A comparison of milk fat composition between pasture based cows supplemented with either canola meal or wheat

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Abstract

Increasing the polyunsaturated fatty acid (PUFA) content of milk has been the focus of much recent research. Milk from pasture based cows has been reported to have a high content of PUFA, which can be further manipulated with the addition of supplements differing in fatty acid composition. This experiment used 980 Holstein-Friesian and Jersey crossbred cows in a split treatment design to determine the effect of canola pulp or wheat supplementation on milk fatty acid composition and milk solid production in a typical large pasture based herd. Principle component analysis was used to determine the most influential factors of change in milk composition. Milk solid production did not differ significantly between treatments ($P > 0.05$), C10:0, C12:0, C14:0, and C16:0 were very significantly ($P < 0.001$) decreased by the canola pulp treatment. C18:0 anteiso and conjugated linoleic acid were not significantly affected by treatment. C18:1 c9 and C18:1 t9 were very significantly increased ($P < 0.001$). C18:1 t11 was significantly increased in the canola pulp treatment ($P < 0.03$). These results show that canola pulp is a potentially valuable supplement for use in grazing based systems. The equivalent response to supplementation compared to wheat would recommend its use if cost on an ME basis was competitive.

Keywords: canola pulp; wheat; milk fat; supplement; grazing based; dairy cows

Introduction

Milk is widely perceived as having high levels of saturated fatty acids, and little of the health beneficial unsaturated long-chain fatty acids (Dewhurst et al. 2006). The increasing evidence for the potential health benefits delivered by altering the fat composition of milk has attracted research towards manipulating the fatty acid composition of milk to make it a more attractive product to consumers, with particular interest on C18:1c9 (Oleic acid) and C18:2 c9 t11 (CLA) (Conte et al. 2010). The New Zealand dairy system overwhelmingly uses ryegrass-clover dominant pasture as the principal feed source, and it has been well documented that grazed pasture generally leads to increased levels of polyunsaturated fatty acids in milk compared with total mixed ration fed cows (Dewhurst et al. 2006; Elgersma et al. 2006; Chilliard et al. 2001). The challenge with increasing the levels of unsaturated fatty acids deposited into milk is having dietary lipids survive the rumen environment and pass through to the intestinal tract where they can be absorbed into the circulation.

Canola meal is a by-product of the production of canola oil by pressing, which is a growing biodiesel industry in New Zealand. While the feed is an oil rich and relatively high protein product that can be eaten by ruminants, the existing literature on the use of canola meal in ruminants has largely been done in confinement dairy systems internationally (Mulrooney et al. 2009; Neves et al. 2009). It is known that canola lipids are high in PUFA, and therefore may be of use in manipulating milk fat composition in cows. However, the effect of canola meal in altering milk fat when used as a supplement to high quality pasture, and the effect on milk solid production, is not known.

With the introduction of electronic identification tags and in-shed feeding, it is now feasible to allocate individual preferential feeding of supplement to co-grazing cows. This study used a large herd of 980 cows grazing irrigated pasture with individual electronic identification of cows in the milking bails to preferentially feed either canola meal or wheat during lactation, to compare the effects of these supplements on milk solids production and milk fat composition.

Materials and methods

Animals, diet and sampling

A total of 980 Holstein-Friesian and Jersey crossbred dairy cows grazing a ryegrass-clover dominant grazing based system in Burnham, Canterbury, New Zealand were supplemented with 2 kg canola pulp or wheat (fresh weight) twice daily during AM and PM milkin for five months from January to May 2011, after blocking the cows into two treatment groups. In blocking the cows were balanced within groups for age, calving date, milk solid production, production worth and breed. Electronic identification enabled the preferential delivery of canola pulp or wheat, via two separate augurs, as they entered their individual bales on a 54 bale rotary platform. The cows were slowly accustomed to the canola meal over the period of November to January by blending wheat and canola pulp in a 4:1 ratio and feeding to the whole herd, then initiating separate supplement feeding from January onwards.

