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Original article

Enhancement of oleic acid and vitamin E concentrations of bovine milk using dietary supplements of whole rapeseed and vitamin E

D. Ian GIVENS[a]*, Richard ALLISON[b], Jonathon S. BLAKE[b]

[a] Nutritional Sciences Research Unit, School of Agriculture, Policy and Development & ADAS, The University of Reading, Earley Gate, PO Box 237, Reading RG6 6AR, UK
[b] ADAS Bridgets Dairy Research Centre, Martyr Worthy, Winchester SO21 1AP, UK

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Abstract — With the aim of reducing the degree of saturation and increasing the C18:1 *cis* fatty acid content of milk fat, the effects of feeding high levels of whole cracked rapeseed to dairy cows was investigated together with the effect of increasing dietary intake of vitamin E on the vitamin E content of milk. Using a 3 × 3 factorial design, 90 Holstein dairy cows were fed one of three levels of whole cracked rapeseed (0 (ZR), 134 (MR) and 270 g·kg^{-1} diet dry matter (DM) (HR)) in combination with one of three intakes of supplementary vitamin E (0 (ZE), 2 (ME) and 4 g·cow^{-1}·d^{-1} (HE)). Supplementing with up to almost 2 kg·d^{-1} of rapeseed oil (diet HR) significantly ($P < 0.001$) increased C18:1*cis* in milk fat, from 181 (ZR) to over 400 g·kg^{-1} (HR) of total milk fatty acids. Concentrations of C18:0, C18:2 and C18:3 fatty acids were also increased ($P < 0.001$) but by a much lesser degree, and the saturated fatty acids C4:0 to C16:0 decreased substantially. Vitamin E supplementation increased ($P < 0.01$) milk vitamin E concentrations from 1.29 (ZE) to 1.68 mg·kg^{-1} whole milk (HE). Thus substantial changes in milk fat composition with potentially beneficial effects on human health were achieved and without any adverse effects on milk taste. However, these improvements must be offset against the substantial reductions ($P < 0.001$) observed in voluntary feed DM consumption (ZR, 20.6; HR, 15.2 kg DM·d^{-1}), milk yield (ZR, 22.9; HR, 13.2 kg·d^{-1}) and milk fat concentration (ZR, 42.1; HR, 33.4 g·kg^{-1}) which would not be commercially sustainable unless a considerable premium was paid for this modified milk. It seems likely that the optimum dose of dietary rapeseed is lower than used in this study.

milk fat / fatty acids / oleic acid / vitamin E

* Corresponding author: d.i.givens@reading.ac.uk

Résumé — Augmentation de la concentration en acide oléique et en vitamine E du lait de bovin en incorporant dans la ration des graines de colza entières et de la vitamine E. Afin de réduire le degré de saturation des acides gras et d'augmenter la teneur en C18:1 *cis* du lait, les effets d'une alimentation avec des niveaux élevés de graines de colza concassées entières ont été étudiés chez des vaches laitières, en relation avec les effets de l'augmentation de l'ingestion de la vitamine E sur la teneur en vitamine E du lait. Au total, 90 vaches laitières de race Holstein ont été alimentées selon un schéma factoriel 3 × 3 avec trois niveaux de graines de colza concassées (0 (ZR), 134 (MR) et 270 g·kg^{-1} de matière sèche (HR)) et trois teneurs en vitamine E (0 (ZE), 2 (ME) et 4 g·vache^{-1}·j^{-1} (HE)). La supplémentation avec près de 2 kg·j^{-1} d'huile de graines de colza (régime HR) a augmenté significativement ($P < 0{,}001$) la concentration du C18:1 *cis* du lait, de 181 (ZR) à plus de 400 g·kg^{-1} d'acides gras totaux du lait (HR). Les concentrations en C18:0, C18:2 et C18:3 ont été également augmentées ($P < 0{,}001$), mais dans des proportions moindres. Dans le même temps, la concentration des acides gras saturés C4:0 à C16:0 a diminué sensiblement. La supplémentation en vitamine E a augmenté les concentrations en vitamine E du lait ($P < 0{,}01$), de 1,29 (ZE) à 1,68 mg·kg^{-1} de lait (HE). Des changements substantiels de la composition en matière grasse du lait avec des effets potentiellement bénéfiques sur la santé humaine ont été réalisés sans aucun effet néfaste sur le goût. Toutefois, ces améliorations sont à revoir à la baisse en raison des réductions substantielles ($P < 0{,}001$) observées pour la consommation volontaire de matière sèche (ZR, 20,6 ; HR, 15,2 kg DM·j^{-1}), la production laitière (ZR, 22,9 ; HR, 13,2 kg·j^{-1}) et la concentration en matière grasse du lait (ZR, 42,1 ; HR, 33,4 g·kg^{-1}) ; ce qui ne serait pas commercialement viable à moins d'attribuer une prime considérable pour ce lait modifié. Il est probable que la dose optimale de graines de colza pour l'alimentation bovine est inférieure à celles de cette étude.

matière grasse du lait / acides gras / graine de colza

1. INTRODUCTION

In recent decades human dietary guidelines have proposed reductions in total fat and in saturated fatty acid consumption as a means of reducing the prevalence of coronary heart disease (CHD). Many guidelines have recommended that saturated fatty acids should provide no more than 10% of dietary energy (e.g. FAO/WHO [9]). In addition, much attention has been recently paid to the beneficial effects of dietary n-3 polyunsaturated fatty acids (PUFA), not only in relation to CHD but also for the immune system and inflammatory responses [8].

