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Effect of forage conservation method, concentrate level and propylene glycol on the fatty acid composition and vitamin content of cows' milk

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Based on potential health benefits, there is a need to develop effective strategies for enhancing milk fat concentrations of *cis*-9 18 : 1, 18 : 3 *n*-3 and conjugated linoleic (CLA) content in milk without compromising the sensory or storage characteristics of processed milk or dairy products. Sixteen Finnish Ayrshire dairy cows were used in a cyclic change-over experiment with four 21-d experimental periods and a $4 \times 2 \times 2$ factorial arrangement of treatments to evaluate the effects of forage conservation method, concentrate level and supplements of propylene glycol (PG), and their interactions on milk fatty acid composition and vitamin content. Experimental treatments consisted of four conserved forages offered *ad libitum*, supplemented with two levels of a standard concentrate (7 or 10 kg/d) and PG (0 and 210 g/d) fed as three equal meals. Primary growths of timothy and meadow fescue sward were conserved by ensiling with none (NA), an inoculant enzyme preparation (IE) or a formic acid based (FORM) additive or as hay 1 week later. Conservation of grass by drying rather than ensiling resulted in lower forage 18 : 2*n*-6, 18 : 3*n*-3, total fatty acid and fat-soluble vitamin concentrations. In spite of lower intakes, milk fat 18 : 2*n*-6 and 18 : 3*n*-3 content was higher ($P<0{\cdot}05$) for hay than for silage diets (12·1, 9·6, 9·6 and 9·3 and 5·00, 3·51, 4·27 and 2·93 g/kg total fatty acids, for hay, NA, IE and FORM silages, respectively). Forage conservation method had no clear effects on milk *trans* 18 : 1 or CLA content. Compared with silage, hay diets resulted in milk containing lower ($P<0{\cdot}001$) riboflavin, α-tocopherol and β-carotene concentrations, but had no effect on ascorbic acid, thiamine, pyridoxine or retinol content. Feeding more concentrates had no effect on milk fatty acid composition or milk vitamin content, other than lowering ($P<0{\cdot}001$) 16 : 0 concentrations from 348 to 338 g/kg fatty acids. Supplements of PG led to small ($P<0{\cdot}05$) increases in milk 13 : 0 *anteiso* and 15 : 0 content from 1·06 and 11·3 to 1·22 and 12·6 g/kg fatty acids and reduced ($P<0{\cdot}05$) the concentrations of ascorbic acid (16·1 *v.* 15·1 g/kg milk).

Keywords: Forage conservation, *trans* fatty acids, conjugated linoleic acid, milk vitamins.

Milk fat is a major source of saturated fatty acids (SFA) in the human diet (Hulsof et al. 1999), and there is evidence to suggest that consumption of foods rich in SFA increases cardiovascular disease risk and the development of insulin resistance and dyslipidaemia (Vessby et al. 2001). The medium-chain fatty acids (12 : 0, 14 : 0 and 16 : 0), which account for the majority of SFA in milk fat, have been implicated in increasing total and low-density lipoprotein cholesterol concentrations (Williams, 2000). However, milk lipids also contain several compounds including butyrate, isomers of conjugated linoleic acid (CLA) and sphingomyelin that are anti-carcinogenic (Parodi, 1999; Kritchevsky, 2000) and *cis*-9 C18 : 1 and C18 : 3 *n*-3 which have putative cardio-protective effects (Williams, 2000). Therefore, there is considerable interest in developing nutritional strategies for enhancing concentrations of beneficial fatty acids and decreasing the SFA content of milk fat for improving long-term human health.

Supplementing diets with free oils, oilseeds or rumen-protected lipids is the most common nutritional means for

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manipulating milk fatty acid composition (Chilliard et al. 2000; Lock & Shingfield, 2004), despite forages often being the major source of fatty acids in the diet (Harfoot & Hazlewood, 1988). Milk from cows eating fresh grass contains less 14 : 0 and 16 : 0 and higher concentrations of 18 : 1, 18 : 3 *n*-3 and CLA than that from cows eating dried or ensiled forages (Aii et al. 1988; Kelly et al. 1998), but the impact of conservation method *per se* on milk fatty acid composition is not well defined (Chilliard et al. 2001).

To meet the nutritional requirements of high-producing dairy cows, forages are fed with concentrated energy and protein supplements. Increasing concentrates in the diet generally reduces forage intake (Huhtanen, 1998), lowers the rate of lipolysis (Gerson et al. 1985) and inhibits ruminal biohydrogenation of dietary unsaturated fatty acids, which results in increased flow of *trans* 18 : 1 at the duodenum (Kalscheur et al. 1997; Loor et al. 2004) and elevated concentrations of *trans* 18 : 1 in milk fat (Gaynor et al. 1995; Griinari et al. 1998).

Supplements of propylene glycol (PG) could potentially be used to improve the efficiency of nitrogen utilization for diets based on restrictively fermented grass silage (Huhtanen, 1998). However, PG alters lipid metabolism in the dairy cow causing an increase in the molar proportions of glucogenic to lipogenic volatile fatty acids in the rumen and a reduction in plasma non-esterified fatty acids and milk fat content (Nielsen & Ingvartsen, 2004). Ruminal metabolism of PG to propionate (Nielsen & Ingvartsen, 2004) would be expected to increase substrate availability for synthesis of odd-chain fatty acids *de novo* by rumen bacteria (Jenkins, 1993) which is the major source of these fatty acids in milk (Harfoot & Hazlewood, 1988). Even though supplements of PG can lower milk fat content, their impact on milk fatty acid composition is unclear.

Enhancing the polyunsaturated fatty acid (PUFA) content of milk is associated with increased susceptibility to auto-oxidation and development of off-flavours (Palmquist et al. 1993; Timmons et al. 2001; Kristensen et al. 2004). PUFA in milk phospholipids and triacylglycerides are protected from oxidation by natural antioxidants located in fat-soluble (α-tocopherol and β-carotene) and water-soluble (ascorbic acid) phases. Concentrations of α-tocopherol and β-carotene in milk are related to the dietary intake (Charmley et al. 1993; Focant et al. 1998), while the amounts of α-tocopherol and β-carotene in grass are substantially reduced during conservation (Weiss, 1998).

In this experiment, the effects of forage conservation method, level of concentrate feeding and PG supplements on the fatty acid composition and vitamin content of milk were examined.

Materials and Methods

Experimental design

Sixteen cows were used in a cyclic change-over design (Davis & Hall, 1969) with four 21-d periods. Cows were allocated at random to experimental treatments. Treatments in a $4 \times 2 \times 2$ factorial arrangement consisted of four forages (hay or silage ensiled with an inoculant enzyme preparation (IE), a formic acid based additive (FORM) or ensiled directly with no additive (NA)), two levels of concentrate (7 and 10 kg fresh weight /d; L and H, respectively) and 0 or 210 g propylene glycol/d (−PG and +PG, respectively).

