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Effect of replacing calcium salts of palm oil distillate with incremental amounts of conventional or high oleic acid milled rapeseed on milk fatty acid composition in cows fed maize silage-based diets

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Based on potential benefits to human health, there is increasing interest in altering the composition of ruminant-derived foods. Including rapeseeds in the dairy cow diet is an effective strategy for replacing medium-chain saturated fatty acids (SFA) with cis*-monounsaturated fatty acids (MUFA) in bovine milk, but there is limited information on the optimum level of supplementation. Decreases in SFA due to plant oils are also accompanied by increases in milk* trans *fatty acid (FA) content and it is possible that high oleic acid rapeseeds may result in a higher enrichment of* cis*-9 18:1 and lower increases in* trans *FAs in milk compared with conventional varieties. Seven multiparous lactating Holstein–Friesian cows were allocated to one of seven treatments in an incomplete Latin square design with five 28-day experimental periods, to evaluate the effect of replacing calcium salts of palm oil distillate (CPO; 41 g/kg diet dry matter, DM) with 128, 168 or 207 g/kg diet DM of conventional (COR) or a high oleic acid (HOR) rapeseed fed as a supplement milled with wheat. Rapeseed variety and inclusion level had no effect (*$P > 0.05$*) on DM intake, milk yield and composition. Both rapeseed varieties decreased linearly (*$P < 0.001$*) milk fat SFA content, which was partially compensated for by a linear increase (*$P < 0.001$*) in* cis*-9 18:1 concentration. Reductions in milk SFA were also associated with increases (*$P < 0.05$*) in* trans *18:1 and total* trans *FA content, with no difference (*$P > 0.05$*) between rapeseed varieties. Replacing CPO in the diet with milled rapeseeds had no effect (*$P > 0.05$*) on total milk conjugated linoleic acid (CLA) concentration. Relative to a COR, inclusion of a high oleic acid variant in the diet increased (*$P = 0.01$*) the ratio of* trans*-MUFA :* trans*-polyunsaturated fatty acids in milk that may have implications with respect to cardiovascular disease risk in humans. In conclusion, data indicated that replacing CPO with milled rapeseeds at levels up to 1150 g oil/day could be used as a nutritional strategy to lower milk SFA content without inducing adverse effects on DM intake and milk production. HOR reduced milk fat SFA content to a greater extent than a conventional variety, but did not minimise associated increases in* trans *FA concentrations. However, the high oleic acid variant did alter the relative abundance of specific* trans *18:1, CLA and* trans *18:2 isomers compared with conventional rapeseeds.*

Keywords: milk fat, rapeseed, oleic acid, *trans* fatty acids, conjugated linoleic acid

Implications

Certain saturated fatty acids (SFA) are thought to increase the risk of cardiovascular disease (CVD). Milk and dairy products are a major source of SFA in the human diet in Europe, and therefore research has been directed towards developing sustainable strategies to decrease milk fat SFA content. This study examined the effect of inclusion level and rapeseed type on milk fatty acid (FA) composition and dairy cow performance. The results showed that replacing calcium salts of palm oil distillate with rapeseed supplements altered milk FA composition without adverse effects on milk production. Inclusion of milled rapeseeds in the diet of lactating cows could be used as part of an overall strategy for decreasing SFA in the human diet with potential to lower CVD risk.

Introduction

Clinical and biomedical studies have shown that cardiovascular disease (CVD) risk factors can be improved by the isoenergetic replacement of saturated fatty acids (SFA) with

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cis-monounsaturated fatty acids (MUFA) in the human diet (Kris-Etherton *et al*., 1999; Mensink *et al*., 2003). Milk and dairy products are the major source of SFA in the European diet (Hulshof *et al*., 1999; Henderson *et al*., 2003). Rather than simply advocating population-wide decreases in milk and dairy product consumption, altering the FA composition of ruminant milk offers the opportunity to lower SFA intake while maintaining the contribution of these foods to high-quality protein, bioactive lipid, mineral and vitamin supply in the human diet (Shingfield *et al*., 2008).

Numerous studies have shown the potential of oilseed and plant oil supplements in the diet to alter bovine milk FA composition (Glasser *et al*., 2008; Shingfield *et al*., 2008). A recent review reported an average decrease in total SFA concentration in milk fat of almost 10 g/100 g FAs when cows consume just over 500 g rapeseed oil/day (average response from a number of studies; Glasser *et al*., 2008). Larger decreases in SFA of almost 20 g/100 g of total FAs have been reported in milk from cows fed diets supplemented with 4.1 kg/day of crushed rapeseeds supplying 1.97 kg of rapeseed oil, but the changes in milk fat composition were accompanied by substantial reductions in nutrient intake and milk yield relative to the control diet (Givens *et al*., 2003). Feeding high levels of supplemental fat to dairy cows can adversely affect rumen function and lower milk production (Palmquist, 1994; Lock and Shingfield, 2004). Givens *et al*. (2009) concluded that rapeseed milled with wheat, which offers a practical solution for processing oilseeds on-farm with the added benefit of minimising oil losses during milling, represented the most ideal compromise for lowering milk fat SFA content and increasing *cis*-MUFA concentrations without affecting animal performance and minimising associated increases in milk fat *trans* FAs.

Including rapeseed-based supplements in the diet of lactating cows also increases milk *trans* FA concentrations (Loor *et al*., 2002; Rego *et al*., 2009). Although some evidence suggests that *trans* FAs from ruminant-derived products may not exert as negative an effect on CVD risk as those from industrial sources (Jakobsen *et al*., 2006; Willett and Mozaffarian, 2008), other evidence is inconsistent (Shingfield *et al*., 2008; Brouwer *et al*., 2010). Several varieties of rapeseed containing higher proportions of oleic acid and lower amounts of polyunsaturated fatty acids (PUFA) have been bred and commercialised primarily for use as industrial frying oils (Schierholt *et al*., 2000 and 2001). High oleic acid rapeseed variants offer the potential to lower milk SFA content, enhance *cis*-9 18:1 concentrations and minimise increases in *trans* FAs compared with conventional rapeseeds. The apparent biohydrogenation of *cis*-9 18:1 in the rumen is not as high as that of 18:2n-6 and 18:3n-3 (Shingfield *et al*., 2010) with the implication that high oleic acid variants would result in a greater proportion of dietary unsaturated FAs escaping the rumen compared with conventional rapeseeds. Furthermore, studies in grazing cows have shown that increases in milk *trans* FA concentrations due to plant oils are directly related to the ratio of PUFA : *cis*-9 18:1 in lipid supplements (Rego *et al*., 2009), with the implication that oilseeds rich in *cis*-9 18:1 rather than PUFA would minimise the appearance of ruminal biohydrogenation intermediates in milk fat triacylglycerides.