Although there is considerable agreement on the adverse effects of the major saturated fatty acids on blood cholesterol concentrations, attempts to reduce consumption of saturated fats has been relatively unsuccessful because of resistance to low fat diets [36]. As a result there has been speculation about the possibility of displacing dietary saturated fatty acids with PUFA or monounsaturated fatty acids (MUFA). Although the cholesterol-lowering effects of PUFA are greater than that of MUFA [36], the potential for reducing saturated fatty acids through substitution with MUFA has increased as a result of the increased ability to manipulate the fatty acid composition of meat and milk (e.g. Chilliard et al. [6]). Dairy products produced using this approach have been shown to successfully reduce blood cholesterol levels in human subjects [26].

Free radicals are highly reactive molecules that are generated during many metabolic processes and are associated with damage to cell membranes. There is increasing interest in the role of free radicals in the aetiology of a number of serious medical conditions, including CHD, thrombosis and some cancers [24, 31]. An important link between free radical mediated cell damage and coronary heart disease is thought to be through oxidative damage of

the PUFA in low density lipoprotein (LDL) cholesterol [31]. Vitamin E is one of the most powerful antioxidants and can prevent the damaging effects of free radicals, most important being the prevention of lipid oxidation in cell membranes. Recent work [4] showed a two fold increase in the concentration of vitamin E in milk when 5 g all-rac-α tocopheryl acetate·d^{-1} was fed to dairy cows.

Although earlier studies (e.g. [7, 23, 34] have shown that feeding moderate quantities of crushed rapeseeds can increase the oleic acid (C18:1, n-9) content of milk fat there are fewer data on the use of whole, cracked rapeseeds which may infer some protection to the lipid from biohydrogenation in the rumen. In addition, the responses to dietary intake of vitamin E on the vitamin E and fatty acid composition of the milk is not clear. The aims of the present study were therefore to investigate the response to feeding to dairy cows increasing high levels of whole rapeseeds on the MUFA and saturated fatty acid concentrations of milk fat and to examine the effect of supplementary vitamin E on the vitamin E concentration of the milk. The effects of increasing whole rapeseeds and vitamin E on the taste of the milk were also assessed.

2. MATERIALS AND METHODS

2.1. Cows and housing

A total of 90 Holstein cows were used in the study, of which 27 were primiparous. Cows were on average 194 days in milk at the start of the study. They were housed in cubicles that were bedded with wood shavings, and slurry was removed at frequent intervals by automatic scrapers. The cows were weighed and condition scored [14] in the covariate week and in week 7. Routine daily health records were kept throughout the study.

2.2. Diets and design

Three diets were formulated to meet the energy and protein requirements for maintenance + 25 kg milk (assuming no loss in liveweight) containing 0, 134 and 270 g·kg^{-1} dry matter (DM) of whole cracked rapeseed (diets ZR, MR and HR respectively). The ingredient composition of these diets is given in Table I. These diets were designed to supply 0, 2.5 and 5.0 kg of whole cracked rapeseed·cow^{-1}·d^{-1}. Within each of these diets the wheat/vitamin E (Lutavit (E 50, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany) premix was adjusted to provide a daily added vitamin E intake of 0, 2 and 4 g·cow^{-1}·d^{-1} (ZE, ME and HE respectively) thus giving a total of nine dietary treatments (ZRZE, ZRME, ZRHE, MRZE, MRME, MRHE, HRZE, HRME and HRHE). All diets were fed as total mixed rations (TMR) on one occasion daily using electronic feeders which allowed diet intake of individual cows to be measured.

Table I. Ingredient composition of three experimental diets ZR, MR and HR (g·kg^{-1} DM).

Ingredient	Diet		
	ZR	MR	HR
Maize silage	429	433	437
Grass silage	143	144	145
Wheat	172	105	37
Rapeseed meal	154	96	37
Soya bean meal	55	53	52
Sugar beet feed (molassed)	25	13	0
Whole cracked rapeseeds	0	134	270
Mineral/vitamin supplement[1]	9	9	9
Wheat-vitamin E premix	13	13	13

ZR, MR, HR: 0, 134 and 270 g·kg^{-1} DM of whole cracked rapeseed, respectively.
[1] Contained 25 mg·kg^{-1} DM vitamin E.

The study was preceded by a one-week covariate period and by a changeover week during which the cows were acclimatised to the experimental diets. The diets were fed in a randomised block, 3 × 3 factorial design with 10 cows per treatment. Cows were formed into blocks on the basis of parity, days calved at the start of study and milk yield during the covariate period. Within each block, cows were allocated at random to one of the nine diets. The effect of the treatments on voluntary DM intake, milk yield, milk composition and milk taste were investigated over a seven week period.