Table 1 Mean (mg/g of freeze dried sample) fatty acid profiles of samples of pasture, canola pulp and wheat collected April and May 2010. SEM = Standard error of the mean. * = Only one measurement available.

Fatty acid	Pasture	SEM	Canola pulp	SEM	Wheat	SEM
C4:0	0.0	0.0	0.0	0.0	0.0	0.0
C6:0	0.0	0.0	0.0	0.0	0.0	0.0
C8:0	0.0	0.0	0.0	0.0	0.0	0.0
C10:0	0.0	0.0	0.0	0.0	0.0	0.0
C10:1	0.0	0.0	0.0	0.0	0.0	0.0
C12:0	0.0	0.0	0.0	0.0	0.0	0.0
C12:1	0.0	0.0	0.0	0.0	0.0	0.0
C14:0	0.1	0.0	0.2	0.0	0.0	0.0
C16:0	5.2	0.4	16.0	0.2	2.9	0.2
C16:1 t9	0.0	0.0	0.0	0.0	0.0	0.0
C16:1 c9	0.8	0.2	0.2	0.0	0.0	0.0
C18:0 anteiso	0.0	0.0	0.4	0.0	0.0	0.0
C18:0	0.5	0.1	3.8	0.0	0.2	0.1
C18:1 t9	0.0	0.0	0.0	0.0	0.0	0.0
C18:1 t11	0.0	0.0	0.0	0.0	0.0	0.0
C18:1 c6	0.0	0.0	0.0	0.0	0.0	0.0
C18:1 c9	0.4	0.0	157	*	2.2	0.5
C18:2 c9,12	4.2	0.4	81.1	0.7	10.6	2.7
C18:3 c6,9,12	0.0	0.0	0.1	0.0	0.0	0.0
C18:3 c9,12,15	23.7	3.5	27.6	0.6	0.7	0.0
CLA c9 t11	0.0	0.0	0.0	0.0	0.0	0.0

Milk samples were collected from twelve individuals of each treatment group in January, April, and May. The January sampling was a Control, with no difference between treatment groups, whilst April and May samplings were from distinct treatment groups. A milk sample (25 mL) was collected from all four quarters of each cow, immediately stored on ice and then frozen. Frozen milk samples were then freeze dried for subsequent milk fat analysis.

The 200 day lactation milk solid production of all cows was estimated from three milk yield tests of all herd cows in spring, summer and autumn, using Livestock Improvement Corporation (Hamilton, New Zealand) figures supplied to the farmer via Minda Pro (v 4.1) herd management software.

Two samples of pasture, canola pulp, and wheat were taken in April and May and freeze dried for subsequent fat analysis.

Chemical analysis

Proximate analysis of crude protein (CP), neutral and acid detergent fibres (NDF and ADF), and organic matter (OM) of pasture, canola pulp, and wheat samples were obtained using near infrared spectroscopy procedures using Foss Systems ISI Scan software (FOSS NIRSystems, Laurel, MD, USA).

The FA composition as the % of fatty acids assessed and as g/ 00g DM, of pasture, canola pulp and wheat, was determined using a modification of the method of Kramer et al. (1997) using gas chromatography. Sub-samples of each material (0.4 g DM) were directly methylated with 4 mL of 0.5 mol/L NaOH/methanol for 15 minutes at 50°C, followed by 4 mL of 5% HCl/methanol for one1 hour at 50°C, extracted with 2 mL of heptane and then introduced onto a capillary Varian CP7420, USA) 100 m x 0.25 mm x 0.25 μm column with a GC-HP6890 (Hewlett Packard, Wilmington, DE, USA) gas chromatograph. The methyl esters were analyzed by gas chromatography with helium as carrier gas. The split ratio was 1:30 and the injector and detector were held at 260°C. A temperature gradient of 166°C for 39 minutes was increased by 10.0°C per minute to 240°C, held for 10 minutes, increased by 3.0°C per minute to 245°C, and held for 10 minutes to optimize the separation of most of the 18:1 FA in the first isothermal range as reported by Ribeiro et al. (2007).