Animals and their management

Sixteen multiparous Finnish Ayrshire cows of mean ± SD live weight 584 ± 64·2 kg, parity 2·4 ± 0·73 and 51 ± 16·3 d into lactation, producing 33·2 ± 3·89 kg of energy-corrected milk/d, were selected from the Jokioinen dairy herd and housed in individual tie stalls. Stalls were fitted with two feeding troughs that allowed forages to be fed separately from concentrate and PG supplements. Each stall was bedded with peat and sawdust. Animals had continuous access to water and were milked at approximately 06·30 and 15·30. Forages were offered at 12·30 to ensure proportionate refusals of 0·05, while concentrates and PG supplements were fed as three equal meals at 05·30, 12·30 and 16·30.

Experimental diets

Forages were produced from primary growths of mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) based grass swards. Grass used to prepare silages was cut and wilted for 6 h before being picked up with precision-chop forage harvesters fitted with gravity-fed applicators. Differences in silage fermentation characteristics were achieved by treating grass with a formic acid based additive (800 g/kg formic acid and 20 g/kg ortho-phosphoric acid; Kemira Agro Ltd., Helsinki, Finland) applied at a rate of 8·3 l /tonne grass, an inoculant enzyme preparation (IE) (Enzymax, Finnfeeds International Ltd., Marlborough, UK) containing both cellulase and hemicellulase, applied at a rate of 3·9 l/tonne grass (corresponding to 5×10^5 pediococci and lactobacilli colony-forming units/g grass) or ensiling grass directly with no additive (NA). The experiment was planned with the intention that hay and silages would be prepared simultaneously but, owing to heavy rainfall (25·7 mm) on the scheduled day of harvesting, grass swards assigned for the production of hay were harvested 7 d later. Once cut, grass was left to dry for 96 h, before baling. Baled hay was transferred to a barn and dried with ambient air for 7 d.

The concentrate supplement was formulated (g/kg on an air-dry basis) from rolled barley (285), rolled oats (284), molassed sugar beet pulp shreds (200) solvent-extracted rapeseed meal (200) of low glucosinolate content (Raisio Feeds Ltd, Raisio, Finland), a proprietary mineral and vitamin supplement (20; Viher-Minera Muro, Suomen Rehu Ltd, Helsinki, Finland), calcium carbonate (8) and sodium chloride (3). The mineral and vitamin supplement

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contained (g/kg) calcium (165), sodium (90), magnesium (80), phosphorus (72) and zinc (3·0); (mg/kg) manganese (980), copper (500), iodine (100), cobalt (12), selenium (10) and molybdenum (8); (i.u./g) retinol (150), cholecalciferol (60) and α-tocopheryl acetate (0·4).

Measurements and sampling

Samples of prewilted chopped grass treated with additive were collected at regular intervals at the time of filling concrete bunker silos and immediately stored at 4 °C. Once ensiling was completed, samples were composited, subsampled and stored at −20 °C, until analysed. Intake and milk production were recorded daily, but only measurements from the last 7 d of each experimental period were used for statistical analysis. During this period, representative samples of fresh silage, hay and concentrates were composited daily. Fresh samples of silages were stored at −20 °C. At the end of the experiment, feed samples were analysed chemically. Milk samples for the measurement of fat content were collected from each cow over four consecutive milkings starting at 16·30 on day 17 and preserved with Bronopol (Valio Ltd, Helsinki, Finland). Unpreserved samples of milk were also collected at 16·30 on day 18 and 06·30 on day 19, and stored at 4 °C before being composited according to yield and analysed for vitamins, fatty acids and sensory qualities.

Chemical analysis

Feed DM content and chemical composition were determined using standard procedures (Shingfield et al. 2001). The fatty acid content and composition of feeds was determined in freeze-dried samples using a one-step extraction and transesterification procedure according to Sukhija & Palmquist (1988) except that methanolic sulphuric acid (20 ml/l) was used as the methylating reagent and tritridecanoin (T-135, Nu-Chek Prep, Elysian MN, USA) was used as an internal standard. Fatty acid methyl esters (FAME) were separated using a GC (6890; Hewlett-Packard, Wilmington DE, USA) equipped with a 100-m fused silica capillary column (i.d. 0·25 mm) coated with 0·2-μm film of cyanopropyl polysiloxane (CP-SIL 88; Chrompack, Middelburg, The Netherlands) using hydrogen as the carrier gas (Shingfield et al. 2003).

Concentrations of α- and γ-tocopherol and β-carotene in feed samples were determined by HPLC after saponification with KOH (500 g/l) at 100 °C for 20 min and extraction with aqueous hexane and ethyl acetate. Fat-soluble vitamins were separated using a liquid chromatograph (model 515; Waters, Millford DE, USA) equipped with a normal phase analytical column (Porasil 10 μm, 300 × 39 mm; Waters), u.v. (model 486; Waters) and fluorescence detector (model 474; Waters) according to Salo-Väänänen et al. (2000).

Milk fat content was determined using a Milko-Scan 133B analyser (Foss Electric, Hillerød, Denmark). Fatty acid composition was determined in lipid extracted in duplicate from 10-ml samples of milk with a mixture of diethylether petroleum ether, ethanol and ammonia (25 : 25 : 10 : 2 (v/v); Antila & Kankare, 1983). Organic extracts were combined and evaporated to dryness at 60 °C under nitrogen for 60 min. Samples were dissolved in hexane and methyl acetate and transesterified to FAME using freshly prepared methanolic sodium methoxide (Christie, 1982). The mixture was neutralized with methanolic HCl, centrifuged and dried using anhydrous calcium chloride. FAME were separated using a GC (8700; Perkin Elmer, Norwalk CN, USA) equipped with a flame-ionization detector, automatic injector, split injection port and a 50-m fused silica capillary column (i.d. 0·25 mm) coated with a 0·2-μm film of cyanopropyl polysiloxane (CP-SIL 88; Chrompack) using helium as the carrier gas. Injector and detector temperatures were 250 °C and 270 °C, respectively. Total FAME profile was determined in a 1-μl sample at a split ratio of 1 : 70 using a temperature gradient programme. Following sample injection, column temperature was held at 90 °C for 1 min, increased at a rate of 5 deg C/min to a 220 °C and held at this temperature for 25 min. Peaks were routinely identified by comparison of retention times with FAME standards (GLC 463; Nu-Check-Prep and US-59-M; Matreya Incorporated, Pleasant Gap PA, USA).

Individual *cis* and *trans* 18 : 1 isomers are not completely resolved using the 50-m CP-SIL 88 column, resulting in an underestimate of *trans* 18 : 1 and overestimate of *cis*-9 18 : 1 content (Molkentin & Precht, 1995). Under these conditions, *trans*-6 to *trans*-11 18 : 1 are detected as a single peak, and *trans*-12, -13, -14, 15 and -16 18 : 1 and *cis*-6 18 : 1 co-elute with *cis*-9 18 : 1. Therefore, to account for these sources of error, measurements of *trans*-6 to *trans*-11 18 : 1 and 16 : 0 were used to estimate total *trans* 18 : 1 content {[total *trans* 18 : 1] = 1·291 × [*trans*-6 to *trans*-11 18 : 1] + 0·0253 × [16 : 0]} according to Molkentin & Precht (1995) and the sum of *trans*-12 to *trans*-16 18 : 1 was used to correct *cis*-9 18 : 1 concentrations.