2.3. Milk yield, composition and taste

The cows were milked twice daily at 06.00 and 15.30 h and individual daily milk yield recorded. Milk samples were taken from each cow on two consecutive milkings in the covariate week and in weeks 3 and 7 of the study. These samples were analysed for fat, protein and lactose content by infrared spectroscopy (Milkoscan, Foss Instruments, UK). Sub-samples from the samples taken in weeks 3 and 7 were frozen (–20 °C) and at the end of the study these were thawed, bulked by yield for each cow and fatty acid content determined by capillary gas chromatography of the derived fatty acid methyl esters. A capillary column 100 m × 0.25 m internal diameter was used (SP-2560, Supelco, Bellefonte, USA) with a 0.2 μm film. Helium was used as the carrier gas. The temperature of the injector (split 1:50) was 250 °C and that of the flame ionisation detector 260 °C. The initial oven temperature was 140 °C increasing at 3 °C·min^{-1} to 215 °C which was held for 40 min. This method allows four *trans* C18:1 isomers to be resolved, i.e. n-11 (*trans*-7-octadecenoic), n-9 (elaidic), n-7 (*trans*-11, vaccenic) and n-6 (*trans*-12-octadecenoic). Conjugated C18:2 fatty acids were estimated as the total of all isomers present. Additional sub-samples of milk from the samples taken in weeks 3 and 7 were analysed for vitamin E content by the UKAS accredited method AM/C/702.

In week 7, a total of 200 kg of milk was collected from each treatment (from all cows) in two consecutive milkings, and pasteurised in the School of Food Biosciences at the University of Reading. Five replicate samples of treatments ZRZE, ZRME, ZRHE and HRHE were taken before and after pasteurisation and analysed for fatty acid content.

Two further samples were taken from treatments ZRZE, ZRME, ZRHE, and HRHE. One sample per treatment was subject to assessment by a taste panel of 13 subjects on day 1 following pasteurisation and the second sample per treatment was assessed by the panel on day 12 after refrigerated storage at 4 °C. On each occasion 500 mL of pasteurised, homogenised whole milk was purchased from a local supermarket as a reference sample. Panellists were asked to indicate whether a range of sensory attributes was present or absent in each milk.

2.4. Feed analysis

During the experiment, the grass and maize silages were sampled weekly and frozen at –20 °C. At the end of the study the accumulated frozen samples were thawed and bulked to give one sample of each silage. Grass silage was analysed for DM content, pH, ammonia N, crude protein, water soluble carbohydrates, neutral detergent fibre (NDF), and total ash by the methods of MAFF [15] and volatile fatty acids and lactic acid by the method of Fussell and McCalley [10]. Maize silage was analysed for DM, pH, ammonia N, crude protein, NDF, and total ash by the methods of MAFF [15] and neutral detergent-cellulase plus gamannase digestibility (NCGD) [16]. Starch was measured as described by Moss and Givens [19] and volatile fatty acids and lactic acid by the method of Fussell and McCalley [10]. Grass and maize silage

DM contents were corrected (C) using measured levels of volatile components and the calculated proportions of volatiles lost during oven drying as described by Porter et al. [30].

Organic matter digestibility (OMD) of the grass silage was predicted by near infrared reflectance spectroscopy (NIRS) [27] and metabolisable energy (ME) content calculated as described by Barber et al. [1].

Each new batch of the other dietary ingredients was sampled, bulked and stored frozen below –12 ºC. These were analysed for DM, crude protein, total ash, acid ether extract, starch, NDF and NCGD by methods used for the forages. In addition, long chain fatty acid analysis was carried out on sub-samples of the whole cracked rapeseed using the method described by Moss et al. [20]. The vitamin E content of the vitamin E supplement was also determined (UKAS method AM/C/702).

The grass and maize silages had the following composition ($g \cdot kg^{-1}$ CDM unless specified) respectively: CDM 281, 300 $g \cdot kg^{-1}$; crude protein 193, 105; NDF 471, 404; total ash 102, 45; pH 4.3, 3.7; ammonia nitrogen 180, 80 $g \cdot kg^{-1}$ total nitrogen and predicted ME 11.0, 11.1 $MJ \cdot kg^{-1}$ CDM. The maize silage had a starch content of 276 $g \cdot kg^{-1}$ CDM. The whole rapeseed used had the following composition ($g \cdot kg^{-1}$ DM unless specified) DM 916 $g \cdot kg^{-1}$, crude protein 196, NDF 139, total ash 39 and acid ether extract 479. The lipid fraction comprised ($g \cdot kg^{-1}$ total lipid) C16:0 50, C18:0 20, *cis* C18:1 (n-7) 30, C18:1 (n-9) 550, C18:2 (n-6) 200, C18:3 (n-3) 110, C20:0 10 and C20:1 10. The finding of small amounts of *cis* C18:1 (n-7) in rape oil products was also reported by Delbecchi et al. [7].

2.5. Statistical methods

Data were analysed by analysis of variance of a randomised block, 3 × 3 factorial design using Statistica 5 (StatSoft Inc, Tulsa, OK, USA). The model included effects caused by block, treatment and cow. Statistical significance was declared at $P < 0.05$ for all comparisons. A correlation (Pearson product moment) matrix amongst milk fat content and milk fatty acids was calculated from individual cow data (mean of weeks 3 and 7) using Minitab 10 (Minitab Inc., State College, PA, USA).

3. RESULTS

3.1. Diet intake, milk yield and composition

There were no significant ($P > 0.05$) effects of level of vitamin E supplementation and no significant ($P > 0.05$) interactions between levels of vitamin E and whole rapeseed on DM intake, milk yield, milk protein, fat and lactose concentrations and yields, body condition score or liveweight. Accordingly, the main treatment effects of diets ZR, MR and HR only are given in Table II. There were no effects ($P > 0.05$) of treatment on liveweight or condition score at week 7.