Heneicosanoic acid methyl ester (C21:0) was used as an internal standard. Retention times and response factors were determined with methyl ester standards (ME61 and ME93, BR3, CLA c9,t11 and CLAt10,c12 methyl esters) (Larodan Fine Chemicals, Malmo, Sweden). The C18:1 (t6/7/8, c12, c15) and C18:2 (t8,c13, c9,t12, t11,c15) isomers that were not available commercially were identified by order of elution as reported by Loor et al. (2004). The response factor for C18:0 was used to quantify those C18:1 and C18:2 isomers that were not available commercially as described by Loor et al. (2003).

Statistical analysis

The estimated milk solids production of all cows in each group was compared using one way ANOVA in Genstat 14.1 software (VSN International, Hemel Hempstead, UK) with treatment as a block and milk solids as a variable. Significance was assumed at $P < 0.05$.

The g/100g values of individual chain fatty acids were standardised through principle component analysis (PCA) in MiniTab 14.0 software (Minitab Pty Ltd: Sydney, Australia) to separate groups with divergence. Principle components with the highest values for the effects of treatment were then used to determine individual fatty acids which contributed most to a change in fatty acid profile using Genstat 14.1 software and one way ANOVA, with treatment as a block and identified fatty acids as variables.

Results

Milk solid production

Estimated 200 day milk solids production did not differ significantly (P > 0.05) between treatments, with canola group at 350.1 kg and wheat group at 346.2 kg.

Table 2 Estimated neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP) and organic matter (OM) of pasture, canola pulp and wheat fed to the lactating cows.

Component	Pasture	Canola pulp	Wheat
NDF (g/100 g DM)	34.2	39.3	36.5
ADF(g/100 g DM)	18.0	21.1	16.5
CP (g/100 g DM)	23.0	31.2	12.8
OM (g/100 g DM)	88.8	94.9	92.2

Table 3 Results of principal component analyses identifying the most influential components of milk. Principle component (PC) 1 represents the effect of treatment and PC2 represents the effect of sampling time.

Fatty acid	Principal components	
	PC1	PC2
	Treatment	Sampling time
C4:0	0.25	-0.33
C6:0	0.61	-0.38
C8:0	0.74	-0.30
C10:0	0.78	-0.19
C10:1	0.88	0.04
C12:0	0.83	-0.09
C12:1	0.83	0.19
C14:0	0.86	-0.08
C16:0	0.85	-0.18
C16:1 t9	-0.54	0.61
C16:1 c9	0.58	0.32
C18:0 anteiso	-0.04	-0.05
C18:0	-0.83	-0.33
C18:1 t9	-0.68	-0.20
C18:1 t11	-0.51	0.42
C18:1 c6	-0.49	0.38
C18:1 c9	-0.84	0.18
C18:2 c9 t12	-0.01	0.23
C18:3 c6,9,12	-0.33	0.49
(ALA) C18:3 c9,12,15	-0.19	0.04
CLA c9 t11	-0.33	0.77

Fatty acid profile of feed

The total measured fatty acid content of pasture, canola pulp, and wheat was 37.8, 312.5, and 17.7 mg/g DM, respectively. The total unsaturated fatty acids for pasture, canola pulp, and wheat are 6.8, 23.6, and 3.5mg/g DM respectively. The fatty acid profile of pasture, canola pulp, and wheat sampled in April and May of the lactation season is shown in Table 1. Canola had the highest content of measured PUFA, largely in C18 lengths.

The NIRS proximate analysis of pasture, canola pulp and wheat are shown in Table 2. The supplements had broadly similar NDF and ADF content, but canola had a higher CP content.

Principal component analysis

PCA showed a divergence between treatments and sampling time. This revealed the principle components most affected by treatment were C10:0, C11:0, C12:0, C12:1, C14:0, C14:1 c9, C16:0, C18:0, and C18:1 c9. Of these C11:0 had the highest influence. Table 3 shows the different values obtained by PCA analyses.

