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Original article

Dietary supplements of whole linseed and vitamin E to increase levels of α-linolenic acid and vitamin E in bovine milk

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Abstract – The potential to increase the concentrations of n-3 polyunsaturated fatty acids (PUFAs) in milk fat was investigated by studying the effects of feeding a xylose-treated, whole cracked linseed supplement (rich in α-linolenic acid) to dairy cows. Also the effect of increasing the dietary intake of vitamin E on the vitamin E status of milk was investigated. The effect of pasteurisation on milk fatty acid composition was also examined. Using a 3 × 2 factorial design, a total of 60 Holstein dairy cows were fed a total mixed ration based on grass silage supplemented with one of three levels of whole cracked linseed (78, 142 or 209 g·kg^{-1} diet dry matter (DM); designated LL, ML or HL, respectively) in combination with one of two levels of additional dietary vitamin E intake (6 or 12 g vitamin E·animal^{-1}·day^{-1}; designated LE or HE, respectively). Increasing lipid supplementation reduced ($P < 0.01$) diet DM intake and milk yield, and increased ($P < 0.001$) the overall content of oleic, vaccenic, α-linolenic and conjugated linoleic acids, and total PUFAs and monounsaturated fatty acids (MUFA). Myristic and palmitic acids in milk fat were reduced ($P < 0.001$) through increased lipid supplementation. While α-linolenic acid concentrations were substantially increased this acid only accounted for 0.02 of total fatty acids in milk at the highest level of supplementation (630 g α-linolenic acid·animal^{-1}·day^{-1} for HL). Conjugated linoleic acid concentrations in milk fat were almost doubled by increasing the level of lipid supplementation (8.9, 10.4 and 16.1 g·kg^{-1} fatty acids for LL, ML and HL, respectively). Although milk vitamin E contents were generally increased there was no benefit ($P > 0.05$) of increasing vitamin E intake from 6 to 12 g·animal^{-1}·day^{-1}. The fatty acid composition of milk was generally not affected by pasteurisation.

milk fat / fatty acids / α-linolenic acid / vitamin E / pasteurisation

Résumé – **Augmentation de la concentration en acide α-linolénique et en vitamine E dans le lait de vache par complémentation de la ration avec des graines de lin entières et de la vitamine E.** La possibilité d'augmenter la concentration en acides gras polyinsaturés n-3 (PUFAs) de la matière grasse du lait a été étudiée en suivant les effets de l'addition de graines entières de lin concassées, traitées au xylose (riches en acide α-linolénique) à la ration de vaches laitières. L'effet de l'augmentation de l'ingestion de la vitamine E sur la concentration de la vitamine E dans le lait,

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et l'effet de la pasteurisation sur la composition en acides gras du lait ont également été examinés. En utilisant un schéma factoriel 3 × 2, un total de 60 vaches laitières Holstein ont reçu une ration complète à base d'ensilage d'herbe complétée par un des trois niveaux de graines de lin entières concassées (78, 142 ou 209 g·kg^{-1} matière sèche (MS), respectivement, LL, ML ou HL), en combinaison avec un des deux niveaux d'apport alimentaire de vitamine E (6 ou 12 g·vache^{-1}·jour^{-1}, respectivement, LE ou HE). La supplémentation croissante en lipides a réduit l'ingestion (MS) du régime ($P < 0,01$) et la quantité de lait produite. Elle a augmenté ($P < 0,001$) la teneur globale en acides linoléique, oléique, vaccénique, α-linolénique et linoléique conjugué, en PUFAs totaux et en acides gras monoinsaturés (MUFA). Les acides myristique et palmitique dans la matière grasse du lait ont été réduits ($P < 0,001$) par la supplémentation accrue en lipides. Tandis que la concentration en acide α-linolénique était sensiblement augmentée, cet acide a seulement représenté 2 % des acides gras totaux du lait pour le niveau de supplémentation le plus élevé (630 g d'acide α-linolénique·vache^{-1}·jour^{-1} pour HL). Les concentrations en acide linoléique conjugué dans la matière grasse du lait ont été presque doublées par l'augmentation du niveau de la supplémentation en lipides (8,9, 10,4 et 16,1 g acide gras·kg^{-1} pour LL, ML et HL respectivement). Bien que la teneur en vitamine E du lait ait été généralement augmentée, il n'y a eu aucun avantage ($P > 0,05$) à augmenter (de 6 à 12 g·animal^{-1}·jour^{-1}) la quantité de vitamine E distribuée. La composition en acides gras du lait n'a pas été affectée par la pasteurisation.