Overall (weeks 1 to 7), increasing intake of whole rapeseed produced a linear decline in DM intake ($P < 0.001$), milk yield ($P < 0.001$) and milk fat content and yield ($P < 0.001$). Milk protein and lactose concentrations were not significantly affected by rapeseed intake and the linear decline in milk protein and lactose yield was primarily a reflection of the decline in milk yield.

The overall DM intakes achieved gave rise to mean intakes of whole rapeseed of 0, 2.53 and 4.10 $kg \cdot cow^{-1} \cdot d^{-1}$ and of rapeseed lipid of 0, 1.21 and 1.97 $kg \cdot cow^{-1} \cdot d^{-1}$ for diets ZR, MR and HR, respectively. Similarly, the mean intakes of the C18:1 (n-9) fatty acid from the added whole rapeseed were 666 and 1081 $g \cdot cow^{-1} \cdot d^{-1}$ for diets MR and HR, respectively.

Table II. Mean dry matter intake, milk yield and milk protein, fat and lactose concentration and yield on diets ZR, MR and HR.

	Treatment			*P* for effect of	
	ZR	MR	HR	Level of WR	Linearity
Animal performance					
Dry matter intake (kg·day^{-1})	20.6	18.9	15.2	<0.001	<0.001
Milk yield (kg·day^{-1})	22.9	19.3	13.2	<0.001	<0.001
Milk composition (g·kg^{-1})					
Fat	42.1	33.5	33.4	<0.001	<0.001
Protein	36.1	37.9	38.4	NS	NS
Lactose	45.3	46.5	42.9	NS	NS
Yield of milk constituents (kg·day^{-1})					
Fat	0.94	0.63	0.42	<0.001	<0.001
Protein	0.80	0.70	0.48	<0.001	<0.001
Lactose	1.02	0.89	0.54	<0.001	<0.001

WR: whole rapeseed, NS: not significant $P > 0.05$.

3.2. Milk fatty acid composition

Table III gives the mean fatty acid concentrations in the milk fat from samples taken in weeks 3 and 7 according to the effects of diets ZR, MR and HR. Concentrations of all fatty acids from C4:0 to C17:1 were significantly ($P < 0.001$) and linearly reduced by increasing inclusion of whole rapeseed apart from C16:1 (n-9) which increased by a factor of 1.4 from diets ZR to HR.

The major effects of treatment were linear increases ($P < 0.001$) in the concentrations of C18:0, C18:1 (n-9), C18:2 (n-6), C18:3 (n-3) and C20:0 fatty acids by factors of 1.9, 2.2, 1.3, 1.3 and 2.3, respectively, between diets ZR and HR. Although there were increases in the concentrations of *trans* C18:1 (n-7) and conjugated C18:2, maximum concentrations occurred on diet MR. Overall, concentrations of saturated fatty acids decreased by 1.5 whilst MUFA and PUFA increased by 1.8 and 1.3, respectively. Correlation coefficients between milk fat content and concentrations of C18 fatty acids, total saturated, total MUFA and PUFA in milk fat are given in Table IV. Milk fat content was negatively correlated with all C18 fatty acids and C18:1 (n-9) in particular (r = –0.91). Milk fat content was somewhat less negatively correlated with *trans* C18:1 (n-7) (r = –0.43) than conjugated C18:2 (r = –0.67), although *trans* C18:1 (n-7) and conjugated C18:2 were highly positively correlated with each other (r = 0.85). Total MUFA and PUFA were strongly negatively correlated with total saturates.

Despite increases in the C18:1 (n-9) concentrations in milk fat, the daily yield of C18:1 (n-9) was lower on treatment HR (mean 165 g·cow^{-1}·d^{-1}) than on MR (mean 219 g·cow^{-1}·d^{-1}) due to the accompanying reductions in milk yield and milk fat content. The calculated mean gross efficiency of transfer of added dietary C18:1 (n-9) from rapeseed to milk fat was 7.3% for MR and –0.47% for HR although these ignore any contributions from other accompanying dietary changes.

The effects of vitamin E supplementation were very small with significant effects

Table III. Mean milk fatty acid composition data on diets ZR, MR and HR (g·kg^{-1} total fatty acids).

	Treatment			*P* for effect of	
Fatty acid	ZR	MR	HR	Level of WR	WR × vitamin E
C4:0	49.9	31.6	27.2	<0.001	<0.001
C6:0	22.6	11.3	9.6	<0.001	<0.001
C8:0	12.5	5.5	4.3	<0.001	<0.01
C10:0	30.7	13.0	9.8	<0.001	<0.05
C10:1	3.7	1.3	0.8	<0.001	NS
C11:0	1.1	0.5	0.5	<0.001	NS
C12:0	39.5	18.9	14.4	<0.001	NS
C14:0	116	78.5	60.0	<0.001	NS
C14:1 (n-5)	14.0	11.5	7.5	<0.001	NS
C15:0	22.8	14.3	9.9	<0.001	NS
C16:0	307	198	180	<0.001	NS
C16:1 (n-9)	1.8	2.3	2.6	<0.001	NS
C16:1 (n-7)	19.2	18.3	15.8	<0.05	NS
C17:0	16.3	11.6	8.9	<0.001	NS
C17:1	3.1	2.2	1.8	<0.001	NS
C18:0	83.0	141	158	<0.001	NS
C18:1 (n-9)	181	347	393	<0.001	NS
C18:1 *trans* (n-7)	19.7	25.8	20.3	<0.001	NS
C18:2 (n-6)	20.8	23.7	27.6	<0.001	NS
C18:2 (conjugated)	6.0	10.2	7.4	<0.001	NS
C18:3 (n-3)	4.5	4.8	6.0	<0.001	NS
C20:0	1.3	2.7	3.0	<0.001	NS
Others	7.4	11.3	12.9	<0.001	NS
Summary					
Total saturates	706	528	486		
Total monounsaturates	242	408	442		
Total polyunsaturates	31.3	38.7	41.0		