Milkfat

Groups did not differ in milk fat composition for the January Control samples. The effects of treatment on milk fatty acid profile are shown in Table 4. Milk fat composition was altered by treatment during the months of May and April. C10:0, C12:0, C14:0 and C16:0 were significantly (P < 0.001) decreased by the canola pulp treatment, with canola pulp having 28.8% and 16% less C12:0 and C14:0, respectively, compared to the wheat treatment. C18:0 anteiso and conjugated linoleic acid were not significantly affected by treatment. C18:1 c9 and C18:1 t9 were significantly increased (P < 0.001), with levels approximately 20% and 80%, respectively, higher compared to wheat. C18:1 t11 was significantly increased in the canola pulp treatment (P < 0.03) with yields almost 30% higher compared to wheat.

The ratios of unsaturated to saturated fatty acid measured in milk samples are shown in Table 5. Both values were higher in the canola treatments compared to the Control and Wheat treatment. Ratio for Wheat treatment was similar to the Control samples.

Discussion

The inclusion of canola pulp compared to wheat in the cow ration at 2 kg daily as in this study, did not significantly alter milk solids production for the lactation season, but increased the proportion of unsaturated fats in the milk, with a significant reduction in C10:0, C12:0, C14:0, C16:0 and a significant increase in C18:1 c9 and C18:1 t9 (Table 4). This is the first report of the effect of canola meal on milk solids production and milk fat composition in high production herds grazing highly managed, quality ryegrass pastures. The results suggest that inclusion of a diet component rich in

Table 4 Mean fatty acid profile in January, April, and May of milk from 12 cows fed either canola pulp or wheat SEM = Standard error of the mean.

Fatty acid	January				April				May			
	Canola pulp	SEM	Wheat	SEM	Canola pulp	SEM	Wheat	SEM	Canola pulp	SEM	Wheat	SEM
C4:0	1.5	0.1	1.5	0.1	1.4	0.1	1.5	0.0	1.4	0.1	1.3	0.0
C6:0	1.6	0.0	1.6	0.1	1.5	0.1	1.7	0.0	1.3	0.1	1.5	0.0
C8:0	1.1	0.0	1.1	0.0	1.0	0.1	1.2	0.0	0.9	0.1	1.1	0.0
C10:0	2.6^{a}	0.1	2.4^{a}	0.1	2.4^{a}	0.1	3.1^{b}	0.2	1.9^{c}	0.2	2.6^{a}	0.1
C10:1(?)	0.2	0.0	0.3	0.0	0.2	0.0	0.3	0.0	0.2	0.0	0.3	0.0
C12:0	2.9^{ab}	0.1	2.8^{a}	0.1	2.8^{ab}	0.2	3.8^{b}	0.2	2.2^{c}	0.2	3.2^{ab}	0.2
C12:1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0
C14:0	10.4^{a}	0.3	10.1^{a}	0.3	10.4^{a}	0.4	12.1^{b}	0.3	8.9^{c}	0.4	10.7^{a}	0.4
C16:0	26.1^{a}	0.9	26.6^{a}	1.1	26.8^{a}	1.1	32.0^{b}	0.9	22.1^{c}	1.0	26.1^{a}	0.8
C16:1 t9	0.2	0.0	0.2	0.0	0.1	0.0	0.1	0.0	0.2	0.0	0.2	0.0
C16:1 c9	1.3	0.1	1.2	0.1	1.1	0.1	1.5	0.1	1.1	0.1	1.4	0.1
C18:0 anteiso	0.0^{a}	0.0	0.0^{a}	0.0	0.0^{a}	0.0	0.0^{a}	0.0	0.0^{a}	0.0	0.0^{a}	0.0
C18:0	12.3^{a}	0.8	12.4^{a}	0.7	13.2^{a}	0.7	9.3^{b}	0.6	14.3^{c}	0.9	11.0^{a}	0.8
C18:1 t9	0.5	0.0	0.4	0.0	0.6	0.0	0.3	0.0	0.7	0.0	0.4	0.0
C18:1 t11	2.5^{a}	0.2	1.9^{b}	0.1	1.7^{bd}	0.2	1.2^{c}	0.1	2.7^{ad}	0.4	2.1^{d}	0.2
C18:1 c6	1.7	0.1	1.7	0.3	1.5	0.2	1.1	0.1	2.0	0.3	1.4	0.1
C18:1 c9	21.5	0.7	22.3	0.7	22.2	0.8	18.1	0.7	25.8	0.9	22.2	0.7
C18:2 c9,12	1.3	0.1	1.3	0.0	1.4	0.3	1.1	0.0	1.3	0.1	1.1	0.0
C18:3 c6,9,12	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
C18:3 c9,12,15	0.7	0.0	0.8	0.0	0.8	0.1	0.7	0.0	0.8	0.1	0.8	0.0
C18:2 c9 t11	1.4	0.1	1.3	0.1	1.1	0.1	0.9	0.1	1.6	0.2	1.5	0.1