Fat-soluble vitamins (α-tocopherol, β-carotene and all-*trans*-retinol) in milk were determined by HPLC according to Salo-Väänänen et al. (2000). Thiamine and riboflavin were measured after autoclaving milk at 120 °C for 15 min and incubation with invertase, α-amylase, porcine pepsin, cellulose and phosphatase (Serva Feinbiochemica GmbH, Heidelberg, Germany) for 120 min at 40 C (Laukkanen et al. 1988) using reversed-phase HPLC under isocratic conditions and fluorescence detection (Fellman et al. 1982). Ascorbic acid was determined as dehydroascorbic acid in milk samples pooled across concentrate treatments by reversed-phase HPLC and fluorescence detection (Speek et al. 1984).

Samples of milk were also pasteurized at 72 °C for 15 s and submitted to an experienced six-member taste panel for the assessment of organoleptic properties. Milk samples presented to the panel were maintained at 15 °C and samples were evaluated using a numerical interval scale

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Table 1. Fatty acid composition and fat-soluble vitamin content of prewilted chopped grass, silage, hay and concentrate supplements

	Pre-wilted chopped grass†			Silage‡			Hay§		
	NA	IE	FORM	NA	IE	FORM	Baled	Dried	Concentrate
No. of determinations	2	2	2	8	8	8	2	8	8
Dry matter (DM), g/kg	238	237	228	233¶	241¶	237¶	724	858	886
Organic matter, g/kg DM	917	924	924	923¶	931¶	931¶	929	928	916
Neutral detergent fibre, g/kg DM	597	609	573	512¶	491¶	512¶	641	640	280
Fatty acids, g/kg DM									
12 : 0	0·00	0·00	0·00	0·13	0·12	0·12	0·09	0·07	0·00
14 : 0	0·10	0·09	0·07	0·13	0·10	0·10	0·08	0·07	0·07
16 : 0	3·91	3·88	3·89	3·30	3·34	3·33	2·92	2·75	4·99
16 : 1 *cis*-9	0·06	0·07	0·07	0·05	0·07	0·05	0·06	0·03	0·08
18 : 0	0·23	0·21	0·23	0·25	0·27	0·26	0·20	0·22	0·46
18 : 1 *cis*-9	0·72	0·75	0·82	0·71	0·80	0·73	0·50	0·38	5·99
18 : 1 *cis*-11	0·13	0·12	0·13	0·12	0·14	0·11	0·06	0·05	0·73
18 : 1 *trans*-9	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·05
18 : 2 *n*-6	3·14	3·36	4·58	2·92	3·06	3·45	1·43	1·10	4·05
18 : 3 *n*-3	10·36	10·60	13·08	9·17	9·52	11·70	2·78	2·28	0·40
20 : 0	0·05	0·05	0·06	0·12	0·12	0·14	0·09	0·16	0·09
20 : 1 *cis*-11	0·03	0·03	0·03	0·03	0·02	0·03	0·04	0·02	0·13
22 : 0	0·12	0·10	0·13	0·21	0·21	0·21	0·29	0·26	0·09
24 : 0	0·06	0·06	0·06	0·14	0·13	0·14	0·14	0·14	0·08
24 : 1 *cis*-15	0·04	0·04	0·05	0·03	0·03	0·04	0·00	0·00	0·03
Total saturates	4·46	4·37	4·44	4·25	4·29	4·29	3·82	3·67	5·77
Total monounsaturates	0·98	1·01	1·09	0·94	1·08	0·96	0·65	0·48	7·00
Total polyunsaturates	13·49	13·96	17·65	12·09	12·58	15·14	4·21	3·38	4·44
Total fatty acids	18·94	19·33	23·19	17·27	17·95	20·39	8·68	7·53	17·21
Fat-soluble vitamins, mg/kg DM									
α-tocopherol	ND	ND	ND	53·8	67·8	60·5	ND	22·3	20·3
γ-tocopherol	ND	ND	ND	10·8	19·6	17·6	ND	9·4	11·8
β-carotene	ND	ND	ND	312	335	302	ND	59	0·00

ND, not determined

† Refers to cut grass wilted for 6 h prior to chopping with a forage harvester and application of no additive (NA), an inoculant enzyme preparation (IE) or a formic acid based ensiling additive (FORM)

‡ Refers to silages prepared using no additive (NA), an inoculant enzyme preparation (IE) or a formic acid based ensiling additive (FORM)

§ Composition of hay at the time of baling or after barn drying with ambient air for 7d

¶ Oven DM corrected for loss of volatiles according to Huida et al. (1986)

from 1 (poor) to 5 (excellent) according to reference procedures (IDF 50C:1995; IDF 99C:1997; International Dairy Federation, Brussels, Belgium). The overall mean ($n=6$) assessment of each sample was used for statistical analysis.

Statistical Analysis

Results were analysed by ANOVA using the General Linear Model procedure (PROC GLM) of Statistical Analysis Systems Institute (SAS®, 1989). The statistical model included the effects of cow, period, treatment and treatment carry-over. Sums of squares for treatment effects were further separated using orthogonal contrasts into single degree of freedom comparisons as: C_1=effect of drying *v.* ensiling (hay *v.* NA, IE and FORM), C_2=comparison of treated *v.* untreated silages (NA *v.* IE and FORM), C_3=effect of biological *v.* chemical additive (IE *v.* FORM), C_4=concentrate level (L *v.* H) and C_5=effect of PG supplementation (−PG *v.* +PG). Relationships between experimental variables were evaluated by Pearson correlation coefficients and regression analysis using SAS®. Least square means are reported and treatment effects were considered significant at $P<0·05$.

Results

Feed composition

Application of a formic acid based additive to prewilted chopped grass resulted in herbage at the time of ensiling that contained higher amounts of unsaturated fatty acids compared with herbage treated with no additive or an inoculant enzyme preparation (Table 1). Irrespective of additive treatment, all silages contained lower amounts of fatty acids than the prewilted chopped grass used for ensiling (Table 1). Use of a relatively high level of formic acid or an inoculant enzyme preparation to control secondary fermentation, resulted in silage containing more tocopherol than untreated silage (Table 1). Conservation of

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Table 2. Effect of forage conservation on dry matter intake (DMI) and the intakes of fatty acids and fat-soluble vitamins