WR: whole rapeseed, NS: not significant $P > 0.05$.

only seen for caproic (C6:0; $P < 0.05$) and palmitoleic (C16:1, n-9; $P < 0.05$). Significant interactions between levels of vitamin E and whole rapeseed were observed for the short chain fatty acids up to capric acid (C10:0) although the effects were very small.

There were no pasteurisation × dietary treatment interactions ($P > 0.05$) on fatty acids in milk taken in week 7 from treatments ZRZE, ZRME and ZRHE. Pasteurisation led to small but significant ($P < 0.05$) increases in concentrations (g·kg^{-1} milk fat) of C4:0 (29 to 32), C6:0 (12 to 13), C8:0

Table IV. Correlation coefficients between milk fat content and concentrations of C18 fatty acids, total saturated (SA), total monounsaturated (MU) and total polyunsaturated (PU) fatty acid in milk fat.

	Milk fatty acid								
Milk	C18:0	C18:1 (n-9)	C18:1 *trans* (n-7)	C18:2 (n-6)	C18:2 (conj)	C18:3 (n-3)	Total SA	Total MU	Total PU
Fat content	–0.85	–0.91	–0.43	–0.76	–0.67	–0.56	+0.92	–0.92	–0.89
C18:0		+0.99	+0.33	+0.86	+0.57	+0.74	–0.99	+0.99	+0.94
C18:1 (n-9)			+0.34	+0.88	+0.59	+0.75	–0.99	+0.99	+0.97
C18:1 *trans* (n-7)				+0.01	+0.85	–0.24	–0.37	+0.39	+0.32
C18:2 (n-6)					+0.22	+0.95	–0.88	+0.87	+0.93
C18:2 (conj)						–0.04	–0.61	+0.63	+0.57
C18:3 (n-3)							–0.74	+0.72	+0.79
Total SA								–1.00	–0.98
Total MU									+0.97
Total PU									

conj: conjugated.

(6.2 to 6.8) and C10:0 (15 to 16) whilst the concentration of *trans* 18:1 (n-7) was slightly ($P < 0.05$) reduced (22 to 20).

3.3. Milk vitamin E concentrations and taste

Mean vitamin E concentrations of milk in weeks 3 and 7 are given in Table V. Increasing intakes of whole rapeseeds increased ($P < 0.05$) milk vitamin E concentration although there was little effect between MR and HR treatments. Increasing dietary intake of vitamin E linearly ($P < 0.01$) increased milk vitamin E concentration by a factor of 1.3 between treatments ZE and HE. There was no interaction between intake of whole rapeseed and vitamin E.

The results of the two taste panels, carried out on the pasteurised milk after 1 and 12 d of storage, showed no differences ($P > 0.05$) between individual milk characteristics on either of the two days for the reference milk, the three levels of whole rapeseed at the zero vitamin E level (treatments ZRZE, MRZE and HRZE) or the high level of whole rapeseed with the high vitamin E

Table V. Mean milk vitamin E concentrations ($mg \cdot kg^{-1}$ whole milk).

Treatment	Milk vitamin E
ZRZE	1.19
ZRME	1.33
ZRHE	1.57
MRZE	1.39
MRME	1.65
MRHE	1.68
HRZE	1.30
HRME	1.55
HRHE	1.78
P for effect of:	
Level of WR	< 0.05
Level of vitamin E	< 0.01
WR × vitamin E	NS

ZE, ME and HE: 0, 2 and 4 $g \cdot cow^{-1} \cdot d^{-1}$ of vitamin E; WS: whole rapeseed; NS: not significant ($P > 0.05$).

level (HRHE). There was no difference ($P > 0.05$) between the total number of favourable and unfavourable scores for the day 1 taste panel, but there were significantly ($P < 0.05$) more unfavourable scores at day 12.

4. DISCUSSION

4.1. Effects on animal performance

The lack of an effect of dietary vitamin E intake on DM intake, milk yield or milk fat, protein or lactose concentration was in contrast with the findings of an earlier study [17] where supplementing dairy cow diets with vitamin E reduced DM intake and milk yield. However, feeding the whole cracked rapeseed caused substantial reductions in DM intake, with consequent large reductions in milk yield and milk fat content. These effects are similar to effects of other whole oilseeds described by Thomas and Chamberlain [33].