matière grasse du lait / acides gras / acide α-linolénique / vitamine E / pasteurisation

1. INTRODUCTION

In the last 20 years reductions in the intake of total fat and saturated fatty acids, to provide no more than 10% of dietary energy intake [7], have been recommended as a means of reducing the prevalence of coronary heart disease (CHD). More recently, much attention has been paid to the putative beneficial effects of dietary n-3 polyunsaturated fatty acids (n-3 PUFA) in relation to CHD, and the immune system and inflammatory responses [5].

Although it is generally agreed that the major saturated fatty acids adversely affect blood cholesterol concentrations, attempts to reduce consumption of saturated fats has been relatively unsuccessful through a resistance to low fat diets [31]. Consequently, it has been speculated that dietary saturated fatty acids could be displaced with n-3 PUFA or monounsaturated fatty acids (MUFA). The effects of n-3 PUFA on lowering blood LDL-cholesterol concentrations are greater than for MUFA. Therefore, a number of dietary strategies have been proposed to increase the dietary consumption of n-3 PUFA [31] including increased intake of eicosapentaenoic (EPA; C20:5n-3) and docosahexaenoic (DHA; C22:6n-3) acids because of their high physiological activity [14, 31]. An increased consumption of α-linolenic acid (C18:3n-3) has also been proposed partly because some of it may be converted to EPA and DHA in vivo [28].

In dairy cows, incorporation of dietary α-linolenic acid into milk fat can be substantial only when feeding rumen protected linseed oil [4]. This affords the possibility of increasing the national dietary intake of n-3 PUFAs via consumption of α-linolenic acid enriched milk. An increase in milk PUFA content may require a concomitantly higher concentration of antioxidants to prevent oxidative damage of the fatty acids. Antioxidants, vitamin E being one of the most powerful, play an important role in preventing the damaging effects of free radicals, particularly the prevention of lipid oxidation in cell membranes. Cheli et al. [3] recently showed a two-fold increase in the concentration of vitamin E in milk when 5 g all-rac-α tocopheryl acetate per day was fed to dairy cows. However the role of vitamin E in promoting changes in milk fatty acid composition which are medically desirable is unclear.

The aim of the present study was to investigate the response to feeding dairy cows increasing quantities of treated linseed on n-3 PUFA concentrations of milk fat. The effect of pasteurisation on milk fat composition was also investigated. In addition, the effect of dietary vitamin E supplementation on milk vitamin E concentration and fatty acid composition was examined.

2. MATERIALS AND METHODS

2.1. Animals and experiment design

A total of 42 multiparous and 18 primiparous Holstein cows were used and housed in cubicles, which were bedded with wood shavings. Slurry was removed at frequent intervals by automatic scrapers. Experimental diets were offered as total mixed rations (TMR) once daily through Calan-Broadbent gates and all cows had continuous access to fresh drinking water. Animals were milked twice daily and daily health records (e.g. lameness, mastitis) kept throughout.

Six dietary treatments were investigated in a 3 × 2 factorial design with level of inclusion of a lipid supplement (3 levels) and synthetic vitamin E (2 levels) as the main factors. Animals were formed into blocks, on the basis of parity (mean 2.3, range 1 to 7) and the number of days in milk (mean 117 d, range 18 d to 189 d) at the start of the experiment, such that there were ten cows per treatment. The study lasted 10 weeks and comprised a one week covariate period, one week change-over period, to allow acclimatisation to the dietary treatments, and eight consecutive weeks for the main experimental period. Individual cow live-weight and body condition score (scale 0 to 5 according to MAFF [16]) were recorded in the covariate week and weeks 1 and 8 of the experiment.