Different letters in a row indicate significance ($P < 0.05$).

Table 5 Ratios of measured unsaturated fatty acid (UFA) to saturated fatty acid (SFA) found in milk samples in January, April and May of cows fed either canola pulp or wheat.

Ratio	January		April		May	
	Canola	Wheat	Canola	Wheat	Canola	Wheat
UFA:SFA	0.57	0.57	0.58	0.45	0.77	0.59

unsaturated fatty acids will still have a significant effect on milk fat composition when the basal diet of the cow is pastures already relatively rich in unsaturated fats.

Both the total fat content and individual fatty acids of a supplement have an influence on microbial populations and rumen metabolism, ultimately affecting milk fat deposition. The basal diet of animals in this experiment was a ryegrass-clover dominant pasture with a total fat content of 3.5% DM (Table 2). The supplementation of 2 kg canola pulp compared to wheat altered the fatty acid profile of the animal's basal diet, with canola pulp in this experiment containing 12.5% DM fats and wheat 3.2% DM (Table 2). The higher fat content of Canola pulp compared to wheat, may have enabled more unbiohydrogenated fat to exit the rumen. As a result supplementation with canola had significantly ($P < 0.001$) reduced levels of saturated short-chain fatty acids (C10:0, C12:0, C14:0, and C16:0) being deposited in milk compared to wheat, which was an indication reduced proportions of fat being saturated in the rumen.

Reduction in proportions of these individual fatty acids is beneficial; however the reduction in these fatty acids was not accompanied by a beneficial increase in conjugated linoleic acid. Liu et al. (2007) reported similar effects with the supplementation of vegetable oilseeds, with a reduction in the proportion of C12:0 and C14:0, however the proportion of conjugated linoleic acid was increased. Work by Flowers et al. (2008) investigating the effects of linseed oil supplementation, to animals grazing lucerne, on milk conjugated linoleic acid, showed a proportional increase in a dose dependant manner to linseed oil supplementation, accompanied with a dose dependant decrease in C14:0. However there was no increase in C18:0 with the addition of linseed oil, as seen in this experiment with the addition of canola pulp. Interestingly the presence of docosahexaenoic acid has been shown to promote the accumulation of C18:1 t11 in the rumen due to inhibiting the reduction of C18:1 t11 to C18:0 (AbuGhazaleh et al. 2004). Very low levels of docosahexaenoic acid of 0.001g/100g, were seen in milk of both treatments, though none was detected in the feed.

In general, vegetable oils rich in C18:2 c9,12 and C18:3 c9,12,15 are biohydrogenated and isomerised in the rumen to C18:1 t11 (van de Vossenberg et al. 2003).The effects of high intake of oils are not completely understood as confusion is

added with an interaction of many factors; such as inhibition of mammary enzymes or regulatory behaviour, caused by different substrates, over mammary gene expression. In this experiment there was a significant ($P < 0.03$) increase in the levels of C18:1 t11 but no significant increase in conjugated linoleic acid with canola pulp compared to wheat supplement. This may suggest that mammary enzymes were not operating at their optimum activity, or were not expressed enough, to convert C18:1 t11 to conjugated linoleic acid. Notably, C18:1 t11 can still be considered a source of CLA for humans as it can be converted by human tissues to conjugated linoleic acid (Turpeinen et al. 2002).