Overall, feeding whole cracked rape at 134 g·kg^{-1} of dietary DM or above, reduced DM intake, milk yield and milk fat concentration. This may have been due to the lipid in the whole rape reducing microbial activity in the rumen with consequent reduced energy intake and hence milk output. The effects of feeding increasing quantities of lipid on rumen function and feed intake are well documented [29], and in the present study, lipid intake from rapeseed in HR diets was in excess of that proposed by Palmquist [29] as desirable. High intakes were, however, chosen to examine the upper limits of response in milk fat composition and notably a number of studies [2, 7, 34] which fed diets containing lower concentrations of rape seeds (33–83 g·kg^{-1} DM) reported few reductions in DM intake, milk yield and milk fat content. The reduction in milk fat concentration in the present study may also have been due to reduced fibre degradation leading to reduced production of fat precursors in the rumen, or to an increased supply of *trans* fatty acids from the rumen. Increased intake of rapeseed did increase the concentration of *trans* C18:1 (n-7) fatty acid in milk fat (Tab. III) probably synthesised in the rumen from the associated increased intake of C18:2 (n-6) and C18:3 (n-3) [6, 28]. *Trans* fatty acids have been shown to have a specific effect on lowering fat production in the mammary gland [11, 37] although it is notable in the present study that the *trans* C18:1 (n-7) fatty acid had a lower negative correlation with milk fat content that did C18:2 conjugated fatty acids (Tab. IV).

4.2. Effects on milk fatty acids

The increases in total *cis* C18 fatty acids in milk fat (ZR, 289; MR, 517; HR, 584 g·kg^{-1} milk fat) were very similar to the values obtained by Murphy et al. [23] when feeding 0, 1 and 2 kg·cow^{-1}·d^{-1} of cracked rapeseed. In the present work approximately 0, 2.5 and 4 kg·cow^{-1}·d^{-1} were fed and it is not clear why greater quantities of cracked rapeseed were required to obtain the same response although different basal diets may have played a part.

Typically total milk fatty acids comprise between 200 and 250 g·kg^{-1} of the MUFA oleic acid (C18:1 n-9) [6]. Whilst some is produced from stearic acid (C18:0) by desaturation in the mammary gland [3], other work [21, 22] has shown that the concentration of C18:1 in milk fat can be increased by up to 30% by feeding diets containing whole cracked rapeseed, and further increases may be possible if whole cracked rapeseed can be protected from rumen biohydrogenation. In the present study increased dietary supply of C18:1 (n-9), the predominant fatty acid in rape oil, produced a linear increase in this fatty acid in milk fat from 181 (treatment ZR) to almost 400 g·kg^{-1} of total fatty acids at the highest level of whole rape inclusion (Tab. III). This concentration is considerably higher than seen (202–319 g·kg^{-1} total fatty acids) in

studies which fed rapeseeds at dietary concentrations between 33 and 83 g·kg^{-1} DM [2, 7, 34]. Although the increases seen in C18:1 (n-9) concentration were substantial, it is noteworthy that the gross apparent efficiency of capture of dietary fatty acids in milk fat was low (7.3% for diet MR), particularly at the highest dietary supplementation rate (–0.47% for diet HR) where yield of milk fat was lowest (Tab. II). Diet MR represented a supplementation of 1.21 kg of rape oil·cow^{-1}·day^{-1} and the gross apparent efficiency of capture of dietary C18:1 was only slightly lower than that (10.6%) found by Chilliard and Doreau [5] following a duodenal infusion of 630 g·d^{-1} of rapeseed oil.

It is recognised that fatty acids with a chain length of 16 or more carbon atoms are potent inhibitors of mammary fatty acid synthesis [6]. This effect, highlighted in Table IV, is mainly as a direct inhibitory effect on acetyl-Co A carboxylase, the enzyme responsible for the initial incorporation of acetate during fatty acid synthesis. This effect is also implied in the model of Hermansen [12], developed to predict the fatty acid composition of milk fat from dietary fatty acids and is borne out in the present study where milk fat concentrations of essentially all fatty acids from C4:0 to C17:1 were reduced significantly as a result of rapeseed supplementation. This effect may have been exacerbated by a reduced supply of acetate and 3-hydroxybutyrate from the rumen due to the substantial associated reduction in DM and hence energy intake.

Although not normally associated with dietary oleic acid, increases in *trans* C18:1 (n-7) and conjugated C18:2 fatty acids were seen (Tab. III). Ward et al. [34] also observed this effect on conjugated C18:2 at lower rapeseed intakes than in the present work. It is likely that these effects are due to enhanced production of *trans* C18:1 (n-7) in the rumen from the associated increased intake of C18:2 (n-6) and C18:3 (n-3) leading increased conversion of *trans* C18:1 (n-7) to conjugated C18:2 in the mammary gland [6]. The relationship between *trans* C18:1 (n-7) and conjugated C18:2 is reflected in their high inter-correlation (Tab. IV).

The effects of vitamin E supplementation were very small and suggest that dietary vitamin E has little role in the manipulation of milk fat composition although some small effects were seen for the short chain fatty acids up to capric acid (C10:0). Similarly, pasteurisation appears to have little effect on the majority of medium and long chain fatty acids which suggests that milk with modified fat composition will maintain this modification to the point of consumption. Some increase (5 to 10%) in the short chain fatty acids was seen after pasteurisation although the reasons are unclear.

