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Running head: Saturated fat-reduced dairy products

ABSTRACT

Lactating cow diets were supplemented with high oleic acid sunflower oil over two production periods spanning two years, to modify the milk fat, partially replacing saturated fatty acids (SFA) with *cis*-monounsaturated fatty acids (MUFA). The resulting milk was used for ultra-high temperature (UHT) milk, butter and Cheddar cheese production, and fatty acid profiles were compared with those of conventionally-produced products. Fat from products made with modified milk had lower SFA and higher *cis*- and *trans*-MUFA concentrations than that of conventional products. This was consistent over production periods, demonstrating that this food chain approach could be adopted on a wider scale.

Keywords: Bovine milk, fatty acids, lipids, milk processing

INTRODUCTION

Milk and dairy products are a major source of fat and fatty acids (FA) in the UK adult diet, contributing to 18 % total fat, 28 % saturated fatty acid (SFA), 38 % *trans* fatty acid (TFA) intake (Bates *et al*., 2016) and 12 % *cis*-monounsaturated fatty acid (MUFA) intake (Hobbs, personal communication). At the population level total SFA intake exceeds current recommendations (12.1 % total energy intake [EI]; Bates *et al*., 2016 vs <10 % total EI; WHO, 2010). A reduction in dietary TFA from industrially hydrogenated food sources has led to an increase in the contribution of these FAs from dairy products but overall TFA consumption has declined (Henderson *et al*., 2003; Bates *et al*., 2016) and is below the maximum recommended intake of 2 % total energy (SACN, 2007).

Replacing dietary SFA with *cis*-MUFA and *cis*-polyunsaturated fatty acids (PUFA) has been shown to reduce cardiovascular disease (CVD) risk factors, including fasting serum total and

low density lipoprotein-cholesterol concentrations and total:high density lipoprotein-cholesterol ratio (Vafeiadou *et al*., 2015). The most effective means of replacing SFA with unsaturated FA in milk fat is by altering the dairy cow diet (Kliem & Shingfield, 2016). Due to the more extensive rumen biohydrogenation of dietary PUFA compared with *cis*-MUFA (Shingfield *et al*., 2008), and the greater proportion of *cis*-MUFA compared with PUFA in milk fat (Kliem *et al*., 2013), replacement of SFA with *cis*-MUFA offers a greater potential for SFA reduction in milk fat. Relatively few studies have assessed the impact of consuming dairy products modified using this dietary strategy (replacing milk SFA with either MUFA or PUFA) on cardiovascular health outcomes in humans (Livingstone *et al*., 2012). Some of these studies used only butter as the test dairy product, thus not representing the nutritional composition of a range of dairy products and food matrices (Livingstone et al., 2012). Two studies (Noakes *et al*., 1996; Seidel *et al*., 2005) included a range of dairy products modified by changing the cow diet in their interventions, but the human interventions only lasted for relatively short periods of time (up to three weeks) and involved small numbers of participants. It is not known whether the FA composition of milk and dairy products produced by changing the cow diet would be consistent over longer periods of time, especially as milk FA response to dietary oilseeds appears to vary according to differences in other dietary nutrients (Lerch *et al*., 2012a).

The main objectives of this study were twofold; firstly to identify whether UHT milk, butter and Cheddar cheese could be produced with the same FA profile over a two-year period, from milk produced using an oleic-acid rich supplementation strategy. This utilised a high oleic acid (*cis*-9 18:1) sunflower oil to maximise the replacement of SFA with MUFA, after previous research highlighted the potential of these oils (Loor *et al*., 2002; Kliem *et al*., 2011). The second objective was to compare the FA composition of the modified dairy products with conventional products containing FA profiles typical of UK retail milk during winter months

(average SFA 71.5 g/100 g total FA, *cis*-MUFA 21.2 g/100 g FA; Kliem *et al*., 2013), and for this to be consistent across products.