Least square means and SEM for $n=16$

	Treatment†					Significance§		
	Hay	NA	IE	FORM	SEM‡	C_1	C_2	C_3
Forage DMI, kg/d	12·01	12·78	12·66	13·02	0·14	**		
Total DMI, kg/d	19·64	20·41	20·30	20·65	0·14	**		
Fatty acids, g/d								
12 : 0	0·9	1·6	1·5	1·6	0·05	***		
14 : 0	1·4	1·8	1·8	1·9	0·03	***		
16 : 0	71·6	80·0	80·3	81·4	0·75	***		
16 : 1 *cis*-9	1·0	1·2	1·5	1·3	0·03	***	***	***
18 : 0	6·2	6·7	7·0	6·9	0·08	***	*	
18 : 1 *cis*-9	50·1	54·1	55·8	55·2	0·45	***		
18 : 1 *cis*-11	6·1	7·1	7·4	7·1	0·07	***		*
18 : 2 *n*-6	44·6	67·7	70·1	76·6	0·94	***	**	**
18 : 3 *n*-3	35	120	127	161	3·6	***	**	***
20 : 0	2·7	2·3	2·2	2·4	0·03	***		*
20 : 1 *cis*-11	1·2	1·3	1·2	1·3	0·01	*		*
22 : 0	3·7	3·3	3·1	3·4	0·06	***		*
24 : 0	2·3	2·4	2·3	2·4	0·05			
24 : 1 *cis*-15	0·18	0·62	0·57	0·71	0·019	***		**
Total saturates	86·1	95·8	95·8	97·6	1·07	***		
Total monounsaturates	58·9	64·6	66·8	65·9	0·52	***		
Total polyunsaturates	79	188	197	237	4·5	***	**	***
Total fatty acids	227	350	362	403	5·9	***	**	**
Fat-soluble vitamins, mg/d								
α-tocopherol	375	824	1055	943	33·4	***	**	
γ-tocopherol	191	226	348	316	7·8	***	***	
β-carotene	547	3921	4287	3942	86·2	***		

† Refers to silages prepared using no additive (NA), an inoculant enzyme preparation (IE) or a formic acid based ensiling additive (FORM)
‡ Standard error of the mean; degrees of freedom of error 15
§ Significance of single degree of freedom orthogonal contrasts comparing hay with silage based diets (C_1), untreated *v.* treated silages (C_2) and inoculant enzyme *v.* formic acid treated silages (C_3). *$P<0·05$, **$P<0·01$, ***$P<0·001$

grass by drying rather than ensiling resulted in marked reductions in the concentration of fatty acids and fat-soluble vitamins in hay compared with silages. Barn drying of baled hay was associated with small oxidative losses of unsaturated fatty acids (Table 1).

Intake

Cows consumed all the concentrate and PG supplements fed. Owing to improved forage DM intakes and higher concentrations in ensiled grass, fatty acid and fat-soluble vitamin intake was higher ($P<0·001$) for silage than for hay diets (Table 2). Compared with untreated silage, use of an ensiling additive enhanced ($P<0·05$) the intakes of 18 : 2 *n*-6, 18 : 3 *n*-3, total fatty acids, α-tocopherol and β-carotene (Table 2). Ingestion of 18 : 2 *n*-6 and 18 : 3 *n*-3 were further increased ($P<0·05$) when grass was ensiled using a formic acid based additive rather than an inoculant enzyme preparation, whilst the intakes of *cis*-9 16 : 1, 18 : 0, α-tocopherol and β-carotene were marginally, but significantly higher ($P<0·05$) for diets based on IE than FORM silage.

Increases in concentrate supplementation increased ($P<0·05$) total DM intake, but caused a mean reduction in forage DM intake of 1·11 kg/d (Table 3). Improvements in total DM intake due to increases in concentrate supplementation for silage diets depended ($P<0·05$) on the use of an ensiling additive (Table 3), while DM intake responses to concentrates were higher ($P<0·05$) in the absence than in the presence of PG (mean 2·05 and 1·05 kg/d, for −PG and +PG, respectively). Feeding higher amounts of concentrates increased ($P<0·05$) 16 : 0, 18 : 0, *cis*-9 18 : 1 and 18 : 2 *n*-6 consumption, but had only minor effects on the intake of fat-soluble vitamins (Table 3). Supplements of PG caused a small, but significant ($P<0·05$) increase in total DM intake, but had no effect ($P>0·05$) on fatty acid or fat-soluble vitamin intake (Table 3).

Milk fatty acid composition

Forage conservation method had no effect ($P>0·05$) on milk yield or milk fat content (Table 4). Compared with silage, milk from hay contained higher ($P<0·05$) concentrations

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Table 3. Effect of level of concentrate and propylene glycol in the diet on the intake of dry matter (DMI), fatty acids and fat-soluble vitamins

Least square means and SEM for $n=32$

	Treatment†					Significance§		
	L	H	−PG	+PG	SEM‡	C_4	C_5	Interactions
Forage DMI, kg/d	13·17	12·06	12·53	12·70	0·10	***		$C_2 \times C_5$*, $C_4 \times C_5$*
Total DMI, kg/d	19·48	21·03	20·06	20·45	0·10	***	*	$C_2 \times C_5$*, $C_4 \times C_5$*
Fatty acids, g/d								
12 : 0	1·5	1·3	1·4	1·4	0·03			
14 : 0	1·7	1·7	1·7	1·7	0·02			
16 : 0	73·4	83·2	78·0	78·7	0·53	***		
16 : 1 *cis*-9	1·1	1·3	1·2	1·2	0·02	**		
18 : 0	6·2	7·2	6·7	6·7	0·06	***		
18 : 1 *cis*-9	46·3	61·3	53·6	53·9	0·32	***		
18 : 1 *cis*-11	6·0	7·8	6·9	6·9	0·05	***		
18 : 2 *n*-6	60·7	68·8	64·3	65·2	0·66	***		
18 : 3 *n*-3	114	107	109	112	2·6			
20 : 0	2·3	2·4	2·4	2·4	0·02			
20 : 1 *cis*-11	1·1	1·4	1·2	1·2	0·01	***		
22 : 0	3·4	3·3	3·3	3·4	0·04			
24 : 0	2·3	2·4	2·3	2·4	0·04			
24 : 1 *cis*-15	0·50	0·54	0·52	0·53	0·014			
Total saturates	88·5	99·2	93·4	94·3	0·687	***		
Total monounsaturates	55·3	72·8	63·9	64·2	0·37	***		
Total polyunsaturates	175	176	173	177	3·209			
Total fatty acids	321	350	333	338	4·2	**		
Fat-soluble vitamins, mg/d								
α-tocopherol	792	806	791	808	23·6			
γ-tocopherol	261	280	269	272	5·5			
β-carotene	3282	3066	3118	3231	60·9			

† Treatments L and H refer to concentrate supplements of 7 and 10 kg (air dry basis)/d, respectively. Treatments −PG and +PG refer to daily propylene glycol supplements of 0 and 210 g/d, respectively

‡ Standard error of the mean; degrees of freedom of error 15

§ Significance of single degree of freedom orthogonal contrasts comparing the level of concentrate (C_4) and propylene glycol in the diet (C_5) and interactions with orthogonal contrasts comparing forage conservation methods (refer to Table 2). *$P<0·05$, **$P<0·01$, ***$P<0·001$

of odd-, branched-chain and PUFA (Table 4). Increases in the PUFA content for hay diets was associated with a higher ($P<0·001$) apparent transfer of 18 : 2 *n*-6 and 18 : 3 *n*-3 from diet to milk (Table 4). Use of an ensiling additive increased ($P<0·05$) milk fat *trans* 18 : 1 content and lowered ($P<0·05$) 15 : 0 concentrations, while milk from FORM silage contained higher ($P<0·05$) levels of *trans* 18 : 1 and CLA and reduced ($P<0·05$) concentrations of 4 : 0 and 18 : 3 *n*-3 than did IE silage (Table 4). Increases in levels of concentrate or PG did not affect ($P>0·05$) milk yield or milk fat content (Table 5). Feeding more concentrates decreased ($P<0·001$) milk fat 16 : 0 concentrations and increased ($P<0·05$) 20 : 3 *n*-6 content, while supplements of PG enhanced ($P<0·05$) the levels of 13 : 0 *anteiso*, 15 : 0, 20 : 3 *n*-6 and total PUFA in milk (Table 5).