Other studies which supplement high levels of long-chain fatty acids have, like this study, shown an efficient reduction in C10-C16 saturated fatty acids, and increasing C18:0 and C18:1 in milk fat. This is with or without protection from rumen biohydrogenation (Delbecchi et al. 2001). Even with a higher content of saturated fatty acids seen in the canola pulp compared to pasture and wheat, the canola pulp treatment maintained higher ratios of unsaturated to saturated fats as shown in Table 5.

Often in experiments where diets with high levels of long-chain fatty acids are fed the ratio of C18:1 c9:C18:0 is considered as it typically remains unchanged. This is thought to be as a result of changes in diet being compensated by regulation of lipogenic enzymes. Sterol CoA desaturase 1 (SCD1) is known to catalyse the desaturation of fatty acids in the cis-Δ^9 position. This enzyme is regulated by a transcription factor named sterol regulatory binding element protein (SREBP-1). Other key enzymes are acetyl CoA carboxylase, fatty acid synthase, and acetyl transferases (Conte et al. 2010). There is also a relationship between C4, C16, and C18 fatty acids. This comes about as fatty acids are bound as triglycerides in the mammary epithelial cells for deposition into milk. Both fatty acids *de novo* synthesized and absorbed from the blood are incorporated bound to a glycerol molecule, so as to appear in the milk as triglycerides. This activity is carried out by different esterifying enzymes which favour binding of particular chain lengths (Conte et al. 2010). At Carbon 3 of the glycerol molecule the enzyme diacylglycerol acyl transferase favours binding of short-chain fatty acids while at the opposite end of the glycerol molecule (Carbon 1) esterification of long-chain fatty acids is favoured (Glasser et al. 2008). However in this experiment C16:0 was very significantly reduced upon feeding of canola pulp, with no complementary decrease in C18 fatty acids (Table 4). The most likely explanation is the substantial increase in C18 substrate supply in the canola pulp treatment initiating a proportionate increase in milk C18:0.

Milk solid yield was not significantly different between canola and wheat treatments ($P > 0.05$). This suggests a potential use for canola pulp in the New Zealand dairy industry on the basis of the energy and utility value. Profitable use of supplements requires them to be less than the value of extra milk produced, if they are to be of value to the producer. Wheat is typically considered a high energy supplement. Secondly, pasture substitution with some supplements can be a disincentive to effective use (Leaver 1985). Pasture intake is a relationship between bite rate, bite size, and grazing time. Generally supplements do not affect the first two but do affect the latter (Bargo et al. 2003). Harvatine et al. (2006) showed that the supplements rich in unsaturated fatty acid reduced normal meal size. Canola pulp with its high fat content would seem likely to show a similar effect. However in this experiment no difference between production was observed between wheat and canola meal supplemented groups, suggesting little difference in practical energy intake in this commercial scenario.

Together, these findings suggest that canola pulp and wheat had broadly equivalent metabolisable energy (ME) values and did not affect dry matter intake of grazed pasture. In two factorial experiments over consecutive years the magnitude of the difference between the cows demand and the actual consumption of dry matter had the predominant effect on the dry matter intake response of cows to supplements (Penno et al. 2006a). In order to get the greatest response, allocation of supplements should be matched by the decline in pasture allowance (Penno et al. 2006b). For milk fat depression to occur dietary components must alter the rumen environment and also contain a modest level of unsaturated fatty acids (Bauman et al. 2011).

Canola pulp can be considered a viable supplement for its use in grazing based systems as it had no adverse effects on milk production, or milk fat concentration even though it had a high oil content. The similar milk solid response to wheat would suggest its use is more viable if its cost were determined on an ME basis and remained competitive.

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