MATERIALS AND METHODS

Production of modified milk

Between December 2013 and May 2015, groups of multiparous Holstein-Friesian cows were fed a diet which resulted in modified milk. For the purposes of the human intervention trial that this study formed part of (Markey et al., 2017), milk production was divided into two periods; Production period 1 (P1) took place between December 2013 and September 2014, involving a total of 58 cows (mean ± standard error parity 4.0 ± 0.12; milk yield at start 35.0 ± 0.77 litres/day, and days in lactation 181 ± 7.7), producing a total of approximately 12,500 litres of milk which were used to produce modified UHT milk, butter and cheese. Production period 2 (P2) took place between November 2014 and May 2015, involving a total of 41 cows (mean ± standard error parity 4.0 ± 0.12; milk yield at start 33.4 ± 0.91 litres/day, and days in lactation 205 ± 7.2) producing a total of 16,350 litres of milk. Cows selected to produce modified milk were adapted to a total mixed ration (TMR) diet, an example of which is presented in Table 1. Dietary ingredients were replicated for each production batch. The diet had a forage:concentrate ratio of 50:50 on a DM basis, with the forage consisting of maize silage, grass silage, grass hay and wheat straw. The diet was supplemented with 43 g/kg dry matter (DM) of high oleic acid sunflower oil (AAK Ltd., Hull, East Yorkshire, HU9 5PX, UK) so that it would supply a cow consuming 23 kg DM per day with 1 kg oil. The cows were adapted to this diet for a period of four weeks before any milk collection was made. Following this adaptation period, subsamples of modified milk were taken and preserved with potassium dichromate (1 mg/ml; Lactabs; Thompson and Capper, Runcorn, UK) for milk compositional

analysis. A further subsample was frozen to measure the FA profile prior to product manufacture.

Manufacture of dairy products

UHT Milk Processing. UHT milk, i.e. both modified and conventionally-produced milk, was produced three times, twice during P1 and once during P2. The first P1 production run was carried out at Reaseheath College (Nantwich, UK) with the remaining production runs carried out at Frampton's Ltd (Shepton Mallet Somerset, UK). Raw conventional milk was provided by Arla Foods UK (Taw Valley Creamery, North Tawton, UK), and represented retail milk with a typical UK winter dairy FA profile. Conventional milk was standardised to match the fat content of the modified milk using skimmed milk provided by A.E Rodda & Son Ltd., (Redruth, Cornwall, UK), for the purposes of the human intervention study. Raw conventional milk was pumped to a tank and skimmed milk, at the level required to match the low SFA milk fat content, was added. The milk was agitated in the tank for 5 minutes and a sample removed and analysed to ensure the fat content was correct. UHT processing was carried out in a pilot scale UHT plant. The milk was preheated to 85ºC using a plate heat exchanger and homogenised. The milk was then heated to 142ºC for 5 seconds using direct steam infusion and cooled to 15ºC and aseptically packaged into 5 kg aseptic bags (Reaseheath) or 330 ml aseptic cartons (Framptons). All UHT milk was stored at 4ºC until required.

Cheddar cheese processing. Conventional Cheddar cheese was provided by Arla Foods UK (Taw Valley Creamery), to represent retail mild (3 month) Cheddar cheese with a typical UK winter dairy FA profile. Modified Cheddar cheese was manufactured at the University of Reading Pilot Plant (Reading, UK). Both cheese types were produced only once during P1 and P2. The processing parameters for the modified cheese were selected to mimic the commercial

process used at Arla. Modified raw milk was pasteurised at 73ºC for 15 seconds in a high-temperature short-time pasteuriser (flow rate 300 L/h) using a plate heat exchanger. The milk was cooled to 32ºC and transferred to 100 L cheese vats. Starter culture **(**R 604, Chr. Hansen) was added (0.15 g/L) to the vat and allowed to ripen under stirring for 50 minutes. Enzyme (CHY-MAX, Chr. Hansen) was added (0.24 ml/L) and stirred for a further 3 minutes, before the stirrers were removed. Cutting time was 40 minutes after enzyme addition and was visually confirmed by the cheesemaker. The coagulum was cut by hand using coagulum cutting knives. Stirring commenced, and the temperature was increased slowly until it reached 38ºC; this scalding process continued for 1 h. Whey was subsequently drawn off the cheese vat. The resulting curd underwent a cheddaring process by piling and turning the curd 4 times. The curd was then milled using a cheese mill and dry salted (0.02 kg/kg) and placed into a stainless steel cheese mould. The mould was placed in a horizontal cheese press and pressed at 7 kPa overnight. The next day the cheese was vacuum packed and placed in an 8ºC ripening room for three months. After ripening, cheese was apportioned into 350 g, vacuum packed and stored at 2ºC.

