent digestibility and rumen degradation characteristics. *Animal Science* **60:** 429–438.

Demeyer, D. and Doreau, M. 1999. Targets and procedures for altering ruminant meat and milk lipids. *Proceedings of the Nutrition Society* **58:** 593–607.

Dimick, P.S., McCarthy, R.D. and Patton, S. 1966. Paths of palmitic acid incorporation into milk fat triglycerides. *Biochimica et Biophysica Acta (BBA) – Lipids and Lipid Metabolism* **116:** 159–162.

EC. 1987. EC Council Regulation No 1898/87 on the protection of designations used in the marketing of milk and milk products. *Official Journal of the European Communities* No L182, 3.7.87.

EC. 1994. EC Council Regulation No 2991/94 laying down standards for spreadable fats. *Official Journal of the European Communities* No L316, 9.12.94.

Fearon, A.M., Mayne, C.S., Beattie, J.A.M. and Bruce, D.W. 2004. Effect of level of oil inclusion in the diet of dairy cows at pasture on animal performance and milk composition properties. *Journal of the Science of Food and Agriculture* **84:** 497–504.

Fearon, A.M., Mayne, C.S., Bruce, D.W. and Moffatt, B. 2003. A scientific approach to producing naturally spreadable butter. In: "British Society of Animal Science and British Grassland Society Joint Meeting – Re-connecting the food chain", British Society of Animal Science Occasional Publication, Penicuik, Midlothian, UK, pages 6–10.

Ferris, C.P., Frost, J.P., Binnie, R.C. and Patterson, D.C. 2006. Dairy cow performance and labour inputs associated with two silage feeding systems. *Grass and Forage Science* **61:** 304–314.

Glasser, F., Ferlay, A. and Chilliard, Y. 2008. Oilseed lipid supplements and fatty acid composition of cow milk: A meta analysis. *Journal of Dairy Science* **91:** 4687–4703.

Gilliland, T.J., Barrett, P.D., Mann, R.L., Agnew, R.E. and Fearon, A.M. 2002. Canopy morphology and nutritional quality traits as potential value indicators for *Lolium perenne* varieties. *Journal of Agricultural Science* **139:** 257–273.

Grummer, R. 1991. Effect of feed on the composition of milk fat. *Journal of Dairy Science* **74:** 3244–3257.

Harfoot, C.G. and Hazelwood, G.P. 1997. Lipid metabolism in the rumen. In: 'The Rumen Microbial Ecosystem' (eds. P.N. Hobson and C.S. Stewart), Chapman & Hall, London, pages 382–426.

Khorasani, G.R., Okine, E.K. and Kennelly, J.J. 2001. Effects of forage source and amount of concentrate on rumen and intestinal digestion of nutrients in late lactation cows. *Journal of Dairy Science* **84:** 1156–1165.

Magowan, E. 2004. Investigation of the contribution of grass and herd management factors to the variation in milk fat composition of cows offered a standard lipid supplement on summer pasture. PhD Thesis, Queen's University Belfast, School of Agriculture and Food Science.

Mayne, C.S. and Laidlaw, A.S. 1999. Managing swards to achieve high grass intakes with dairy cows. In: "Agricultural Research Institute of Northern Ireland, 72nd Annual Report 1998-1999", pages 31–39.

Mayne, C.S. and Wright, I.A. 1988. Herbage intake and utilization by the grazing dairy cow. In: 'Nutrition and Lactation in the Dairy Cow' (ed. P.C. Garnsworthy), Butterworths, London, page 280.

Morrison, S.J. and Patterson, D.C. 2007. The effects of offering a range of forage and concentrate supplements on milk production and dry matter intake of grazing dairy cows. *Grass and Forage Science* **62:** 332–345.

Murphy, J.J. 2000. Synthesis of milk fat and opportunities for nutritional manipulation. In: "Milk Composition, Occasional Publication No 25, British Society of Animal Science 2000" (eds. R.E. Agnew, K.W. Agnew and A.M. Fearon), British Society of Animal Science Occasional Publication, Penicuik, Midlothian, UK.

Murphy, J.J., Connolly, J.F. and McNeill, G.P. 1995a. Effects on milk fat composition and cow performance of feeding concentrates containing full fat rapeseed and maize distillers grains on grass-silage based diets. *Livestock Production Science* **44:** 1–11.

Murphy, J.J., Connolly, J.F. and McNeill, G.P. 1995b. Effects on cow performance and milk fat composition of feeding full fat soyabeans and rapeseeds to dairy cows at pasture. *Livestock Production Science* **44:** 13–25.

Pantoja, J., Firkins, J.L., Eastridge, M.L. and Hull, B.L. 1996. Fatty acid digestion in lactating dairy cows fed fats varying in degree of saturation and different fibre sources. *Journal of Dairy Research* **79:** 575–584.

Parodi, P.W. 1981. Relationship between triacylglycerol structure and softening point of milk fat. *Journal of Dairy Science* **48:** 131–138.

Petursson. 2002. Clarification and expansion of formulas in AOCS recommended practice Cd 1c-85 for the calculation of iodine value from FA composition. *Journal of American Oil Chemist's Society* **79:** 737–738.

Vlaeminck, B., Fievez, V., Cabrita, A.R.J., Fonseca, A.J.M. and Dewhurst, R.J. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. *Animal Feed Science and Technology* **131:** 389–417.

Wright, A.J., Scanlon, M.G., Hartel, R.W. and Marangoni, A.G. 2001. Rheological properties of margarine and butter. *Journal of Food Science* **66:** 1056–1071.

Received 16 June 2010