2.2. Treatment diets

The experimental treatment diets (see Tab. I for ingredient composition) were based on grass silage which had the following composition (as $g \cdot kg^{-1}$ DM unless specified): DM 218 $g \cdot kg^{-1}$, crude protein (CP) 129, NDF 545, total ash 86, pH 3.7, WSC 5.7, ammonia N 102 $g \cdot kg^{-1}$ total N and vitamin E 43.4 $mg \cdot kg^{-1}$ DM. The grass silage was well fermented (lactic acid content 178 $g \cdot kg^{-1}$ DM) and had a predicted

Table I. Ingredient composition of the six experimental diets used in the study ($g \cdot kg^{-1}$ diet dry matter).

	Experimental diet					
Ingredient	LLLE	MLLE	HLLE	LLHE	MLHE	HLHE
Grass silage	474	487	500	474	487	500
Wheat	177	173	168	177	173	168
Molassed sugar beet feed	116	80	41	116	80	41
Rapeseed meal	99	49	–	99	49	–
LinPreme lipid supplement	78	142	209	78	142	209
Soyabean meal	38	49	59	38	49	59
Wheat/vitamin E Premix[1]	9	10	10	9	10	10
Mineral/vitamin supplement[2]	9	9	9	9	9	9
Limestone	–	1	2	–	1	2

LL, ML and HL: levels of lipid supplementation (78, 142 or 209 $g \cdot kg^{-1}$ diet dry matter, respectively); LE and HE: levels of vitamin E supplementation (6 or 12 g additional vitamin $E \cdot animal^{-1} \cdot day^{-1}$, respectively); [1]vitamin E supplied as Lutavit E-50; [2] supplement supplies 500 mg vitamin $E \cdot day^{-1}$.

ME value of 10.5 MJ·kg^{-1} DM. The experimental diets were supplemented with 78, 142 or 209 g lipid supplement·kg^{-1} diet DM (designated LL, ML or HL, respectively) and 6 or 12 g additional vitamin E·animal^{-1}·day^{-1} (designated LE or HE, respectively). The six treatments (LLLE, MLLE, HLLE, LLHE, MLHE and HLHE) were formulated to be isoenergetic and isonitrogenous, and to meet the animals' energy and protein requirements for maintenance + 25 kg milk (assuming no loss in live-weight). The lipid supplement (LinPreme, Borregaard UK Ltd.), comprised of cracked, whole linseed which had been mixed with xylose and heated according to the process described by Mansbridge et al. [20]. The lipid supplement had the following composition (as g·kg^{-1} DM unless specified): DM 908 g·kg^{-1}, CP 188, NDF 253, total ash 42.0, NCGD 818 and AEE 301. The lipid fraction comprised predominantly of the *cis* fatty acids C18:1(n-9), C18:2(n-6) and C18:3(n-3) (140, 180 and 580 g·kg^{-1} total fatty acids, respectively). For treatments LL, ML and HL, the AEE concentrations were 23.5, 42.7 and 62.9 g·kg^{-1} TMR DM, respectively. Vitamin E (Lutavit E-50, 50% DL-α-tocopheryl acetate, BASF) was added in the form of a wheat-based premix, which was given as a top dressing to the TMR once daily.

2.3. Feed intake and milk yield and composition

Total feed intake (fresh-weight basis) was recorded daily for individual cows and using the DM content of the diets, determined twice weekly, total daily DM intake was calculated. Daily milk yield was recorded automatically for individual animals and samples of milk were bulked from two consecutive milking times in the covariate week and in weeks 2, 4 and 8 of the experimental period. These samples were used in the estimation of fat, protein and lactose content by mid-infrared analysis (Milkoscan, Foss Instruments, York, UK). Sub-samples of the samples taken in weeks 4 and 8 of the experimental period were frozen (–20 °C), without preservative, and at the end of the study these were thawed and analysed for individual fatty acid content by capillary gas chromatography (using a SP-2560 column; 100 m × 0.25 mm × 0.2 μm) of the derived fatty acid esters. Helium was used as the carrier gas and hydrogen (FID; 30 mL·min^{-1}), air (FID; 400 mL·min^{-1}) and nitrogen (auxiliary; 5–15 mL·min^{-1}). The temperature of the FID detector was set at 260 °C, the injector (split 1:50) at 250 °C and the oven at 140 °C initially, increasing at 3 °C·min^{-1} to 215 °C and then held for 40 minutes. Sub-samples of the milk samples taken in weeks 4 and 8 were analysed within 24 hours of sampling for vitamin E content by the UKAS accredited method AM/C/702.