4.3. Effects on milk vitamin E

Milk in the UK contains on average 0.9 mg·L^{-1} of vitamin E [18]. Work in Italy [4] showed a two fold increase in vitamin E concentration in milk when 5 g all-rac-α tocopheryl acetate·cow^{-1}·d^{-1} was fed. Although the present study confirmed that dietary supplements of vitamin E significantly increase vitamin E concentrations in milk (Tab. V) the increases were smaller than reported by Cheli et al. [4] with the highest level of supplementation (4 g all-rac-α tocopheryl acetate·cow^{-1}·d^{-1}) only giving an increase of 1.3 times that of the control. Perhaps surprisingly, milk vitamin E content increased with increasing levels of whole rapeseed, although the level of increase (1.2 times the level of ZR diets) was modest, but only slightly less than from vitamin E supplementation. This may be simply a concentration effect of reduced milk output with increasing whole rapeseed consumption.

Low recovery in milk of dietary vitamin E supplements has been observed earlier [17]. Whilst Shin and Owens [32] reported that between 39 to 52% of supplemental

dietary vitamin E was degraded in the rumen, a number of other studies have shown that degradation is minimal [13, 35]. This suggests that the source of the low efficiency of transfer of vitamin E to milk lies beyond the rumen.

4.4. Milk and taste

The results from the taste panels were inconclusive, with no clear effects of dietary treatments on individual milk acceptability scores at either one or 12 days post-pasteurisation. There was a difference between the total number of favourable and unfavourable scores at day 12, perhaps indicating that feeding whole rapeseed may increase the rate of oxidation and decrease milk palatability as storage time increases. Overall, however, there was little evidence that high levels of rapeseed supplementation adversely affected taste.

4.5. Implications for human health

The key strategy of substituting MUFA for saturates [36] was achieved with the ratio of saturates to MUFA falling from 2.91 in milk fat from the control diet (ZR) to 1.1 in milk fat from diet HR. In particular, it is now clear that it is mainly lauric, myristic and palmitic fatty acids that are responsible for increasing total and LDL cholesterol concentrations in plasma. All three fatty acids were substantially reduced in milk fat from rapeseed supplemented diets. A decrease in the C16:0 to C18:0 ratio in milk fat has also been shown to be beneficial to human health [25] and between diets ZR and HR this ratio was reduced from 3.7 to 1.1. Similarly, another goal for human health and to increase the spreadability of butter would be to decrease the C18:0 to C18:1 (n-9) ratio [6] and between diets ZR and HR this ratio was reduced from 0.46 to 0.40.

The use of rape oil supplements produced a number of changes in milk fat composition with potentially far reaching health benefits. However, these improvements must be offset against the decline in milk yield and milk fat concentration which would probably not be commercially viable. Based on other work it would seem that the optimum dose of rapeseed is lower than in the present work.

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REFERENCES

[1] Barber G.D., Givens D.I., Kridis M.S., Offer N.W., Murray I., Prediction of the organic matter digestibility of grass silage, Anim. Feed Sci. Technol.28 (1990) 115–128.

[2] Bayourthe C., Enjalbert F., Moncoulon R., Effects of different forms of Canola oil fatty acids plus Canola meal on milk composition and physical properties of butter, J. Dairy Sci. 83 (2000) 690–696.

[3] Bickerstaff R., Annison E.F., The desaturation of stearic acid by mammary-gland tissue of the lactating goat and sow, Biochem. J. 107 (1968) 27.

[4] Cheli F., Baldi A., Bontempo V., Cattaneo D., Fabbri B., Effect of vitamin E administration on milk and plasma α-tocopherol levels in dairy cows, in: Proceedings of 45th Annual Meeting of the EAAP, paper No. NC1.25, 1995, p. 123.

[5] Chilliard Y., Doreau M., Influence of a duodenal infusion of rapeseed oil and amino acids on cow milk yield and composition, in: Proceedings of 42nd Annual Meeting of the EAAP, Vol. 2, Berlin, 1991, pp. 26–27.

[6] Chilliard Y., Ferlay A., Mansbridge R.M., Doreau M., Ruminant milk plasticity: nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids, Ann. Zootech. 49 (2000) 181–206.

[7] Delbecchi L., Ahnadi C.E., Kennelly J.J., Lacasse P., Milk fatty acid composition and mammary lipid metabolism in Holstein cows fed protected or unprotected Canola seeds, J. Dairy Sci. 84 (2001) 1375–1381.

[8] DOH, Department of Health Report on Health and Social Subjects No. 46, Nutritional Aspects of Cardiovascular Disease, Report of the Cardiovascular Review Group Committee on Medical Aspects of Food Policy, HMSO, London, 1994, 186 p.

[9] FAO/WHO, General conclusions and recommendations of the consultation, in: Expert Consultation on Fats and Oils in Human Nutrition, Food and Agriculture Organisation of The United Nations IFAO/WHO, Rome, 1998.

[10] Fussell R.J., McCalley D.V., Determination of volatile fatty acids (C2-C5) and lactic acid in silage by gas chromatography, Analyst 112 (1987) 1213–1216.

[11] Gaynor P.J., Waldo D.R., Capuco A.V., Erman R.A., Douglass L.W., Teter B.B., Milk fat depression, the glucogenic theory, and *trans*-C18:1 fatty acids, J. Dairy Sci. 78 (1995) 2008–2015.