Butter processing. Conventional butter was provided by Arla Foods UK (Taw Valley Creamery) from winter butter stocks during P1 and P2. Modified P1 butter was manufactured at Reaseheath College (Nantwich, UK) and modified P2 butter was manufactured at Ty Tanglwyst Dairy (Bridgend, South Wales). Two batches of modified butter were manufactured in each period. In both cases the cream was separated from the milk using a disc bowl separator, pasteurised, and aged at 4ºC overnight. The cream was transferred to a churn and churned until butter grains were formed. The buttermilk was drained off and the butter grains were further worked to create a continuous emulsion. Salt was then added (1.7 g/100 g) and the butter was

150 further worked to ensure even distribution of the salt. The butter was apportioned into 250 g,
151 packaged in butter wrap and stored at -20°C until required.
152
153 **Chemical analysis of milk and dairy products**
154 A sample of high oleic acid sunflower oil used during P1 and P2 and subsample of the TMR
155 diet were analysed in duplicate for FA profile using a modified version of the one step
156 transesterification method of Sukhija & Palmquist, (1988). Briefly, 50 mg oil or 300 mg TMR
157 was incubated with an internal standard (methyl heneicosanoate, Sigma Aldrich Company Ltd.,
158 Dorset, UK) at 60°C in the presence of 0.4 M sulphuric acid in methanol and toluene as an
159 extraction solvent, for 2 h (oil) or 3 h (TMR). Following neutralisation, the resulting fatty acid
160 methyl esters (FAME) in toluene were allowed to stand over sodium sulphate for 30 min to
161 remove methanol residues before being quantified by gas chromatography (GC; Bruker 350,
162 Bruker, Germany). The GC was equipped with a flame ionisation detector and 100 m fused
163 silica capillary column (CP-SIL 88, Agilent Technologies, Cheshire, UK), and GC conditions
164 were as published previously (Kliem *et al*., 2013). Carbon deficiency in the flame ionization
165 detector response for FAME containing 4- to 10-carbon atoms was accounted for using a
166 combined correction factor which also converted FAME to FA (Ulberth *et al*., 1999). FA were
167 quantified using internal standard peak area, and the results were also expressed as g/100 g FA.
168
169 Samples of milk taken just prior to dairy product production (modified and conventional) were
170 analysed for FA profile according to the method of Kliem *et al*. (2013). Briefly, lipid in 1 ml
171 thawed, warmed (to 40°C) milk was extracted in duplicate using a mixture of diethyl ether and
172 hexane (IDF 1: 2010 [E], International Dairy Federation, 2010, Brussels, Belgium) and extracts
173 were transesterified to FAME according to previously described procedures (Kliem *et al*.,
174 2013). GC conditions and FAME identification were as described above. Methyl esters not

175 available as authentic standards were identified by gas chromatography-mass spectrometry
176 (GC-MS; Thermo Trace GC coupled to ITQ 1100 mass spectrometer using helium as a carrier
177 gas) analysis of 4, 4-dimethyloxazoline (DMOX) derivatives prepared from FAME.
178 Preparation of DMOX derivatives and parameters used for GC-MS analysis were largely in
179 accordance with earlier reports (Shingfield *et al.*, 2006), however a split ratio of 1:14 was used
180 for injection and the online reference library of DMOX electron impact ionisation spectra was
181 http://lipidlibrary.aocs.org. The results were expressed as g/100 g FA. Lipid in 50 mg of
182 conventional and modified butter was first warmed to 40°C before 1 ml distilled water (room
183 temperature) added, and mixed vigorously to emulsify the butter fat. Extraction and
184 methylation continued as with milk and the results were expressed as g/100 g total FA. The
185 lipid in 3 g of conventional and modified cheese was firstly hydrolysed using 100 ml 3M HCl,
186 and the resulting residue filtered through Whatman 1 filter paper prior to drying at 60°C for 18
187 h. The lipid was extracted from the residue using petroleum ether (Brown & Mueller-Harvey,
188 1999), and the amount of lipid calculated gravimetrically. The lipid was then gently warmed
189 before 50 mg was transferred to clean glass tubes, and methylation of extracted FA was
190 conducted as for milk and butter.
191
192 Nutritional analysis (energy, protein, fat, carbohydrate, ash and moisture) of the dairy products
193 from each cohort was conducted in duplicate by SGS United Kingdom Ltd. (ISO 17025
194 accredited laboratory; Ellesmere Port, Cheshire, UK). To calculate protein content, the
195 obtained nitrogen result was multiplied by the standard dairy nitrogen conversion factor (6.38)
196 to account for the fraction of non-protein nitrogen in each sample (Maubois & Lorient,
197 2016). Micronutrient content analysis (calcium, magnesium, sodium and phosphorus) was
198 conducted in duplicate by inductively coupled plasma-optical emission spectrometry

(Quaternary Scientific [QUEST], University of Reading, Berkshire, UK). The results were expressed on a dry and fresh weight basis.