2.4. Milk pasteurisation

Milk samples were taken from two consecutive milking times in week 8 of the experimental period and bulked within treatments (from all cows) to give a total of 100 kg of milk for treatments LLLE, HLLE, LLHE and HLHE. These samples were then pasteurised (72 °C for 15 s) in the School of Food Biosciences, University of Reading. Five samples of each treatment were taken before and following pasteurisation and used to determine fatty acid composition.

2.5. Feed analysis

During the experiment, a representative sample of the grass silage was taken weekly and then stored frozen (–20 °C). At the end of the study the accumulated frozen samples were thawed, bulked into a single sample and then analysed for DM content, pH, total and ammonia nitrogen (N), water soluble carbohydrates (WSC, following drying at 100 °C for 2 hours), and total ash by the methods of MAFF [17]. Neutral detergent fibre (NDF) was determined according to Van Soest et al. [30] but without the use of amylase or sodium sulphite.

Short chain fatty acids (SCFA) and lactic acid were analysed for by the method of Fussell and McCalley [8]. The organic matter digestibility (OMD) of the grass silage was predicted by near infrared spectroscopy (NIRS, [25]) and metabolisable energy (ME) content calculated according to Barber et al. [1].

Each new batch of the other dietary ingredients (see Tab. I) was sampled, bulked and stored frozen (< –12 °C) pending analysis. These were analysed for DM, total N, acid ether extract (AEE) and total ash contents [17]. Cell wall content was determined as NDF according to Van Soest et al. [30]. Except for the lipid supplement, the starch content of the dietary ingredients was measured using the method described by Moss and Givens [24]. Neutral detergent-cellulase plus gamannase digestibility (NCGD) was completed by the method of MAFF [18].

The grass silage and lipid supplement were analysed for individual fatty acids, by capillary gas chromatography, and vitamin E content (UKAS accredited method AM/C/702).

2.6. Statistical analysis

Milk yield and composition data recorded during the covariate week were used as covariates in the subsequent statistical analysis. The effects of the level of inclusion of the lipid supplement (treated whole linseed) and vitamin E, and their interaction on milk yield and composition was measured using repeated measures analysis of variance (ANOVA) (Statistica 5, StatSoft Inc, Tulsa, OK, US). The effect of pasteurisation on milk composition was also analysed by repeated measures ANOVA. Condition score (a discrete scoring system) was analysed using Kruskal-Wallis analysis of variance by ranks.

3. RESULTS

3.1. Dry matter intake, milk yield and composition

The effects of treatment on overall DM intake, and milk yield and composition are summarised in Table II. Increasing

Table II. Effect of diet on dry matter intake, milk yield, milk composition and yield of milk constituents.

	Experimental diet						Significance[1]	
Parameter	LLLE	MLLE	HLLE	LLHE	MLHE	HLHE	Level of lipid	Lipid × vitamin E
DM intake ($kg \cdot d^{-1}$)	19.0	18.4	17.2	19.6	18.5	17.3	**	ns
Milk yield ($kg \cdot d^{-1}$)	28.4	26.2	25.2	28.0	26.3	26.0	**	ns
Milk composition								
Fat ($g \cdot kg^{-1}$)	41.0	44.9	43.8	44.4	45.5	42.8	ns	ns
Protein ($g \cdot kg^{-1}$)	33.0	33.0	32.0	32.7	33.3	31.4	**	ns
Vitamin E ($mg \cdot kg^{-1}$)	1.94	1.66	0.99	1.76	1.65	1.74	***	***
Yield of milk constituents ($kg \cdot d^{-1}$)								
Fat	1.17	1.18	1.09	1.30	1.13	1.19	ns	ns
Protein	0.91	0.88	0.80	0.93	0.84	0.86	**	ns

LL, ML and HL: levels of lipid supplementation (78, 142 or 209 $g \cdot kg^{-1}$ diet dry matter, respectively); LE and HE: levels of vitamin E supplementation (6 or 12 g additional vitamin $E \cdot animal^{-1} \cdot day^{-1}$, respectively); [1] the effect of level of vitamin E was not significant for all parameters; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant ($P > 0.05$).