Previous studies have examined the effects of high oleic acid rapeseed oil on milk fat composition (Jenkins, 1998; Loor *et al*., 2002), but direct comparisons with conventional sources of rapeseed oil have not been reported. In addition, relatively few studies (Collomb *et al*., 2004; Givens *et al*., 2009; Rego *et al*., 2009) have examined the effect of dietary rapeseed inclusion on milk FA composition in sufficient detail to allow definitive conclusions on the impact of the distribution of *trans* FA isomers to be drawn. In this experiment, nutrient intake, milk production and milk FA composition responses to replacing calcium salts of palm oil distillate (CPO) with incremental amounts of milled conventional or high oleic acid rapeseeds were evaluated in high-yielding lactating cows fed maize silage-based diets.

Material and methods

Experimental design, animals and management

A total of seven multiparous Holstein–Friesian cows of (mean ± s.e.) live weight 633 ± 18.3 kg, parity 5.6 ± 0.53, 79 ± 7.9 days in lactation and producing 44.7 ± 1.39 kg milk/day were used. Animals were randomly allocated to one of seven treatments in an incomplete 7 × 7 Latin square design with five 28-day experimental periods (Table 1). Cows were housed in individual tie stalls equipped with a rubber mattress and bedded with wood shavings. Clean water and trace mineralised blocks (Rockies (red), Tithebarn Ltd, Cheshire, UK) were available *ad libitum*. Cows were milked *in situ* at 0400 and 1600 h.

Experimental diets

Diets were offered as total mixed rations (TMR; forage : concentrate ratio, 50 : 50 on a dry matter (DM) basis with the forage component consisting of maize silage and grass silage, 750 and 250 g/kg of forage DM, respectively). Treatments consisted of a control diet containing 41 g/kg DM of CPO (Megalac®, Volac International Ltd, Royston, UK) or the same

Table 1 *Allocation of experimental treatments according to an incomplete 7 × 7 Latin square design*

	Period				
Cow	1	2	3	4	5
1	COR2	HOR2	COR3	CPO	COR1
2	HOR3	COR1	HOR2	COR3	HOR1
3	HOR2	HOR1	CPO	COR1	COR3
4	COR3	CPO	HOR3	HOR1	COR2
5	HOR1	HOR3	COR2	HOR2	CPO
6	CPO	COR2	COR1	HOR3	HOR2
7	COR1	COR3	HOR1	COR2	HOR3

COR1, COR2 and COR3 = conventional oleic acid rapeseed at three inclusion levels; HOR1, HOR2 and HOR3 = high oleic acid rapeseed at three inclusion levels; CPO = calcium salts of palm oil distillate-based diet.

Table 2 *Ingredients and chemical composition of experimental diets (g/kg DM or as stated)*

	Treatments[1]						
	CPO	COR1	COR2	COR3	HOR1	HOR2	HOR3
Ingredients							
Maize silage	373	375	375	375	375	375	375
Grass silage	124	125	125	125	125	125	125
Wheat straw	10	10	10	10	10	10	10
Milled rapeseed/wheat[2]	0	127.8	168	206.5	127.8	168	206.5
CPO[3]	41	0	0	0	0	0	0
Rapeseed meal	77	49	21.2	0	77	49	21.2
Soyabean meal	72	60	74.8	84	60	74.8	84
Sugar beet feed (molassed)	90	80	80	80	80	80	80
Milled wheat	91	68.2	41	14.5	68.2	41	14.5
Wheat feed[4]	15	20	20	20	20	20	20
Maize gluten meal	20	20	20	20	20	20	20
Soyabean hulls	45	20	20	20	20	20	20
Calcined magnesite	7	10	10	10	10	10	10
Minerals and vitamins[5]	15	15	15	15	15	15	15
Blended molasses and urea[6]	20	20	20	20	20	20	20
Chemical composition							
DM (g/kg fresh)	595	594	595	595	594	596	596
CP	150	153	160	153	154	161	154
NDF	368	370	358	357	366	354	351
Starch	145	169	160	165	167	157	161
Water-soluble carbohydrates	37	35	32	35	37	34	38
ME (MJ/kg DM)	11.3	11.6	11.9	12.2	11.6	12.0	12.3
Fatty acids							
16:0	16.2	3.9	4.1	4.5	3.6	3.8	4.1
18:0	1.54	0.81	0.95	1.11	0.81	0.95	1.10
18:1 *cis*-9	14.6	19.8	24.6	29.4	23.6	29.5	35.5
18:2n-6	10.3	12.9	14.2	15.8	11.7	12.6	13.8
18:3n-3	1.8	4.1	4.8	5.5	2.3	2.4	2.6
Total fatty acids	48	46	54	62	47	55	63

DM = dry matter; CPO = calcium salts of palm oil distillate; COR1, COR2 and COR3 = conventional oleic acid rapeseed; HOR1, HOR2 and HOR3 = high oleic acid rapeseed; ME = metabolisable energy.
[1]1, 2 and 3, rapeseed inclusion levels of 73, 96 and 118 g/kg DM, respectively.
[2]Added as a single mixture.
[3]Megalac® (Volac International Ltd, Royston, UK) declared composition (g/kg DM) as containing oil (840), calcium (90) and ME 33.25 MJ/kg DM.
[4]Wheat feed (GP Feeds Ltd, Cheshire, UK) declared composition (g/kg DM) as containing CP (175), NDF (400), starch and sugars (340) and ME 11.5 MJ/kg DM.
[5]Proprietary mineral and vitamin supplement (Rockies (Red), Tithebarn Ltd, Cheshire, UK) declared composition as containing 380 g/kg sodium and (mg/kg) magnesium (5000), iron (1500), cobalt (50), copper (300), iodine (150), manganese (200), zinc (300) and selenium (10).
[6]Regumaize 44 (SvG Intermol Ltd, Bootle, Merseyside, UK) declared composition (g/kg DM) as containing CP (440), water-soluble carbohydrates (550), ME 11.8 MJ/kg DM.

basal diet with CPO replaced by 35, 46 or 57 g/kg DM of lipid derived from two different rapeseed varieties milled with wheat. Wheat was used as a binding agent to facilitate the milling of rapeseeds and also prevent oil losses following the rupturing of the seed coat. The milled rapeseed blend was prepared by processing the whole seeds with wheat grain (50 : 50, fresh weight basis) in a machine, which uses a combination of hammer (12 mm) and roller mills (processor model AFM 998Q; Buschhoft, GmbH & Co, Ahlen, Germany). Rapeseed treatments were comprised of a mixture of conventional varieties (Mozart and Astrid; COR) or a high oleic acid rapeseed (Nexera 160; Dow AgroSciences LLC, Hitchin, UK) variant. Diets were formulated to be isoenergetic and isonitrogenous (Thomas, 2004) and supply 750, 1000 or 1250 g rapeseed oil/day assuming a predicted mean DM intake of 22 kg/day. Ingredients and chemical composition of the experimental diets are shown in Table 2. Cows were offered diets as equal meals at 0830 and 1600 h. Feed refusals were removed and weighed before the morning milking.