[12] Hermansen J.E., Prediction of milk fatty acid profile in dairy cows fed dietary fat differing in fatty acid composition, J. Dairy Sci. 78 (1994) 872–879.

[13] Leedle R.A., Leedle J.A.Z., Butine M.D., Vitamin E is not degraded by ruminal microorganisms – assessment with ruminal contents from a steer fed a high-concentrate diet, J. Anim. Sci. 71 (1993) 3442–3450.

[14] MAFF, Condition Scoring of Dairy Cows, Publication No. 612, HMSO, London, 1986.

[15] MAFF, The Analysis of Agricultural Materials, Reference Book 427, HMSO, London, 1986.

[16] MAFF, Prediction of The Energy Value of Compound Feedingstuffs for Farm Animals, Publication No. 1285, MAFF Publications, Alnwick, 1993.

[17] Mansbridge R.J., To determine the effect of feeding high levels of fish oil and additional vitamin E on the fatty acid composition and shelf life of bovine milk, Report to MAFF on Project Number MS1805, MAFF, London, 1997.

[18] McCance R.A., Widdowson E.M., The Composition of Foods, 5th ed., The Royal Society of Chemistry and MAFF, London, 1991, pp. 73–77.

[19] Moss A.R., Givens D.I., The chemical composition, digestibility, metabolisable energy content and nitrogen degradability of some protein concentrates, Anim. Feed Sci. Technol. 47 (1994) 335–351.

[20] Moss A.R., Givens D.I., Grundy H.F., Wheeler K.P.A., The nutritive value for ruminants of lupin seeds from determinate plants and their replacement of soya bean meal in diets for young growing cattle, Anim. Feed Sci. Technol. 66 (1997) 247–256.

[21] Murphy J.J., McNeill G.P., Connolly J.F., Gleeson P.A., Effect on cow performance and milk fat composition of including full fat soyabeans and rapeseeds in the concentrate mixture for lactating dairy cows, J. Dairy Res. 57 (1990) 295–306.

[22] Murphy J.J., Connolly J.F., McNeil G.P., Effects on milk fat composition and cow performance of feeding concentrates containing full fat rapeseed and maize distillers' grains on grass silage based diets, Livest. Prod. Sci. 44 (1995) 1–11.

[23] Murphy M., Udén P., Palmquist D.L., Wiktorsson H., Rumen and total diet digestibilities in lactating cows fed diets containing full-fat rapeseed, J. Dairy Sci. 70 (1987) 1572–1582.

[24] National Heart Forum, in Preventing Coronary Heart Disease: The Role of Antioxidants, Vegetables and Fruit, in: Rogers L., Sharp I. (Eds.), HMSO, London, 1997.

[25] Ney D.M., Potential for enhancing the nutrition properties of milk fat, J. Dairy Sci. 74 (1991) 4002–4012.

[26] Noakes M., Nestel P.J., Clifton P.M., Modifying the fatty acid profile of dairy products through feedlot technology lowers plasma cholesterol of humans consuming the products, Am. J. Clin. Nutr. 63 (1996) 42–46.

[27] Offer N.W., Cottrill B.R., Thomas C., The relationship between silage evaluation and animal response, in: Proceedings of 11th International Silage Conference, University of Wales, Aberystwyth, 1996, pp. 26–38.

[28] Offer N.W., Marsden M., Dixon J., Speake B.K., Thacker F.E., Effect of dietary fat supplements on levels of n-3 polyunsaturated fatty acids, *trans* acids and conjugated linoleic acid in bovine milk, Anim. Sci. 69 (1999) 613–625.

[29] Palmquist D.L., The role of dietary fats in efficiency of ruminants, J. Nutr. 124 (Suppl.) (1994) 1377S–1382S.

[30] Porter M.G., Patterson D.C., Steen R.W.J., Gordon F.J., Determination of dry matter and gross energy concentration of fresh silage, in: Proceedings of 7th International Silage Conference, Queen's University, Belfast, 1984, pp. 89–90.

[31] Riemersma R.A., Wood D.A., Macintyre C.C., Elton R.A., Gey K.F., Oliver M.F., Antioxidants and pro-oxidants in coronary heart disease, Lancet 337 (1991) 1–5.

[32] Shin I.S., Owen F.N., Ruminal and intestinal disappearance of several sources of vitamin E, J. Anim. Sci. 68 (1990) 544–545.

[33] Thomas P.C., Chamberlain D.G., Manipulation of milk composition to meet market needs, in: Haresign W., Cole D.J.A. (Eds.), Recent Advances in Animal Nutrition, Butterworths, London, 1984, pp. 219–243.

[34] Ward A.T., Wittenberg K.M., Przybyiski R., Bovine milk fatty acid profiles produced by feeding diets containing Solin, Flax and Canola, J. Dairy Sci. 85 (2002) 1191–1196.

[35] Weiss W.P., Smith K.L., Hogan J.S., Steiner T.E., Effect of forage to concentrate ratio on disappearance of vitamin A and vitamin E during in vitro ruminal fermentation, J. Dairy Sci. 78 (1995) 1837–1842.

[36] Williams C.M., Dietary fatty acids and human health, Ann. Zootech. 49 (2000) 165–180.

[37] Wonsil B.J., Herben J.H., Watkins B.A., Dietary and ruminally derived *trans*-18:1 fatty acids alter bovine milk lipids, J. Nutr. 124 (1994) 556–565.