the level of lipid supplement, equivalent to 0.45, 0.79 and 1.09 kg linseed oil·animal^{-1}·day^{-1} for treatments LL, ML and HL respectively, significantly ($P < 0.01$) reduced total DM intake (19.3, 18.5 and 17.3 kg DM·animal^{-1}·day^{-1} for LL, ML and HL respectively). Overall increase in liveweight between weeks 1 and 8 was significantly ($P < 0.05$) affected by the level of lipid supplement (34, 19 and 19 kg liveweight gain for LL, ML and HL, respectively). There was no significant ($P > 0.05$) effect of treatment on body condition score.

Overall, milk yield was significantly ($P < 0.01$) reduced by increasing the level of the lipid supplement in the diet (28.2, 26.3 and 25.6 kg·animal^{-1}·day^{-1} for LL, ML and HL, respectively). Milk fat content was higher for ML than for LL and HL but treatment differences were not significant ($P > 0.05$). Milk fat yield was highest for LL and tended to decrease with higher levels of lipid supplementation (1.24, 1.16 and 1.14 kg·day^{-1} for LL, ML and HL, respectively). The milk protein content and milk protein yield decreased significantly ($P < 0.01$) with increasing levels of the lipid supplement.

Level of vitamin E supplementation had no significant ($P > 0.05$) effect on overall total DM intake, milk yield, milk composition or yield of milk constituents. Overall vitamin E content of whole milk declined significantly ($P < 0.001$) with increasing inclusion of the lipid supplement (1.85, 1.66 and 1.37 mg vitamin E·kg^{-1} for LL, ML and HL respectively). There was a significant ($P < 0.001$) interaction between the levels of the lipid and vitamin E supplements on whole milk vitamin E content (1.94, 1.66, 0.99, 1.76, 1.65 and 1.74 mg vitamin E·kg^{-1} for LLLE, MLLE, HLLE, LLHE, MLHE and HLHE, respectively).

3.2. Milk fatty acid composition

The effects of the treatments on overall milk fatty acid composition for C14:0, C16:0, C18:1(n-9), C18:1*trans* (n-7), C18:2(n-6), C18:3(n-3), conjugated C18:2 (estimated as the total of all isomers present), and total MUFAs and PUFAs are summarised in Table III. Increasing lipid supplementation significantly ($P < 0.001$) reduced the content of myristic acid (C14:0) and palmitic acid (C16:0) in milk fat (102, 91.0 and 79.3 g·kg^{-1} fatty acids

Table III. Effect of diet on fatty acid concentrations in milk fat (g fatty acid·kg^{-1} total fatty acids).

	Experimental diet						Significance[1]
Milk fatty acid	LLLE	MLLE	HLLE	LLHE	MLHE	HLHE	Level of lipid
C14:0	101	89.8	80.2	103	92.1	78.3	***
C16:0	231	211	184	237	214	174	***
C18:1(n-9)	193	209	224	188	202	231	***
C18:1*trans* (n-7)	15.4	17.9	29.3	16.5	19.3	29.4	***
C18:2(n-6)	16.0	16.3	15.7	16.4	15.4	16.9	ns
C18:3(n-3)	13.7	17.8	18.6	14.2	16.9	20.5	***
Conjugated C18:2	8.6	10.3	16.2	9.1	10.5	16.0	***
Total MUFA	276	300	336	273	293	345	***
Total PUFA	50.4	57.3	64.7	51.7	55.3	67.7	***

LL, ML and HL: levels of lipid supplementation (78, 142 or 209 g·kg^{-1} diet dry matter respectively); LE and HE: levels of vitamin E supplementation (6 or 12 g additional vitamin E·animal^{-1}·day^{-1}, respectively); [1] the effect of level of vitamin E and the interaction between level of lipid × vitamin E was not significant for all parameters; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ***: $P < 0.001$; ns: not significant ($P > 0.05$).

and 234, 213 and 179 g·kg^{-1} fatty acids for LL, ML and HL, respectively). The overall content of oleic acid (C18:1(n-9)), vaccenic acid (C18:1*trans* (n-7)), α-linolenic acid (C18:3(n-3)) and conjugated linoleic acid (C18:2) significantly ($P < 0.001$) increased in milk fat with increasing inclusion of the lipid supplement. Total MUFA and PUFA also significantly ($P < 0.001$) increased (275, 297 and 341 g·kg^{-1} fatty acids and 51.1, 56.3 and 66.2 g·kg^{-1} fatty acids for LL, ML and HL, respectively). Across all treatments, no EPA or DHA fatty acids were detected in the milk.