Data analysis

FA composition of all products was analysed using an ANOVA (Minitab17), and included effects of product type, production period, treatment, and period by treatment interactions. Product type was not significant for every FA, so it was removed from the model. Least squares means (± pooled standard error of the mean) are reported, and differences were considered significant at $P < 0.01$ to account for multiplicity.

RESULTS

The FA profile of the high oleic acid sunflower oil used during production P1 and P2 is presented in Table 2. There was a minimal change in FA profile during P2, when it contained a lower proportion of *cis*-9 18:1 and higher proportion of both 16:0 and 18:2 n-6.

The micronutrient composition of the dairy products is presented in Table 3. Although differences between periods could not be statistically analysed due to sample size, there was numerically little difference between the two periods.

The dairy product FA data presented are means across milk, cheese and butter. Lipid from modified dairy products had a lower ($P<0.001$) total SFA content than lipid from conventional dairy products, including a lower (12 g/100 g FA) concentration of 16:0, but also all SFA ≤ 14:0 including branched chain SFA such as 13:0 anteiso, 14:0 iso and 15:0 anteiso (Table 4). In contrast, the concentration of 18:0 was higher ($P<0.001$) in the modified dairy products.

There was no effect of production period on SFA content apart from 17:0 iso and 20:0 which were more abundant in P1 products ($P<0.01$).

Treatment had an effect on both *cis*- and *trans*- MUFA (Table 4), with lipid from modified dairy products having a higher ($P<0.001$) concentration of both. Of the *cis*-MUFA, *cis*-9 18:1 was the most abundant (Table 5), and concentration was at least 50 % greater in modified dairy compared with conventional products (Table 5). There were also notable differences in most of the other 18:1 isomers identified, modified products containing higher ($P<0.05$) lipid concentrations of *cis*-11, *cis*-13, *trans*-6-8, *trans*-9, *trans*-10, *trans*-12, *trans*-15 and *trans*-16 18:1 (the predominant isomer switching from *trans*-11 18:1 in control products to *trans*-10 18:1 in modified products). Aside from the 18:1 isomers, there were treatment differences in *cis*-9 10:1, *cis*-9 12:1, *trans*-9 14:1, *cis*-13 16:1 and *cis*-9 17:1, all of which were lower ($P<0.01$) in modified dairy products (Table 4).

Total n-6 and n-3 PUFA concentrations were not different between lipid from conventional and modified products. In contrast, the concentration of total conjugated linoleic acid (CLA) isomers was greater ($P=0.001$) in modified products (Table 4). Of the non-methylene interrupted 18:2 isomers, *cis*-9, *trans*-13 18:2, *cis*-9, *trans*-14 18:2 and *cis*-9, *trans*-12 18:2 were higher ($P<0.01$) in concentration in modified products (Table 6). There was an effect of period for *cis*-9, *trans*-13, which was more abundant ($P<0.05$) in P1 products (Table 6).

DISCUSSION

One of the main challenges of food chain interventions is to maintain a supply of the test food that is consistently different to the conventional product over the period of the study. The current study produced greater volumes of modified dairy products over a longer period of time

than other published studies (Livingstone *et al*., 2012). It has been reported previously that the milk FA profile resulting from supplementation with oilseeds can be affected by the chemical composition of other dietary constituents such as starch, or changes in DM intake (Lerch *et al*., 2012a). Therefore, it is important to demonstrate that these strategies produce similar products over time, for the purposes of both controlled human dietary intervention studies, and also for future commercial application and consumer consumption.