Milk organoleptic properties

Taste panel scores showed forage conservation method to have no effect ($P>0·05$) on the organoleptic properties of milk (mean scores 3·80, 4·02, 3·94 and 3·88; SEM 0·072 for hay, NA, IE and FORM silages, respectively). Increases in level of concentrate level or PG also had no effect ($P>0·05$) on taste panel scores (mean 3·91, 3·91, 3·90 and 3·92; SEM 0·051 for L, H, −PG and +PG, respectively).

Milk vitamin content

Relative to silages, milk from hay diets contained lower ($P<0·05$) amounts of riboflavin, α-tocopherol and β-carotene, but the concentrations of ascorbic acid were independent of forage conservation method (Table 6). Use of an ensiling additive enhanced ($P<0·05$) milk thiamine content, while concentrations of α-tocopherol and β-carotene were higher ($P<0·05$) in milk from IE than from FORM silage. Increases in concentrate had no effect ($P>0·05$) on the concentration or secretion of vitamins in milk, while PG supplements decreased ($P<0·05$) milk ascorbic acid content (Table 7). Across all diets a close

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Table 4. Effect of forage conservation method on milk fatty acid composition

Least square means and SEM for n = 16

	Treatment †					Significance§		
	Hay	NA	IE	FORM	SEM‡	C_1	C_2	C_3
Milk yield, kg/d	27·9	28·2	28·6	27·9	0·28			
Milk fat, g/kg	42·4	43·3	43·5	45·8	0·66			
Fatty acid composition, g/kg fatty acids								
4 : 0	25·1	28·9	29·4	25·8	0·69	*		*
6 : 0	21·6	22·3	23·4	22·1	0·51			
8 : 0	14·7	14·9	15·3	15·0	0·35			
10 : 0	34·1	33·1	34·3	34·3	0·75			
12 : 0	39·7	37·9	39·0	39·9	0·71			
12 : 1 *cis*-11	1·25	1·13	1·11	1·35	0·045	*		
13 : 0 *iso*	1·44	1·35	1·17	1·23	0·060			
13 : 0 *anteiso*	1·24	1·27	1·00	1·05	0·063		*	
14 : 0	133	129	131	132	1·2			
14 : 0 *iso*	1·79	1·29	1·22	1·34	0·053	***		
14 : 1 *cis*-9	12·4	11·9	11·8	12·4	0·38			
15 : 0	12·2	12·4	11·5	11·7	0·19		*	
15 : 0 *iso*	3·04	2·35	2·39	2·35	0·073	***		
15 : 0 *anteiso*	5·73	4·60	4·51	4·73	0·070	***		
16 : 0	345	347	338	342	2·7			
16 : 1 *cis*-9	18·0	18·1	17·7	17·9	0·38			
17 : 0	7·66	7·17	6·75	7·14	0·108	**		
17 : 0 *iso*	1·55	1·43	1·50	1·45	0·033			
17 : 0 *anteiso*	4·31	3·54	3·65	3·76	0·050	***		
17 : 1 *cis*-10	2·03	2·92	2·36	2·33	0·127	*	*	
18 : 0	91·7	97·5	100	100	1·49	**		
18 : 0 *iso*	1·28	1·34	1·40	1·42	0·076			
18 : 1 *cis*-9 ¶	152	151	153	145	2·0			
18 : 1 *cis*-11	4·17	2·81	3·10	3·24	0·185	**		
18 : 1 *trans* ††	37·8	36·2	37·1	42·5	0·88		*	*
18 : 1 total	186	184	187	184	2·14			
18 : 2 *cis*-9, *cis*-12	12·1	9·61	9·61	9·31	0·33	***		
18 : 2 *trans*-9, *trans*-12	1·39	1·82	1·92	1·91	0·053	***		
CLA	4·46	4·13	4·10	4·90	0·152			*
18 : 3 *n*-3	5·00	3·51	4·27	2·93	0·108	***		***
20 : 0	1·80	1·33	1·47	1·44	0·095	*		
20 : 1 *cis*-11	1·76	1·35	1·73	1·76	0·090		*	
20 : 2 *n*-6	0·72	1·14	0·57	1·00	0·123			
20 : 3 *n*-6	0·11	0·32	0·39	0·16	0·074			
20 : 4 *n*-6	0·76	0·49	0·58	0·47	0·104			
20 : 5 *n*-3	0·26	0·31	0·11	0·14	0·066			
22 : 0	1·57	1·08	1·16	1·08	0·081	**		
24 : 0	0·61	0·70	0·26	0·42	0·117			
Total ⩽C14	289	286	291	289	3·9			
Total saturates	743	745	743	746	3·1			
Total monounsaturates	228	226	227	224	2·6			
Total polyunsaturates	29·3	25·6	25·9	24·8	0·60	**		
Total fatty acids, g/kg fat	871	864	866	882	0·4			
Ratio								
14 : 1 *cis*-9/14 : 0	0·094	0·093	0·090	0·095	0·0032			
16 : 1 *cis*-9/16 : 0	0·052	0·052	0·052	0·052	0·0011			
18 : 1 *cis*-9/18 : 0	1·696	1·576	1·547	1·473	0·0231	**		
Transfer [1]								
18 : 2 *n*-6	0·293	0·150	0·150	0·140	0·0060	***		
18 : 3 *n*-3	0·172	0·031	0·042	0·027	0·0033	***		

†,‡ and § refer to Table 2

¶ Concentrations corrected for *trans*-12 to *trans*-16 18 : 1 content

†† Total *trans* 18 : 1 fatty acids concentrations predicted based on the sum of measured *trans*-6 to *trans*-11 18 : 1 and 16 : 0 content according to Molkentin & Precht (1995)

[1] Apparent transfer from the diet into milk. *P<0·05, **P<0·01, ***P<0·001. CLA, total conjugated linoleic acid content

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Table 5. Effect of level of concentrate and propylene glycol in the diet on milk fatty acid composition