There was no significant ($P > 0.05$) effect of the level of vitamin E supplementation or level of lipid × vitamin E interaction on milk fatty acid composition.

3.3. Milk pasteurisation

With the exception of C18:2(n-6), there was no overall significant ($P > 0.05$) effect of pasteurisation on milk fatty acid composition. The content of C18:2(n-6) was slightly, but significantly ($P < 0.05$) decreased by the pasteurisation process (16.6 and 16.3 g·kg^{-1} total fatty acids for pre- and post-pasteurisation respectively). There were a number of interactions between pasteurisation × level of lipid, pasteurisation × level of vitamin E and pasteurisation × level of lipid × level of vitamin E on milk fatty acid composition, especially for C18:2(n-6).

4. DISCUSSION

Milk in the UK typically contains α-linolenic acid (C18:3n-3) at a concentration of 5 g·kg^{-1} milk fat [22]. In this experiment all treatments produced higher values than this figure and furthermore concentrations increased with increasing intakes of dietary α-linolenic acid from the treated whole linseed supplement. The highest level of lipid supplementation (HL: 209 g lipid supplement·kg^{-1} diet DM) represented an intake of approximately 1.1 kg linseed oil or 630 g α-linolenic acid·animal^{-1}·day^{-1}. At this level of supplementation, α-linolenic acid accounted for almost 0.02 of total fatty acids in milk. However, increasing the supply of α-linolenic acid in the diet from 263 (LL) to 630 (HL) g·animal^{-1}·day^{-1} resulted in a very low partial efficiency of incorporation into milk fat of about 0.014 (representing a decrease in efficiency of incorporation from 0.066 to 0.035 for LL and HL, respectively). Mansbridge et al. [20] compared the effect of feeding untreated and xylose-treated (as in the present study) whole ground linseed, as rumen unprotected and rumen protected sources of α-linolenic acid respectively, on C18:3, C20:5 and C22:6 fatty acids in milk fat. By feeding approximately 343 (untreated linseed) and 362 (treated linseed) g α-linolenic acid·animal^{-1}·day^{-1} they increased the α-linolenic acid content of milk fat to 12 and 15 g·kg^{-1} milk fatty acids respectively, compared with the control value of 7 g·kg^{-1} milk fatty acids. Mansbridge et al. [20] also reported a similar efficiency of incorporation of α-linolenic acid into milk fat from treated linseed as in the present study (0.034 of additional α-linolenic acid). Chilliard et al. [4] highlighted, however, that the incorporation of dietary α-linolenic acid into milk fat may be high, and reported transfer efficiencies from 0.35 to 0.70, from studies where small amounts of α-linolenic acid (less than 40 g per day) were infused into the abomasum/duodenum. α-linolenic acid accounted for more than 0.2 of milk fat when high levels (0.2 of the total diet) of a protein-protected linseed oil was fed [23] and 0.064 of milk fat when Goodridge and Ingalls [11] fed 410 g protected linseed oil·animal^{-1}·day^{-1}.

The lower rate of incorporation of α-linolenic acid into milk fat reported in the present study may in part suggest that the lipid supplement was not effectively protected from undergoing biohydrogenation in the rumen. The decrease in total DM intake with increasing lipid supplementation supports this view and may suggest

inhibition of fibre digestion/rumen microbes by dietary fatty acids [27].