Experimental measurements, sample collection and chemical analysis

Individual animal dietary intake was recorded daily. Samples of fresh TMR, subsamples of individual dietary components and refused feeds were collected daily during each sampling week, and pooled to provide weekly composite samples. Composite feed samples were stored at −18°C until analysed for chemical composition. Oven-dried (60°C), milled (1 mm screen) samples of forages, concentrates, conventional and high oleic acid rapeseed/wheat mixes were submitted for

neutral detergent fibre, organic matter, crude protein (CP), water-soluble carbohydrates, ether extract, starch, metabolisable energy and FA determinations according to procedures described elsewhere by Kliem *et al.* (2008).

Milk yields were recorded twice daily for individual animals during the last 7 days of each experimental period. Samples of milk preserved with potassium dichromate (1 mg/ml; Lactabs, Thomson and Capper, Runcorn, UK) were collected during this period and submitted for fat, CP and lactose determinations based on infrared spectroscopy (Foss Electric Ltd, York, UK). Additional samples of unpreserved milk were collected during the last 24 h of each experimental period, stored at −20°C until composited according to milk yield and submitted for FA analysis.

Lipid in 1 ml milk and appropriate sample weight of forage, concentrate and lipid supplements was extracted and transesterified to fatty acid methyl esters (FAME) according to standard methods (Kliem *et al.*, 2008). The distribution of conjugated linoleic acid (CLA) isomers in milk FAME was determined by HPLC using four silver impregnated silica columns (ChromSpher 5 Lipids, 250 × 4.6 mm; 5 μm particle size, Varian Ltd, Oxford, UK) coupled in series using 0.1% (v/v) of acetonitrile in heptane as the mobile phase (Shingfield *et al.*, 2003). Isomers were identified using an authentic CLA methyl ester standard (O-5632, Sigma-Aldrich, YA-Kemia Limited, Helsinki, Finland) and chemically synthesised *trans*-9, *cis*-11 CLA (Shingfield *et al.*, 2005). Identification was verified using *cis*-9, *trans*-11 CLA as a landmark isomer.

Milk FA composition was expressed as a weight percentage of total FAs using response factors derived from the analysis of a butter oil reference standard (CRM 164; Community Bureau of Reference, Brussels, Belgium). Concentrations of specific conjugated isomers in milk fat were calculated based on proportionate peak area responses determined by HPLC and the sum of *trans*-7, *cis*-9 CLA, *trans*-8, *cis*-10 CLA and *cis*-9, *trans*-11 CLA weight percentage determined as a single peak by gas chromatography.

Statistical analysis

Intake, milk production and milk FA composition data were analysed using a general linear model with effects for cow, period and treatment (Statistical Analysis Systems software package version 8.2, SAS Institute, Cary, NC, USA). Treatment carry-over effects were also included in the statistical model but were removed when declared non significant ($P < 0.10$). Preplanned contrasts included (i) CPO *v.* COR1, (ii) CPO *v.* HOR1, (iii) linear component of the response to incremental amounts of milled conventional rapeseeds in the diet, (iv) linear component of the response to incremental amounts of milled high oleic acid rapeseeds in the diet and (v) comparison of mean responses to milled conventional and high oleic acid rapeseeds in the diet. Single degree of freedom contrasts were also included in the statistical model to test for the significance of non-linear (quadratic) components of the response to incremental amounts of both types of milled rapeseed supplements in the diet. Least square means are reported and treatment effects were considered significant at $P < 0.05$.

Results

Analysis of the milled rapeseed/wheat supplements confirmed the higher *cis*-9 18:1 content of HOR compared with COR (68 and 57 g/100 g FAs, respectively). Lipid derived from the COR supplement compared with the HOR supplement contained 16:0 (5 *v.* 4 g/100 g FAs), 18:0 (2 *v.* 2 g/100 g FAs), 18:2n-6 (20 *v.* 16 g/100 g FAs) and 18:3n-3 (8 *v.* 2 g/100 g FAs). The higher enrichment of *cis*-9 18:1 in HOR was therefore associated with a lower sum of 18:2n-6 and 18:3n-3 relative to COR (18 *v.* 29 g/100 g FAs). The total FA content of the COR and HOR supplements were 230 and 236 g/kg DM, respectively.

By design, all seven experimental diets were of similar chemical composition. However, total dietary FA content was increased with incremental inclusion of milled rapeseeds (Table 1). The predominant FA in the CPO diet was 16:0 contributing to more than one-third of total FAs, whereas *cis*-9 18:1 was the major dietary FA for all other treatments. For each inclusion level, the amount of *cis*-9 18:1 in the diet was approximately 5 g/kg DM higher on the HOR than COR treatments. Increasing inclusion level of rapeseed also increased dietary 18:2n-6 and 18:3n-3 content, but the total amount of PUFA at each level of inclusion was consistently higher for COR than HOR treatments.

There was no effect ($P > 0.05$) of replacing CPO with milled rapeseeds on DM intake, milk yield, milk constituent content or output (Table 3). FA intake varied in direct relation to milled rapeseed inclusion, with both varieties resulting in lower ($P < 0.001$) intake of 16:0 and 18:0 and higher ($P < 0.001$) intake of *cis*-9 18:1, 18:2n-6 and 18:3n-3 than the CPO diet. Intake of *cis*-9 18:1, 18:2n-6 and 18:3n-3 increased linearly ($P < 0.001$) with incremental milled rapeseed inclusion. Consumption of *cis*-9 18:1 was on average 117 g/day higher ($P < 0.001$) for cows fed HOR than COR treatments, whereas ingestion of PUFA was higher ($P < 0.001$) by cows fed the COR than HOR diets.

Replacing CPO with both rapeseed supplements altered milk FA composition changes that were characterised as a decrease ($P < 0.01$) in total SFA, mainly due to lowered ($P < 0.001$) 16:0 concentrations, and an increase ($P < 0.05$) in total *cis*- and *trans*-16:1, total 18:1 and total *trans* FA content (Table 4). Furthermore, supplements of milled rapeseeds also resulted in a decrease ($P < 0.001$) in *cis*-9 16:1, 18:2n-6, *trans*-8, *cis*-10 CLA and *trans*-9, *trans*-11 CLA and an increase ($P < 0.01$) in 18:0, 20:0, *cis*-9 and -11 20:1 (Table 4), *cis* (Δ9, 13 and 16) and *trans* (Δ6 to Δ9, 12 and 16) 18:1 (Table 5), *cis*-9, *trans*-13 18:2 and *trans*-10, *trans*-15 18:2 (Table 6). Relative to the CPO treatment, the COR1 treatment resulted in specific changes in 13:0, 14:0, 18:3n-3, *cis*-12 18:1, *trans*-13, -14 18:1, *cis*-9, *cis*-15 18:2 and *trans*-10, *cis*-12 CLA concentrations, whereas the HOR1 diet increased ($P < 0.01$) milk *trans*-4 and *trans*-5 18:1 content compared with CPO (Table 5).