Least square means and SEM for $n=32$

	Treatment †					Significance§		
	L	H	−PG	+PG	SEM ‡	C_4	C_5	Interactions
Milk yield, kg/d	28·1	28·9	28·2	28·8	0·20			
Milk fat, g/kg	43·9	43·6	44·4	43·0	0·47			
Fatty acid composition, g/kg fatty acids								
4 : 0	26·8	27·8	27·7	26·9	0·49			
6 : 0	22·3	22·4	22·5	22·2	0·36			
8 : 0	14·9	15·0	14·9	14·9	0·25			
10 : 0	33·8	34·1	33·6	34·3	0·53			
12 : 0	38·6	39·6	38·6	39·6	0·50			
12 : 1 *cis*-11	1·21	1·21	1·17	1·25	0·032			
13 : 0 *iso*	1·24	1·36	1·31	1·29	0·042			$C_3 \times C_5$*
13 : 0 *anteiso*	1·04	1·25	1·06	1·22	0·044		*	$C_3 \times C_5$**
14 : 0	132	130	132	131	0·8			
14 : 0 *iso*	1·46	1·37	1·41	1·41	0·037			
14 : 1 *cis*-9	12·2	12·0	12·3	11·9	0·27			
15 : 0	12·2	11·7	11·3	12·6	0·13		***	
15 : 0 *iso*	2·63	2·43	2·51	2·55	0·052			
15 : 0 *anteiso*	4·86	4·93	4·91	4·88	0·050			
16 : 0	348	338	343	343	1·94	***		$C_3 \times C_5$*
16 : 1 *cis*-9	18·2	17·6	17·9	17·9	0·27			
17 : 0	7·30	7·07	7·12	7·25	0·076			$C_3 \times C_5$*
17 : 0 *iso*	1·48	1·47	1·50	1·46	0·023			
17 : 0 *anteiso*	3·83	3·80	3·87	3·77	0·035			$C_1 \times C_4$*
17 : 1 *cis*-10	2·44	2·38	2·51	2·31	0·090			
18 : 0	95·3	99·3	98·6	96·0	1·05			
18 : 0 *iso*	1·38	1·34	1·40	1·32	0·054			
18 : 1 *cis*-9 ¶	147	153	150	150	1·4			
18 : 1 *cis*-11	3·01	3·65	3·43	3·23	0·131			
18 : 1 *trans* ††	39·5	37·3	38·6	38·2	0·62			
18 : 1 total	184	187	185	185	1·52			
18 : 2 *cis*-9, *cis*-12	9·8	10·6	10·1	10·3	0·24			
18 : 2 *trans*-9, *trans*-12	1·70	1·81	1·71	1·81	0·038			$C_1 \times C_4$*; $C_2 \times C_4$*
CLA	4·45	4·34	4·42	4·38	0·108			
18 : 3 *n*-3	4·06	3·80	3·80	4·05	0·076			
20 : 0	1·56	1·45	1·50	1·52	0·067			
20 : 1 *cis*-11	1·67	1·63	1·66	1·64	0·064			$C_1 \times C_4$*; $C_2 \times C_4$*; $C_3 \times C_4$*
20 : 2 *n*-6	0·81	0·90	0·78	0·93	0·087			
20 : 3 *n*-6	0·07	0·42	0·15	0·34	0·052	*	*	$C_4 \times C_5$*
20 : 4 *n*-6	0·48	0·67	0·57	0·58	0·074			
20 : 5 *n*-3	0·26	0·16	0·23	0·18	0·047			
22 : 0	1·32	1·12	1·23	1·21	0·057			
24 : 0	0·36	0·63	0·44	0·55	0·082			
Total ⩽C14	289	289	289	288	2·8			
Total saturates	747	741	745	743	2·2			
Total monounsaturates	224	228	226	226	1·8			
Total polyunsaturates	25·7	27·1	25·6	27·2	0·42		*	
Total fatty acids (g/kg fat)	871	870	870	871	0·3			
Ratio								
14 : 1 *cis*-9/14 : 0	0·093	0·093	0·094	0·092	0·0023			
16 : 1 *cis*-9/16 : 0	0·052	0·052	0·052	0·052	0·0008			$C_2 \times C_5$*
18 : 1 *cis*-9/18 : 0	1·572	1·574	1·558	1·588	0·0163			
Transfer[1]								
18 : 2 *n*-6	0·187	0·179	0·183	0·183	0·0042			
18 : 3 *n*-3	0·067	0·069	0·066	0·071	0·0024			

†, ‡ and § refer to Table 3

¶ Concentrations corrected for *trans*-12 to *trans*-16 18 : 1 content

†† Total *trans* 18 : 1 fatty acids concentrations predicted based on the sum of measured *trans*-6 to *trans*-11 18 : 1 and 16 : 0 content according to Molkentin & Precht (1995)

[1] Apparent transfer from the diet into milk. *$P<0·05$, **$P<0·01$, ***$P<0·001$. CLA, total conjugated linoleic acid content

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Table 6. Effect of forage conservation method on the concentration and secretion of vitamins in milk

Least square means and SEM for n = 16

	Treatment†					Significance§		
	Hay	NA	IE	FA	SEM‡	C_1	C_2	C_3
Concentration, mg/kg milk								
Ascorbic acid	15·6	15·7	15·6	15·6	0·45¶			
Thiamine	0·27	0·26	0·29	0·30	0·006		**	
Pyridoxine	0·33	0·36	0·34	0·36	0·010			
Riboflavin	1·13	1·48	1·51	1·53	0·036	***		
α-Tocopherol	0·54	1·14	1·15	1·10	0·033	***		
β-Carotene	0·14	0·22	0·26	0·19	0·008	***		**
Retinol	0·26	0·28	0·33	0·24	0·016			*
Yield, mg/d								
Thiamine	7·57	7·54	8·30	8·88	0·167	*	**	
Pyridoxine	9·10	10·07	9·68	10·46	0·304			
Riboflavin	31·16	41·42	43·06	44·94	0·748	***		
α-Tocopherol	15·45	31·47	33·42	32·21	1·013	***		
β-Carotene	3·92	6·08	7·42	5·59	0·293	***		**
Retinol	7·51	7·89	9·45	7·22	0·504			*

†,‡ and § refer to Table 2
¶ degrees of freedom of error 21
* $P<0·05$, ** $P<0·01$, *** $P<0·001$

linear relationship existed between the intake and secretion of α-tocopherol:

α-tocopherol in milk (mg/d) = 5·57 ± 1·935 + 0·028 ± 0·0023 × α-tocopherol intake (mg/d) (n = 16, r^2 = 0·91; $P<0·001$)

Discussion

Forages are often the main source of fatty acids in the diet (Harfoot & Hazlewood, 1988), but the effects of conservation method on milk fatty acid composition are not well defined (Chilliard et al. 2001). Exposure to solar radiation and the duration of wilting are the most important factors affecting oxidative losses of unsaturated fatty acids from cut grass (Dewhurst & King, 1998; Dewhurst et al. 2002), while ensiling method also affects the fatty acid content of grass silages (Dewhurst & King, 1998; Boufaïed et al. 2003a). In the present experiment, hay contained lower amounts of all measured fatty acids than silages, but the comparison between drying and ensiling conservation methods is confounded by the 7-d delay in harvesting. Advancing maturity is often reported to reduce the lipid content of fresh grasses (Dewhurst et al. 2001; Elgersma et al. 2003), but the extent to which the delay in harvesting contributed to the lower fatty acid content of hay compared with silage in this experiment remains unclear. However, direct comparisons show that wilting or drying of timothy causes more extensive losses of 18 : 2 *n*-6 and 18 : 3 *n*-3 than direct ensiling (Boufaïed et al. 2003a).