Concentrations of C18:1(n-9), C18:1*trans* (n-7), conjugated C18:2 and total MUFAs and PUFAs were also increased, and the saturated fatty acids (C14:0 and C16:0) decreased substantially as α-linolenic acid intake increased. This decrease in myristic (C14:0) and palmitic (C16:0) acid supports the observations of Doreau and Chilliard [6] that the inclusion of long chain fatty acids in the diet (particularly C18:1(n-9) and C18:3(n-3)) reduces the concentration of myristic and palmitic acids in the milk mainly by an inhibition of mammary de novo fatty acid synthesis [4]. The failure to detect either EPA or DHA in milk fat is in agreement with the fact that these fatty acids are essentially supplied by dietary inclusion of fish oil or algae [10] and suggests that little in vivo synthesis from α-linolenic acid occurs. Offer et al. [26] suggested that little n-3 long chain PUFAs (defined as α-linolenic acid + EPA + DHA) is absorbed from the small intestine, suggesting that enhancing the EPA and DHA content of milk fat is principally limited by their low rate of incorporation from blood plasma. The changes in milk fatty acid composition were generally achieved while maintaining both milk fat content and milk fat yield although milk protein concentration was significantly reduced.

Interest in conjugated linoleic acid is increasing as it is thought to possess properties for promoting human health [31]. Increasing the inclusion of the lipid supplement almost doubled the level of conjugated linoleic acid in milk fat (8.9 and 16.1 $g \cdot kg^{-1}$ fatty acids for LL and HL respectively). The level of conjugated dienoic acids in cow's milk has been correlated positively with dietary intake of linoleic acid [15], indicating that conjugated linoleic acid formed in the rumen is incorporated into milk fat. However, it is also known that C18:1*trans* (n-7) (vaccenic acid) is an intermediary in the biohydrogenation of dietary α-linolenic acid and that synthesis of *cis*-9, *trans*-11 conjugated linoleic acid from it by Δ-9 desaturase occurs in the mammary gland [2, 4, 13]. This may be the primary source of *cis*-9, *trans*-11 conjugated linoleic acid in milk and it is suggested that this synthetic route is responsible for up to 90% of conjugated linoleic acid in milk [3, 12]. The results of this study support the idea that dietary α-linolenic acid can be a valuable means of increasing conjugated linoleic acid in milk.

In the UK, milk contains approximately 0.9 mg vitamin $E \cdot l^{-1}$ [21]. With the exception of diet HLLE, milk vitamin E contents were higher that this value. Work in Italy [3] showed a two-fold increase in vitamin E concentration in milk when 5 g dl-α tocopherol$\cdot cow^{-1} \cdot day^{-1}$ were fed. Although the present work confirmed that dietary supplementation of vitamin E increased the vitamin E concentration in milk above the UK average value, the increase was smaller than that reported by Cheli et al. [3]. In the present study there was no significant effect of increasing the vitamin E supplementation from 6 $g \cdot animal^{-1} \cdot day^{-1}$ to 12 $g \cdot animal^{-1} \cdot day^{-1}$. Unexpectedly, milk vitamin E content was generally reduced by increasing the level of dietary α-linolenic acid supplementation. This effect on milk vitamin E content is the reverse of that found when increasing amounts of whole rapeseed were fed [9]. These authors suggested this phenomenon may be due either to the natural vitamin E in rapeseed, an improved uptake of vitamin E, or some other metabolic change which increased the partition of vitamin E into milk when high levels of rape oil are included in the diet. It may also, of course, simply reflect a concentration effect of reduced milk output associated with increasing whole rapeseed consumption.

Low recovery in milk of dietary vitamin E supplements has been observed earlier [19]. Shin and Owens [29] also showed that 0.39 to 0.52 of supplemental vitamin E disappeared before reaching the duodenum of steers. This suggests substantial rumen vitamin E degradation and the need therefore, for methods of rumen protection in

order to substantially increase the vitamin E content of milk.

It is concluded that the fatty acid composition of milk can be manipulated by dietary means to increase the potentially beneficial supply of α-linolenic and conjugated linoleic acids, and total MUFAs and PUFAs to the human diet. Fatty acids which are potentially harmful to human health including, myristic and palmitic acids, can also be concomitantly reduced. Although substantial amounts of dietary α-linolenic acid can be incorporated into milk from protected linseed sources, the present study suggests an incomplete protection of the lipid in the treated linseed supplement, and that further research in this area is needed to optimise the manipulation process. Unlike the use of supplemental fish oil or algae, linseed supplementation is not an efficient means of increasing either EPA or DHA in milk lipid, and highlights that little in vivo synthesis from α-linolenic acid occurs. The effects on fatty acid composition were not influenced to any degree by vitamin E supplementation although this did increase milk vitamin E concentrations. The fatty acid composition of raw milk is generally not affected by pasteurisation which is vital if milk is to be used to alter fatty acid profile of the human diet.