Incremental inclusion of milled rapeseeds in the diet resulted in linear ($P < 0.05$) decreases in total short- and medium-chain SFA with between 4 and 14 carbon atoms, 16:0 and total SFA and elicited linear ($P < 0.05$) increases in 18:0, *cis*-9 18:1 and total *cis*- MUFA concentrations.

Table 3 *Effect of replacing CPO with incremental amounts of milled conventional or high oleic acid rapeseeds on DM and fatty acid intake, milk yield and composition*

	Treatments[1]								$P^{\dagger}$				
	CPO	COR1	COR2	COR3	HOR1	HOR2	HOR3	s.e.m.[‡]	1	2	3	4	5
DM intake (kg/day)	23.7	24.2	24.5	23.6	24.7	24.2	23.6	0.53	ns	ns	ns	ns	ns
Fatty acid intake (g/day)													
16:0	387	91.4	103	108	90.0	91.6	98.8	8.22	***	***	ns	ns	ns
18:0	36.7	19.5	23.3	26.2	20.1	23.0	25.9	0.74	***	***	***	***	ns
18:1 *cis*-9	344	477	602	694	583	720	822	13.4	***	***	***	***	***
18:2n-6	243	314	347	374	290	306	325	6.8	***	***	***	**	***
18:3n-3	43.0	101	117	131	57.3	58.6	62.2	2.44	***	***	***	ns	***
Yield													
Milk (kg/day)	41.2	40.9	41.0	41.7	41.3	41.6	41.5	1.01	ns	ns	ns	ns	ns
Fat (g/day)	1540	1490	1439	1451	1487	1450	1460	54.7	ns	ns	ns	ns	ns
Protein (g/day)	1232	1246	1285	1229	1245	1280	1256	41.0	ns	ns	ns	ns	ns
Lactose (g/day)	1845	1897	1917	1889	1912	1926	1944	66.0	ns	ns	ns	ns	ns
Concentration (g/kg)													
Fat	37.2	36.3	34.9	34.7	36.0	34.6	35.1	0.88	ns	ns	ns	ns	ns
Protein	30.0	30.5	31.6	29.4	30.0	30.8	30.2	0.69	ns	ns	ns	ns	ns
Lactose	44.7	46.3	46.6	45.3	46.4	46.3	46.8	0.68	ns	ns	ns	ns	ns

CPO = calcium salts of palm oil distillate-based diet; COR1, COR2 and COR3 = conventional oleic acid rapeseed at three inclusion levels; HOR1, HOR2 and HOR3 = high oleic acid rapeseed at three inclusion levels; DM = dry matter.
[†]Refers to the significance of five preplanned comparisons; 1 – CPO diet compared with 73 g/kg of milled COR; 2 – CPO diet compared with 73 g/kg of milled HORs; 3 – linear component of the response to incremental amounts of milled CORs in the diet; 4 – linear component of the response to incremental amounts of milled HORs in the diet; 5 – comparison of mean responses to milled COR and HOR in the diet. *, ** and ***$P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.
[‡]s.e.m. for $n = 35$ measurements, 13 error d.f.
[1]1, 2 and 3, refers to rapeseed inclusion levels of 73, 96 and 118 g/kg DM, respectively.

The decrease in milk fat 8:0 and *trans*-9 14:1 content was also non-linear ($P < 0.05$). Linear responses ($P < 0.05$) to incremental amounts of milled HOR were also observed for milk fat *trans*-16:1 (Table 4), *trans*-4, -9 and -10 18:1 (Table 5), 18:2n-6, *cis*-9, *cis*-15 18:2 and *trans*-9, *cis*-12 18:2 (Table 6), several isomers of CLA (Table 6) and 18:3n-3 (Table 4) concentrations.

The type of rapeseed in the diet also influenced milk FA composition. Milk from cows fed milled CORs contained higher ($P < 0.05$) 14:0, 16:0, 18:3n-3, ⩽C14 FAs, total SFA (Table 4), *cis*-12 18:1 (Table 5) and specific 18:2 isomers including 18:2n-6 and *cis*-9, *cis*-15 18:2 (Table 6) compared with milled HORs. In contrast, HOR treatments resulted in higher ($P < 0.05$) mean milk fat 18:0, 20:0, *cis*-20:1, *cis*-9 18:1, *trans*-4 18:1, *trans*-5 18:1 and *trans*-6-8 18:1 contents than the COR treatments. Increases in the 18:3n-3 content of milk from cows fed the COR diet resulted in a lower ($P < 0.05$) n-6 : n-3 ratio than the control and HOR treatments supplying the same amount of rapeseed oil.

Discussion

This experiment examined the effects of replacing CPO with incremental amounts of two varieties of milled rapeseeds on milk production and milk FA composition in high-yielding dairy cows fed a maize silage-based diet. Supplements of milled high oleic acid rapeseed and wheat contained lower concentrations of *cis*-9 18:1 relative to milled conventional rapeseed and wheat than anticipated. Previous studies have examined the effects of a rapeseed oil, which contained 77 g *cis*-9 18:1/100 g FAs in lactating cows (Jenkins, 1998; Loor *et al*. 2002), whereas the *cis*-9 18:1 content of oil from high oleic acid mutants can vary from 70% to 81% total FAs (Schierholt *et al*., 2000). No measurements of the lipid composition of whole rapeseeds were made in this study, but the oil of the 'Nexera' high oleic acid rapeseed variety typically contains 73 g *cis*-9 18:1 and 17 g of PUFA/100 g total FAs (Dow AgroSciences LLC).

Including both types of rapeseed in the diet even at the highest inclusion level supplying in excess of 1100 g oil/day had no significant effect on DM intake, milk yield or milk constituent composition compared with the CPO diet, with the exception of a marginal decrease in milk fat concentrations. Previous studies have shown that supplementing the diet with supplements providing 1000 g rapeseed oil/day have no significant impact on DM intake and milk yield of cows when compared with a control diet, (Chelikani *et al*., 2004; Givens *et al*., 2009), but may, depending on the composition of the basal diet, lower milk fat synthesis (Chelikani *et al*., 2004). However, supplementing the diet with 1214 g/day of rapeseed oil in the form of whole cracked rapeseeds was shown to decrease DM intake, milk yield and fat yield in mid-lactation cows (Givens *et al*., 2003). It is generally accepted that high levels of plant oils or oilseeds in the diet induce adverse effects on rumen function and cow performance (Palmquist, 1994; Lock and Shingfield, 2004). Results from this study as well as earlier studies highlight that a form of lipid in the diet is an important determinant of the potential effects on intake and milk production.