Most reports of the impact of ensiling on forage lipids are based on the comparison of fresh herbage and the corresponding silage (Dewhurst & King, 1998; Boufaïed et al. 2003a; Elgersma et al. 2003; Whiting et al. 2004) and it remains unclear whether the lower amounts of unsaturated fatty acids reported for silages is due to oxidative processes initiated by the cutting of herbage or whether they arise from losses incurred during the ensiling process. Anaerobic conditions are rapidly established in well-compacted and tightly sealed silos, and therefore fatty acid peroxidation is assumed to be arrested soon after ensiling (Dewhurst & King, 1998). In the present experiment, prewilted chopped grass, irrespective of the additive treatment applied, contained higher amounts of unsaturated fatty acids than the corresponding silage, with the implication that at least a proportion of unsaturated fatty acids are lost during the ensiling process. Losses of 18 : 3 *n*-3, which is the PUFA in grass that is most susceptible to peroxidation, were similar across treatments, suggesting that oxidative losses of unsaturated fatty acids during ensiling are not substantially altered by the type or extent of silage fermentation. The higher PUFA content of chopped prewilted grass treated with a formic acid based additive may relate to a more rapid decrease in herbage pH resulting in partial or complete inhibition of indigenous plant or microbial lipases. Ensiling with formic acid results in marginally higher fatty acid concentrations in laboratory-scale silages prepared from perennial ryegrass (Dewhurst & King, 1998), but this is not substantiated in studies with timothy grasses (Boufaïed et al. 2003a).

Despite the lower intake, milk from hay contained more 18 : 2 *n*-6 and 18 : 3 *n*-3 than that from silage diets. Even though unsaturated fatty acids in the diet are extensively metabolized in the rumen (Harfoot & Hazlewood, 1988;

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Table 7. Effect of level of concentrate and propylene glycol in the diet on the concentration and secretion of vitamins in milk

Least square means and SEM for $n=32$

	Treatment †					Significance§		
	L	H	−PG	+PG	SEM‡	C_4	C_5	Interactions
Concentration, mg/kg milk								
Ascorbic acid	ND	ND	16·1	15·1	0·32¶		*	
Thiamine	0·29	0·27	0·29	0·27	0·005			
Pyridoxine	0·35	0·34	0·35	0·34	0·007			$C_1 \times C_4$*
Riboflavin	1·46	1·37	1·41	1·42	0·025			$C_3 \times C_4$*
α-Tocopherol	0·97	1·00	0·97	1·00	0·023			
β-Carotene	0·21	0·20	0·20	0·21	0·006			
Retinol	0·27	0·28	0·28	0·28	0·012			
Yield, mg/d								
Thiamine	8·26	7·89	8·20	7·94	0·118			
Pyridoxine	9·84	9·82	9·83	9·83	0·215			$C_1 \times C_4$*
Riboflavin	40·80	39·49	39·68	40·61	0·529			$C_3 \times C_4$*
α-tocopherol	27·37	28·90	27·39	28·88	0·716			
β-carotene	5·80	5·70	5·51	5·99	0·207			
Retinol	7·73	8·30	7·81	8·22	0·356			

ND, not determined
†,‡ and § refer to Table 2
¶ degrees of freedom of error 21
* $P<0·05$, ** $P<0·01$, *** $P<0·001$

Doreau & Ferlay, 1994), a small proportion escapes and can be incorporated into milk triacylglycerides. The differences in milk 18 : 2 *n*-6 and 18 : 3 *n*-3 content are consistent with more PUFA leaving the rumen for hay than for silage diets. Most of the lipid in fresh grass is in the form of phospholipids and glycolipids located within thylakoid membranes of chloroplasts, such that the cell wall has to be ruptured through mastication and microbial digestion before lipolysis and biohydrogenation can take place. Lipolysis is believed to be rate-limiting for biohydrogenation (Harfoot & Hazlewood, 1988), but lipids in grass are also hydrolysed to a varying extent during ensiling (Elgersma et al. 2003). Incubations *in vitro* show that the rate and extent of 18 : 2 *n*-6 and 18 : 3 *n*-3 biohydrogenation is higher for ensiled than for dried grass (Boufaïed et al. 2003b), while lipolysis and biohydrogenation of PUFA in ryegrass are reduced by advances in maturity (Gerson et al. 1986). Particle size is also known to affect bacterial colonization and lipolysis of lipids in dried grass (Gerson et al. 1988). In the present experiment, it is possible that changes in the form of lipid ingested, differences in forage particle size distribution in the rumen and variations in grass maturity due to the delay in harvesting all contributed to the effects of forage conservation method on milk PUFA content.

Conservation method had no clear effects on milk fat *trans* 18 : 1 and CLA concentrations. Differences in milk *trans*-18 : 1 and total CLA content between hay and silage diets were much smaller than would be expected based on 18 : 2 *n*-6 and 18 : 3 *n*-3 intakes. Earlier studies show the rate of 18 : 0 formation during incubations with mixed rumen bacteria *in vitro* to be higher for silage than for hay prepared from the same grass (Boufaïed et al. 2003b), which suggests that both lipolysis and biohydrogentation of forage lipids are lower when grass is dried rather than ensiled. Ensiling method resulted in differences in the intake of unsaturated fatty acids, but had no consistent effects on the concentration of *trans* 18 : 1, 18 : 2 *n*-6, CLA or 18 : 3 *n*-3 in milk. Rates of disappearance of 18 : 2 *n*-6 and 18 : 3 *n*-3 during incubations with mixed rumen bacteria *in vitro* are higher for formic acid than for untreated or inoculant treated silage, but the rate of accumulation of 18 : 0 is lower (Boufaïed et al. 2003b).

Increases in the proportion of concentrates in the diet typically have negligible effects on milk fat content of 4 : 0, but generally decrease 6 : 0–16 : 0 and enhance C18 concentrations (Palmquist et al. 1993). In the present experiment, increases in the amount of concentrates led to small increases in 4 : 0–12 : 0, 18 : 0, cis-9 18 : 1 concentrations, minor decreases in 14 : 0 and a significant reduction in milk fat 16 : 0 content, changes that are consistent with responses to increased concentrate supplementation from 4 to 8 kg/d for diets based on ryegrass silage (Dewhurst et al. 2003). Synthesis *de novo* in the mammary gland accounts for all 4 : 0–12 : 0, most of the 14 : 0 and approximately half of the 16 : 0 secreted in milk, while the remaining 16 : 0, and all C18 and longer-chain fatty acids are derived entirely from circulating blood lipids (Lock & Shingfield, 2004). The reductions in milk fat 16 : 0 content in response to increased concentrate supplementation

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occurred in spite of higher 16 : 0 intake, with no evidence of a significant reduction in synthesis of 12 : 0 or 14 : 0. It is possible that the decreases in milk fat 16 : 0 concentration related to changes in the lipid metabolism of rumen bacteria due to increased concentrate supplementation. Recent studies of rumen bacteria isolated from duodenal digesta of goats fed at maintenance show that decreases in the forage:concentrate ratio of the diet lower the proportion and amount of 16 : 0 in bacterial lipids (Bas et al. 2003).