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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] Bauman D.E., Griinari J.M., Regulation and nutritional manipulation of milk fat: low-fat milk syndrome, Livest. Prod. Sci. 70 (2001) 15–29.

[3] 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, Proceedings of 45th Annual Meeting of the EAAP, paper No. NC1.25, 1995, p. 123.

[4] 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.

[5] 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.

[6] Doreau M., Chilliard Y., Fat digestion and metabolism in farm animals, Brit. J. Nutr. 78 (Suppl. 1) (1997) S15–S35.

[7] 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, FAO/WHO, Rome, 1998.

[8] 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.

[9] Givens D.I., Allison R., Blake J.S., Enhancement of oleic acid and vitamin E concentrations of bovine milk using dietary supplements of whole rapeseed and vitamin E, Anim. Res. (2003) 531–542.

[10] Givens D.I., Cottrill B.R., Davies M., Lee P.A., Mansbridge R.J., Moss A.R., Sources of n-3 polyunsaturated fatty acids additional to fish oil for livestock diets, a review, Nutr. Abstr. Rev. (Ser. B) Livest. Feeds and Feed. 70 (2000) 1–19.

[11] Goodridge J., Ingalls J.R., Transfer of omega-3 linolenic acid from flaxseed to milkfat, J. Anim. Sci. 76 (Suppl. 1) (1998) 353 (Abstr.).

[12] Griinari J.M., Corl B.A., Lacy S.H., Chouinard P.Y., Nurmela K.V.V., Bauman D.E., Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by delta 9-Desaturase, J. Nutr. 130 (2000) 2285–2291.

[13] Lawson R.E., Moss A.R., Givens D.I., The role of products in supplying conjugated linoleic acid to man's diet: a review, Nutr. Res. Rev. 14 (2001) 153–172.

[14] Kromhout D., Fish (oil) consumption and coronary heart disease, in: Galli C., Simopoulos A.P. (Eds.), Dietary w3 and w6 fatty acids.

Biological effects and nutritional essentiality, New York, USA, Plenum Publishing, 1989.

[15] Loor J.J., Quinlan L.E., Bandara A.B.P.A., Herbein J.H., Distribution of *trans*-vaccenic acid and *cis*9, *trans*11-conjugated linoleic acid (rumenic acid) in blood plasma lipid fractions and secretion in milk fat of Jersey cows fed canola or soybean oil, Anim. Res. 51 (2002) 119–134.

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

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

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

[19] 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.

[20] Mansbridge R.J., Blake J.S., Collins C., The effect of whole linseed or xylose treated whole linseed on dairy cow performance and level of the fatty acids C18:3, C20:5 and C22:6 in milk fat, in: Agnew R.E., Agnew K.W., Fearon A.M. (Eds.), Milk Composition, BSAS Occasional Publication No. 25, British Society of Animal Science, Penicuik, UK, 2000.

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

[22] McCance R.A., Widdowson E.M., Fatty Acids, Seventh Supplement to the 5th Edition of the Composition of Foods, The Royal Society of Chemistry, Cambridge and MAFF, London, 1998.

[23] McDonald I.W., Scott T.W., Foods of ruminant origin with elevated content of polyunsaturated fatty acids, World Rev. Nutr. Diet. 26 (1977) 107–144.

[24] 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.

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

[26] 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.

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

[28] Scollan N.D., Fisher W.J., Davies D.W.R., Fisher A.V., Enser M., Wood J.D., Manipulating the fatty acid composition of muscle in beef cattle, Proceedings of the British Society of Animal Science, 1997, p. 22.

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

[30] Van Soest P.J., Robertson J.B., Lewis B.A., Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition, J. Dairy Sci. 74 (1991) 3583–3597.

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