Table 4 *Effect of replacing CPO with incremental amounts of milled conventional or high oleic acid rapeseeds on milk fatty acid composition (g/100 g fatty acids)*

	Treatments[1]								$P^{\dagger}$				
	CPO	COR1	COR2	COR3	HOR1	HOR2	HOR3	s.e.m.[‡]	1	2	3	4	5
4:0	3.5	3.2	3.0	3.2	3.3	2.8	2.9	0.19	ns	ns	ns	ns	ns
6:0	2.4	2.4	2.3	2.2	2.4	2.3	1.9	0.07	ns	ns	*	***	ns
8:0	1.2	1.3	1.2	1.1	1.2	1.2	0.9	0.04	ns	ns	**	***	*
10:0	2.4	2.6	2.5	2.1	2.4	2.4	1.9	0.11	ns	ns	**	**	ns
10:1 *cis*-9	0.25	0.29	0.26	0.23	0.27	0.24	0.19	0.014	ns	ns	*	**	*
12:0	2.5	2.9	2.7	2.3	2.6	2.6	2.1	0.13	ns	ns	**	**	ns
13:0[2]	0.11	0.16	0.15	0.13	0.15	0.14	0.12	0.014	*	ns	ns	ns	ns
13:0 anteiso	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.003	ns	ns	ns	ns	ns
14:0	9.2	10.3	9.9	8.8	9.7	9.2	8.3	0.25	**	ns	***	**	*
14:0 iso	0.09	0.10	0.11	0.09	0.10	0.08	0.08	0.014	ns	ns	ns	ns	ns
14:1 *cis*-9	0.80	0.97	0.92	0.82	0.90	0.81	0.80	0.052	*	ns	*	ns	ns
14:1 *trans*-9	0.19	0.19	0.19	0.19	0.19	0.19	0.17	0.003	ns	ns	ns	**	*
15:0	0.74	0.85	0.82	0.77	0.89	0.81	0.79	0.046	ns	*	ns	ns	ns
15:0 anteiso	0.37	0.39	0.41	0.38	0.41	0.40	0.36	0.010	ns	*	ns	**	ns
16:0	33.7	24.3	21.2	20.2	21.9	20.0	19.2	0.51	***	***	***	**	**
16:0 iso	0.24	0.22	0.27	0.20	0.24	0.23	0.21	0.020	ns	ns	ns	ns	ns
16:1 *cis*-9[3]	1.27	0.99	0.90	0.88	0.93	0.81	0.89	0.041	***	***	ns	ns	ns
16:1 *cis*-11	0.002	0.010	0.019	0.010	0.008	0.013	0.017	0.006	ns	ns	ns	ns	ns
16:1 *cis*-13	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.004	ns	ns	ns	ns	ns
16:1 *trans*-6/7/8	0.04	0.04	0.05	0.05	0.06	0.05	0.06	0.005	ns	*	ns	ns	ns
16:1 *trans*-9[4]	0.27	0.30	0.32	0.32	0.28	0.30	0.32	0.014	ns	ns	ns	*	ns
16:1 *trans*-10	0.000	0.009	0.004	0.007	0.002	0.012	0.005	0.003	ns	ns	ns	ns	ns
16:1 *trans*-11	0.02	0.04	0.04	0.04	0.03	0.03	0.04	0.003	ns	ns	ns	*	ns
16:1 *trans*-12	0.17	0.14	0.14	0.15	0.14	0.15	0.16	0.005	ns	ns	ns	**	ns
16:1 *trans*-13	0.34	0.37	0.40	0.35	0.37	0.36	0.33	0.012	ns	ns	ns	ns	ns
17:0	0.40	0.43	0.43	0.41	0.41	0.41	0.39	0.012	ns	ns	ns	ns	ns
17:1 *cis*-9	0.13	0.15	0.14	0.14	0.14	0.13	0.14	0.007	ns	ns	ns	ns	ns
18:0 iso	0.03	0.02	0.02	0.01	0.02	0.01	0.01	0.005	ns	ns	ns	ns	ns
18:0	9.1	13.3	15.2	16.1	14.8	17.0	16.7	0.55	***	***	**	*	*
18:1 *trans* total	3.3	4.8	5.2	5.7	4.9	5.1	5.9	0.40	*	*	ns	ns	ns
18:1 *cis* total	22.2	24.5	26.5	28.5	26.7	27.6	30.6	0.45	**	***	***	***	***
Non-CLA[5] 18:2 total	3.0	2.7	2.6	2.5	2.5	2.3	2.3	0.08	*	***	ns	ns	**
CLA total	0.59	0.62	0.67	0.72	0.59	0.63	0.63	0.038	ns	ns	ns	ns	ns
18:3n-3	0.23	0.29	0.29	0.28	0.22	0.19	0.17	0.014	*	ns	ns	*	***
19:0[6]	0.04	0.05	0.05	0.05	0.05	0.06	0.05	0.005	ns	ns	ns	ns	ns
20:0	0.11	0.20	0.23	0.24	0.23	0.26	0.26	0.007	***	***	***	*	***
20:1 *cis*-5	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.003	ns	ns	ns	ns	ns
20:1 *cis*-9	0.10	0.18	0.21	0.22	0.21	0.22	0.25	0.006	***	***	***	**	***
20:1 *cis*-11	0.05	0.09	0.10	0.10	0.11	0.11	0.13	0.008	**	***	ns	ns	*

Table 4 *Continued*

	Treatments[1]								$P^{\dagger}$				
	CPO	COR1	COR2	COR3	HOR1	HOR2	HOR3	s.e.m.[‡]	1	2	3	4	5
20:2n-6	0.04	0.03	0.02	0.03	0.03	0.03	0.02	0.004	*	*	ns	ns	ns
20:3n-3	0.001	0.007	0.011	0.011	0.043	0.046	0.051	0.004	ns	***	ns	ns	***
20:3n-6	0.03	0.05	0.05	0.04	0.05	0.06	0.06	0.006	*	*	ns	ns	*
20:5n-3	0.03	0.03	0.02	0.02	0.02	0.02	0.01	0.003	ns	*	ns	ns	**
22:0	0.02	0.02	0.02	0.01	0.01	0.02	0.003	0.003	ns	ns	ns	*	ns
22:1 *cis*-13	0.11	0.09	0.08	0.07	0.08	0.08	0.07	0.005	*	**	*	ns	ns
22:2n-6	0.03	0.02	0.03	0.03	0.03	0.02	0.01	0.003	ns	ns	ns	**	*
22:4n-6	0.009	0.02	0.02	0.01	0.01	0.01	0.002	0.004	ns	ns	ns	*	ns
22:5n-3	0.04	0.04	0.04	0.03	0.03	0.04	0.02	0.006	ns	ns	ns	ns	ns
24:0	0.01	0.01	0.02	0.02	0.02	0.02	0.01	0.003	ns	**	ns	*	ns
$\Sigma \leqslant 14{:}0$	21.5	23.2	22.1	19.9	22.0	20.9	18.3	0.57	ns	ns	***	***	*
Σ saturates	66.5	63.3	61.0	58.5	61.2	60.4	56.6	0.55	**	***	***	***	**
Σ *cis* MUFA	24.9	27.4	29.4	31.2	29.4	30.1	33.2	0.46	**	***	***	***	**
Σ *trans* MUFA	4.2	5.8	6.2	6.7	5.8	6.0	6.9	0.41	*	*	ns	ns	ns
Σ *trans* total	5.3	7.2	7.6	8.1	7.1	7.3	8.2	0.43	**	*	ns	ns	ns
n-6 : n-3 PUFA	9.1	5.9	5.7	5.8	6.2	6.1	6.7	0.18	***	***	ns	ns	**
Total fatty acids (g/100 g milk fat)	94.4	94.5	94.6	94.6	94.5	94.3	94.4	0.11	ns	Ns	ns	ns	ns