Increasing the amount of concentrates reduces the rate of lipolysis *in vitro* (Gerson et al. 1985) and inhibits ruminal biohydrogenation of dietary unsaturated fatty acids to 18 : 0, causing an increase in amount of *trans* 18 : 1 at the duodenum (Kalscheur et al. 1997; Loor et al. 2004) and in milk (Gaynor et al. 1995; Grinnari et al. 1998), but there was no evidence of such changes in the present experiment. Forage to concentrate ratio also affects the profile of *trans* 18 : 1, and increases in concentrates often shift ruminal biohydrogenation towards formation of *trans*-10 18 : 1 in the rumen (Piperova et al. 2002; Loor et al. 2004) and enhance levels of this isomer in milk (Griinari et al. 1998; Piperova et al. 2002). Mammary conversion of *trans*-11 18 : 1 is thought to be the main source of *cis*-9, *trans*-11 CLA in milk (Griinari et al. 2000) and a direct relationship exists between the concentrations of these fatty acids in milk fat (Piperova et al. 2002; Shingfield et al. 2003). Individual *trans* 18 : 1 and isomers of CLA in milk are not resolved by the analytical techniques used in this experiment, but a close association between the concentration of total CLA and total *trans* 18 : 1 in milk across all diets {[CLA (g/kg fatty acids)] = 0·35 (SE 0·506) + 0·18 (SE 0·022) [*trans* C18 : 1 (g/kg fatty acids)], $r = 0{\cdot}907$, $n = 16$, $P < 0{\cdot}001$] suggests that increases in concentrate supplementation did not cause substantial changes in milk *trans* 18 : 1 profile.

Supplements of PG resulted in a mean increase in the molar proportion of propionate from 170 to 178 mmol/mol (Shingfield et al. 2002) consistent with PG being metabolized, at least in part, in the rumen (Nielsen & Ingvartsen, 2004). Propionate is a substrate for synthesis of odd-chain fatty acids *de novo* by rumen bacteria (Jenkins, 1993), which would account for PG supplements enhancing milk fat 13 : 0 *anteiso* and 15 : 0 concentrations and causing a small increase in 15 : 0 *iso* and 17 : 0 content. Ruminal infusions of propionate can also increase milk fat 13 : 0, 15 : 0 and 17 : 0 concentrations (Rigout et al. 2003) providing further evidence of propionate utilization for odd-chain fatty acid synthesis by rumen bacteria.

For all diets, the output of C18 fatty acids in milk exceeded ingestion, but the net synthesis was not related to concentrate level or PG supplements, but was significantly ($P < 0{\cdot}001$) higher for hay than for silage diets (−178, −74, −71 and −52 g/d, for hay, NA, IE and FA silages, respectively). For high-forage diets, the flow of 18 : 0 and total C18 fatty acids in the duodenum often exceeds intake, with the net C18 fatty acid synthesis being higher than for low-forage diets, irrespective of the lipid content of concentrate supplements (Doreau & Ferlay, 1994). In goats, the proportion and content of 18 : 0 in microbial lipid is higher when maize stover rather than lucerne hay is fed alone or with concentrates (Bas et al. 2003), and it is possible that drying rather than ensiling grass has similar effects.

Even though transport of α-tocopherol and β-carotene from plasma lipoproteins into the mammary gland conforms to Michaelis-Menten kinetics and is breed-dependent (Jensen et al. 1999), concentrations in milk are thought to be a function of dietary consumption (Weiss, 1998). In the present experiment, secretion of α-tocopherol in milk was related to dietary intake and it was transferred into milk with an mean efficiency of 2·8%. Secretion of β-carotene in milk was also related to intake, but it was transferred at a much lower efficiency of 0·07%. This lower transfer of β-carotene from diet into milk can be attributed in part to more extensive ruminal catabolism and conversion of absorbed β-carotene to retinol (Weiss, 1998), but also reflects the lower maximal secretory capacity of β-carotene than α-tocopherol (Jensen et al. 1999). The effect of diet on milk α-tocopherol and β-carotene concentrations reflected more extensive losses of these vitamins during the drying than during the ensiling of grass. Hidiroglou et al. (1994) also report higher concentrations of α-tocopherol in silage than in hay (35 *v.* 15 mg/kg DM) prepared from the same mixed timothy-lucerne swards. Losses of α-tocopherol during the drying or ensiling of grass are known to vary between 20 and 80% depending on the exposure to incident radiation (Weiss, 1998). Earlier reports show the levels of water-soluble vitamins in milk to be relatively constant throughout the year (Laukkanen et al. 1988), but in the present work riboflavin concentrations were lower for hay than for silage diets, while PG supplements reduced milk ascorbic acid content.

The changes in milk fatty acid compostion and vitamin content have several implications for nutritient content, organoleptic attributes and storage properties of milk. Consumption of milk or dairy products from the hay diets fed in this experiment would supply lower amounts of riboflavin, α-tocopherol and β-carotene and higher amounts of PUFA compared with those from the silage diets. Even though taste panel scores were comparable across diets, it is important to recognize that these assessments were made on fresh samples and do not give an indication of the longer-tem storage characteristics of milk. The shelf-life of milk and dairy products depends on complex interactions between pro- and anti-oxidative processes that are influenced by the degree of fatty acid unsaturation, concentration of transition metal cations and levels of anti-oxidants (Barrefors et al. 1995; Granelli et al. 1998; Timmons et al. 2001; Havemose et al. 2004). High concentrations of α-tocopherol in milk are associated with a reduction in the development of spontaneous oxidized flavour (St-Laurent et al. 1990; Charmley et al. 1993; Barrefors et al. 1995), while enriching 18 : 2 *n*-6 and 18 : 3 *n*-3 contents increases the susceptibility of milk to

oxidation (Barrefors et al. 1995; Granelli et al. 1998; Timmons et al. 2001). Furthermore, β-carotene is a scavenger of singlet oxygen and peroxyl radicals and ascorbic acid is thought to be involved in the regeneration of tocopherol radicals produced during reactions with lipid free radicals (Timmons et al. 2001). Even though no significant effects on taste panel scores were detected in this experiment, it is probable that milk from hay diets, which contained higher PUFA concentrations and lower levels of α-tocopherol and β-carotene, would be more susceptible to oxidation and the development of off-flavours compared with that from silage diets. It is also possible that PG supplements, which lowered the concentrations of ascorbic acid and enhanced milk fat PUFA content, may also have compromised the oxidative stability and organoleptic properties of milk and dairy products.

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