CPO = calcium salts of palm oil distillate-based diet, COR1, COR2 and COR3 = conventional oleic acid rapeseed at three inclusion levels, HOR1, HOR2 and HOR3 = high oleic acid rapeseed at three inclusion levels; CLA = conjugated linoleic acid; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; DM = dry matter.

[†]Refers to the significance of five comparisons; 1 – CPO diet *v.* COR diet level 1; 2 – CPO *v.* HOR diet level 1; 3 – linear response to COR inclusion level; 4 – linear response to HOR inclusion level; 5 – average of COR diet responses *v.* average of HOR responses. *, ** and *** $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

[‡]s.e.m. for $n = 35$ measurements, 13 error d.f.

[1]1, 2 and 3, refers to rapeseed inclusion levels of 73, 96 and 118 g/kg DM, respectively.

[2]Co-elutes with *cis*-9 12:1 as a minor isomer.

[3]Co-elutes with 17:0 anteiso.

[4]Co-elutes with 17:0 iso.

[5]All 18:2 isomers excluding CLA.

[6]Co-elutes with *cis*-15 18:1.

Table 5 *Effect of replacing CPO with incremental amounts of milled conventional or high oleic acid rapeseeds on milk 18:1 isomer composition (g/100 g fatty acids)*

	Treatments[1]								$P^{\dagger}$				
	CPO	COR1	COR2	COR3	HOR1	HOR2	HOR3	s.e.m.[‡]	1	2	3	4	5
cis-9 18:1[2]	21.1	22.7	24.8	26.9	25.1	26.1	28.9	0.44	*	***	***	***	***
cis-11 18:1	0.59	0.65	0.63	0.69	0.69	0.66	0.74	0.031	ns	ns	ns	ns	ns
cis-12 18:1	0.25	0.33	0.28	0.28	0.29	0.24	0.23	0.015	**	ns	*	*	**
cis-13 18:1	0.07	0.10	0.10	0.10	0.10	0.10	0.11	0.006	*	**	ns	ns	ns
cis-16 18:1	0.09	0.11	0.12	0.11	0.11	0.11	0.11	0.005	*	**	ns	ns	ns
trans-4 18:1	0.04	0.04	0.05	0.05	0.05	0.06	0.07	0.004	ns	**	ns	*	**
trans-5 18:1	0.03	0.04	0.04	0.05	0.05	0.05	0.05	0.003	ns	***	ns	ns	***
trans-6,-7,-8 18:1	0.37	0.59	0.65	0.76	0.72	0.77	0.85	0.052	*	***	*	ns	*
trans-9 18:1	0.31	0.48	0.51	0.55	0.52	0.53	0.66	0.036	**	**	ns	*	ns
trans-10 18:1	0.37	0.76	0.89	1.00	0.64	0.69	1.3	0.210	ns	ns	ns	*	ns
trans-11 18:1	0.95	1.13	1.25	1.31	1.04	1.26	1.12	0.068	ns	ns	ns	ns	ns
trans-12 18:1	0.37	0.56	0.59	0.64	0.62	0.62	0.62	0.039	**	***	ns	ns	ns
trans-13,-14 18:1	0.72	1.00	0.96	1.10	1.03	0.88	1.06	0.068	**	ns	ns	ns	**
trans-16 18:1[3]	0.28	0.44	0.45	0.48	0.43	0.44	0.47	0.016	***	***	ns	ns	ns

CPO = calcium salts of palm oil distillate-based diet, COR1, COR2 and COR3 = conventional oleic acid rapeseed at three inclusion levels, HOR1, HOR2 and HOR3 = high oleic acid rapeseed at three inclusion levels; DM = dry matter.
[†]Refers to the significance of five comparisons; 1 – CPO diet *v.* COR diet level 1; 2 – CPO diet *v.* HOR diet level 1; 3 – linear response to COR inclusion level; 4 – linear response to HOR inclusion level; 5 – average of COR diet responses *v.* average of HOR responses. *, ** and *** $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.
[‡]s.e.m. for $n = 35$ measurements, 13 error d.f.
[1]1, 2 and 3, refers to rapeseed inclusion levels of 73, 96 and 118 g/kg DM, respectively.
[2]Co-elutes with *cis*-10 18:1 as a minor isomer.
[3]Co-elutes with *cis*-14 18:1.

Table 6 *Effect of replacing CPO with incremental amounts of milled conventional or high oleic acid rapeseeds on milk 18:2 isomer composition (mg/100 g fatty acids)*

	Treatments[1]								$P^{\dagger}$				
	CPO	COR1	COR2	COR3	HOR1	HOR2	HOR3	s.e.m.[‡]	1	2	3	4	5
cis-9, *cis*-12 18:2	2563	1970	1869	1817	1850	1671	1605	74.8	***	***	ns	*	*
cis-9, *cis*-15 18:2	10.7	23.2	23.2	22.5	13.6	10.6	4.4	2.11	**	ns	ns	**	***
cis-9, *trans*-12 18:2	88.5	106.3	99.0	72.6	118.3	75.5	67.0	13.31	ns	ns	ns	*	ns
cis-9, *trans*-13 18:2	181	299	300	326	285	256	315	16.3	***	***	ns	ns	ns
trans-9, *cis*-12 18:2	22.8	16.1	13.5	8.6	12.7	4.0	0.0	3.85	ns	ns	ns	*	*
trans-11, *cis*-15 18:2	68.4	103.8	121.0	113.8	60.6	52.5	67.3	13.20	ns	ns	ns	ns	**
trans-10, *trans*-15 18:2	94.2	144.5	151.2	156.6	131.9	131.6	158.2	7.94	***	**	ns	*	ns
trans-11, *trans*-15 18:2	34.6	51.4	44.1	46.7	30.7	36.7	45.2	8.64	ns	ns	ns	ns	ns
trans-13, *trans*-15 CLA	0.81	1.2	1.1	1.1	1.5	0.97	0.54	0.447	ns	ns	ns	ns	ns
trans-12, *trans*-14 CLA	5.3	8.0	6.9	7.7	5.9	4.6	4.2	1.11	ns	ns	ns	ns	**
trans-11, *trans*-13 CLA	13.5	15.6	15.8	18.4	12.0	7.9	9.8	1.92	ns	ns	ns	ns	***
trans-10, *trans*-12 CLA	9.2	6.7	6.6	7.0	7.8	6.5	6.3	0.91	*	ns	ns	ns	ns
trans-9, *trans*-11 CLA	21.5	15.1	14.9	14.8	16.0	13.6	11.8	1.36	**	**	ns	*	ns
trans-8, *trans*-10 CLA	12.6	2.3	3.6	3.5	4.1	4.4	3.2	0.89	***	***	ns	ns	ns
trans-7, *trans*-9 CLA	3.7	2.8	3.6	3.5	3.7	4.5	4.1	0.68	ns	ns	ns	ns	ns
trans-12, *cis*-14 CLA	4.3	5.9	4.1	4.8	4.5	1.8	2.4	1.07	ns	ns	ns	ns	*
trans-11, *cis*-13 CLA	6.7	5.5	4.7	5.2	5.5	5.0	2.6	1.09	ns	ns	ns	*	ns
cis-11, *trans*-13 CLA	1.90	1.50	1.64	1.46	2.11	1.13	0.82	0.483	ns	ns	ns	*	ns
trans-10, *cis*-12 CLA	12.6	7.2	8.5	8.1	7.0	4.6	11.0	3.16	ns	ns	ns	ns	ns
cis-9, *trans*-11 CLA	597	580	658	707	570	642	610	49.8	ns	ns	*	ns	ns
trans-9, *cis*-11 CLA	15.6	17.3	23.9	23.8	18.6	18.6	36.4	7.51	ns	ns	ns	*	ns
trans-8, *cis*-10 CLA	5.1	5.4	5.4	4.6	4.3	5.7	7.6	1.23	ns	ns	ns	*	ns
trans-7, *cis*-9 CLA	62.1	126	141	162	144	144	206	24.4	ns[2]	**	ns	*	ns

CPO = calcium salts of palm oil distillate; COR1, COR2 and COR3 = conventional oleic acid rapeseed at three inclusion levels; HOR1, HOR2 and HOR 3 = high oleic acid rapeseed at three inclusion levels; DM = dry matter.
[†]Refers to the significance of five comparisons; 1 – CPO diet *v.* COR diet level 1; 2 – CPO diet *v.* HOR diet level 1; 3 – linear response to COR inclusion level; 4 – linear response to HOR inclusion level; 5 – average of COR diet responses *v.* average of HOR responses. *, ** and *** $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.
[‡]s.e.m. for $n = 35$ measurements, 13 error d.f.
[1]1, 2 and 3, refers to rapeseed inclusion levels of 73, 96 and 118 g/kg DM, respectively.
[2]$P = 0.05$.

Ground rapeseeds have been shown to reduce fat corrected milk yield, milk fat and protein content in the absence of changes in DM intake (Chichlowski *et al.*, 2005). However, supplements of milled rapeseeds and wheat were reported to stimulate higher DM intake compared with the same amount of lipid in the diet as rapeseed oil (Givens *et al.*, 2009). Overall, available data suggest that inclusion of milled rapeseeds in the diet minimises potential negative effects of rapeseed oil on rumen function and animal performance.

Replacing CPO with both rapeseed varieties resulted in a decrease in milk fat 16:0 and total SFA content. This is partially due to the presence of a palm oil-based rumen-protected lipid supplement in the CPO ration that was used to ensure that treatments were isoenergetic. However, earlier studies have shown that compared with a diet control containing no additional lipid, rapeseed oil lowers milk fat 16:0 and total SFA concentrations (Collomb *et al.*, 2004; Ryhänen *et al.*, 2005; Rego *et al.*, 2009), indicating that irrespective of the composition of the basal diet, rapeseed-based lipid supplements can be expected to decrease milk SFA content. Rapeseed oil in the diet leads to an increase in long-chain unsaturated FAs available for absorption in the small intestine of lactating cows (Loor *et al.*, 2002) leading to an inhibition of FA synthesis *de novo* in the mammary gland (Chilliard *et al.*, 2000). In this study, rapeseed inclusion did not appear to affect other short- and medium-chain SFA concentrations, although the COR1 treatment increased milk fat 14:0 content compared with the CPO diet.

Incremental inclusion of rapeseeds in the diet resulted in a linear decrease in milk SFA concentrations, 16:0 in particular, with no evidence that the changes in milk FA composition reached a plateau under the specified conditions of this experiment. Furthermore, marginal decreases in milk SFA content were greater for the HOR compared with the COR rapeseed with HOR treatments resulting in lower milk fat concentrations of SFA synthesised *de novo* and higher 18:0 concentrations. It is probable that the differences in the extent of changes in milk SFA due to rapeseed variety is at least in part due to a concentration effect, as HOR treatments induced a higher enrichment of *cis*-9 18:1. It is also possible that the larger decreases in milk SFA concentration following HOR rapeseed supplementation reflects direct competition between *cis*-9 18:1 and newly synthesised SFA for esterification with glycerol at the *sn*-2 and *sn*-3 during mammary triacylglycerol synthesis (Hansen and Knudsen, 1987).

Despite diets containing rapeseeds resulting in a lower 18:0 intake than the CPO diet, both COR and HOR treatments enhanced milk fat 18:0 concentration. This can be explained by rapeseed diets resulting in a higher 18-carbon unsaturated FA intake than the CPO diet, which, following complete hydrogenation in the rumen (Harfoot and Hazlewood, 1997) increases the availability of 18:0 for absorption in the bovine small intestine. Post-ruminal flow of 18:0 has been found to be approximately 11 times greater than intake (on a g/day basis) in lactating cows fed diets containing rapeseed oil (Loor *et al.*, 2002). An increase in milk 18:0 concentrations is a typical response to dietary rapeseed supplements (Givens and Shingfield, 2006; Glasser *et al.*, 2008).

Replacing CPO with rapeseed enhanced *cis*-MUFA in milk fat. The second most abundant *cis*-MUFA, *cis*-9 16:1, had a lower concentration in milk fat from cows consuming rapeseed diets when compared with the CPO diet, which is in agreement with earlier studies examining the effect of rapeseed in the diet in lactating cows (DePeters *et al.*, 2001; Collomb *et al.*, 2004). A lower concentration of *cis*-9 16:1 can be attributed to decreased supply of 16:0 available for conversion to *cis*-9 16:1 via the action of Δ^9-desaturase in the mammary gland. Use of ^{13}C-labelled FA substrates indicate that around 50% *cis*-9 16:1 secreted in milk is synthesised endogenously via the action of Δ^9-desaturase on 16:0 (Mosley and McGuire, 2007).

Enhanced *cis*-MUFA concentration in milk fat from all of the rapeseed diets was primarily due to increases in *cis*-9 18:1 concentration. This can be explained by an increased intake of *cis*-9 18:1, and also due to increased mammary availability of 18:0, which is desaturated at the mammary gland level. Around 50% of 18:0 taken up by the mammary gland is desaturated (Enjalbert *et al.*, 1998), and all studies examining responses to supplemental rapeseeds or rapeseed oil in the diet report an increase in milk *cis*-9 18:1 concentration (Givens and Shingfield, 2006; Glasser *et al.*, 2008). Increases in milk fat *cis*-9 18:1 concentrations to both rapeseeds were linear across the range of supplements fed in this experiment, indicating the potential to further enhance milk *cis*-9 18:1 content. Rapeseed inclusion also increased the concentration of several other *cis*-18:1 (Δ13 and 16) isomers. It is probable that these FAs originate from the rumen as a result of incomplete biohydrogenation of 18:3n-3 contained in rapeseeds (Jouany *et al.*, 2007).

Addition of both rapeseed types to the diet resulted in numerical increases in milk *trans*-16:1 and *trans*-18:1 content compared with the CPO diet. All rapeseed diets resulted in significant increases in *cis*-9 18:1, 18:2n-6 and 18:3n-3 intake when compared with the CPO diet, but there was no significant increase in milk fat *trans*-11 18:1 concentration, the major *trans* FA in milk fat that is a common intermediate formed during the penultimate step of PUFA biohydrogenation in the rumen (Harfoot and Hazlewood, 1997; Shingfield *et al.*, 2010). A lack of change in milk fat *trans*-11 18:1 content has been reported in cows fed milled rapeseeds even when a similar amount of lipid in the form of rapeseed oil enhanced the concentration of this biohydrogenation intermediate in milk (Givens *et al.*, 2009). Previous studies have also reported that ground rapeseeds have no effect (Collomb *et al.*, 2004) or result in marginal increases in milk fat *trans*-11 18:1 concentrations (Chichlowski *et al.*, 2005).

In spite of cows consuming more PUFA on the COR than HOR treatments, there was no significant difference between rapeseed types with respect to *trans*-MUFA or total *trans* FAs in milk indicating that biohydrogenation of *cis*-9 18:1 as well as 18:2n-6 and 18:3n-3 in the rumen results in the formation and accumulation of substantial amounts of *trans* FAs that are incorporated into milk fat. Total *trans*-MUFA secretion in milk was more closely associated with *cis*-9 18:1 intake ($R^2 = 0.785$; $y = 0.05x + 45.83$) than with

PUFA intake ($R^2 = 0.528$; $y = 0.09x + 40.60$). *In vitro* studies have shown that *cis*-9 18:1 is converted to a range of *trans* 18:1 isomers during incubation with rumen-derived microorganisms (Mosley *et al*., 2002; McKain *et al*., 2010). In particular, around 70% to 100% of *trans*-7, *trans*-9, *trans*-10 and *trans*-12 18:1 were thought to originate from the isomerisation of *cis*-9 18:1 (Mosley *et al*., 2002). Furthermore, *trans*-9 18:1 can be further isomerised during incubations with mixed rumen bacteria to yield a range of positional *trans* 18:1 isomers (Proell *et al*., 2002). Supplying higher amounts of *cis*-9 18:1 in the diet in the form of the HOR supplement in this study resulted in a higher enrichment of *trans*-4, *trans*-5 and *trans*-6-8 18:1 in milk fat, and a numerical but non-significant ($P = 0.09$) enrichment of milk *trans*-9 18:1 content compared with the COR diet. Earlier studies also reported a significant increase in milk *trans*-6-8, *trans*-9 18:1, *trans*-12, *trans*-13-14 and *trans*-16 18:1 in cows fed when rapeseed supplements were fed compared with a control diet (Collomb *et al*., 2004; Givens *et al*., 2009).

Trans-7, *cis*-9 CLA is the second most abundant CLA isomer in bovine milk fat on most diets (Shingfield *et al*., 2008), that is synthesised exclusively via the action of Δ^9 desaturase on *trans*-7 18:1 in ruminant tissues (Corl *et al*., 2002; Piperova *et al*., 2002). Enhanced concentrations of this isomer in milk fat following replacement of CPO with milled rapeseeds can be attributed to an increased availability of *trans*-7 18:1 at the mammary gland, following the formation of this intermediate during biohydrogenation of *cis*-9 18:1 in the rumen (Mosley *et al*., 2002). Consistent with products formed during incubations of *cis*-9 18:1 with mixed rumen bacteria (Mosley *et al*., 2002), HOR enhanced milk fat *trans*-6-8 18:1 content and resulted in numerically higher concentrations of *trans*-7, *cis*-9 CLA compared with COR treatments.

Milk fat *cis*-9, *trans*-13 18:2 content was substantially enhanced when rapeseed lipids replaced CPO in the diet. This FA is at least partially synthesised endogenously in the mammary gland via the action of Δ^9-desaturase on *trans*-13 18:1 formed in the rumen (Shingfield *et al*., 2008). Both *trans*-13 18:1 and *cis*-9, *trans*-13 18:2 tend to increase following the feeding of 18:2n-6 or 18:3n-3 – rich plant oils (Collomb *et al*., 2004; Shingfield *et al*., 2008). Including rapeseed lipids at the expense of CPO resulted in reduced concentrations of *trans*-8, *trans*-10 CLA and *trans*-9, *trans*-11 CLA in milk. Incubations of 18:2n-6 with mixed rumen microbes (Jouany *et al*., 2007), measurements of FA flows at the omasum (Shingfield *et al*., 2008) and milk FA composition in cows fed plant oils (Shingfield *et al*., 2008; Rego *et al*., 2009) indicate that *trans*-8, *trans*-10 CLA and *trans*-9, *trans*-11 CLA accumulate in the rumen in direct relation to 18:2n-6 intake. The results of this study for milk fat CLA isomer concentrations appear to be consistent with the higher 18:2n-6 intake of cows fed diets containing milled rapeseeds than CPO.

In conclusion, replacing CPO with milled rapeseeds at inclusion levels up to 1150 g oil/day decreased milk fat SFA, enhanced milk *cis*-MUFA and increased *trans* FA concentrations without inducing adverse effects on DM intake and milk production. Use of a HOR variety induced larger decreases in milk fat SFA, increased ($P < 0.001$) the ratio of *trans*-MUFA : *trans*-PUFA and altered the relative abundance of specific *trans* FA isomers compared with isoenergetic amounts of CORs.

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