Overall there was little difference in most micronutrient contents of both the conventional and modified milk and dairy products, and this was the same over the two production periods. However, the modified milk had a low fat content (average 28 g/kg during period 1, 23 g/kg during period 2) prior to processing, which meant it was necessary to standardise the raw conventional milk prior to UHT so that the fat content matched that of the modified milk for the purposes of the human intervention study. Feeding unsaturated oils to dairy cows often suppresses milk fat concentration (Halmemies-Beauchet-Filleau *et al*., 2011), mainly due to the inhibitory effect of intermediates of rumen biohydrogenation on mammary FA synthesis (Bauman *et al*., 2011). In the current study, modified cheese was numerically lower (-5.6 g/100 g) in fat content than the conventional cheese, probably due to the raw modified milk being lower in total fat. A study reporting the fat content of Mozzarella cheese made from milk where cows were fed incremental amounts of a linseed supplement reported no linear effect on cheese lipid content (Oeffner *et al*., 2013). In that study an extruded linseed supplement was fed, which may have afforded the constituent oil some degree of protection from rumen biohydrogenation. This suggests that a more suitable approach for production of modified milk on a commercial scale may be to use oilseed supplements that are protected from rumen biohydrogenation.

Despite being produced across two production periods, there was no effect of production period on the main FA in modified and conventional products. Decreases in milk fat concentrations of *de novo*-synthesised SFA and increases in 18:0 and *cis*-9 18:1 following oilseed supplementation were found to be comparable over two consecutive lactations, but changes in most *trans*-18:1 and 18:2 isomers varied depending on year (Lerch *et al*., 2012b). This was probably due to differences in starch contents of the experimental diets across the two years (Lerch *et al*., 2012a). Transient changes in concentration of certain CLA and *trans*-18:1 isomers have been observed in response to oilseed supplementation over shorter periods of time (Roy *et al*., 2006). However the current study employed a dietary adaptation period of 4 weeks prior to milk collection, which should have minimised any variation and successfully produced consistent modified milk essential for effective utilisation in the following human intervention study.

There were differences in most individual FA concentrations between conventional and modified products. All short (4:0-10:0) and medium (12:0-16:0) chain SFA were lower in modified products, which was balanced by higher 18:0, *cis*-9 18:1 and intermediates of biohydrogenation concentrations which supports previous studies (Loor *et al*., 2002; Kliem *et al*., 2011). A meta-analysis of recent data reported that the majority of the milk SFA responses to plant oil supplements in dairy cow diets is due to decreases in 12:0, 14:0 and 16:0 concentrations (Kliem & Shingfield, 2016). This can mainly be attributed to increases in the supply of $\geq$ 16 carbon FA leaving the rumen and inhibiting acetyl CoA carboxylase transcription and activity in the mammary gland (Barber *et al*., 1997).

SFA in modified milk were mostly replaced with *cis*-9 18:1, which was the predominant FA in the oil supplement. The current study aimed to feed 1 kg oil/cow/day, which equated to around

800 g additional *cis*-9 18:1. In this unprotected oil form it would be expected that some rumen biohydrogenation of *cis*-9 18:1 would occur as has been reported in detailed *in vivo* studies (e.g. Loor *et al*., 2002), however there was a 10 g/100 g FA difference in *cis*-9 18:1 concentration between conventional and modified products in the current study, in line with previous studies (Kliem *et al*., 2011). Milk fat *cis*-9 18:1 is derived from two sources – diet and endogenous desaturation of 18:0 by mammary epithelial cell Δ^9-desaturase. One *in vivo* study estimated that this enzyme was responsible for almost 60 % of *cis*-9 18:1 present in milk fat (Mosley & McGuire, 2007). Dairy products from the current study contained a higher concentration of 18:0 than conventional products, which may have contributed to the increase in milk fat *cis*-9 18:1.

A proportion of dairy SFA was also replaced by TFA, which are intermediates of rumen biohydrogenation of dietary unsaturated FA (Harfoot & Hazlewood, 1997). The majority of TFA identified in the current study were *trans* 18:1 isomers. *In vitro* studies have established that *cis*-9 18:1 is converted to a range of *trans* 18:1 isomers during incubation with rumen-derived microorganisms (McKain *et al*., 2010). Furthermore, *trans*-9 18:1 has been shown to further isomerise during *in vitro* incubation with rumen bacteria to a range of positional *trans* 18:1 isomers (Proell *et al*., 2002). The predominant isomer in the conventional dairy products was *trans*-11 18:1, but in the modified products, *trans*-10 18:1 predominated. *Trans*-10 18:1 is thought to arise as an intermediate of 18:2 n-6 biohydrogenation in response to changed rumen conditions on certain diets (Bauman *et al*., 2011), such as those which lower rumen pH (Palmquist *et al*., 2005). A recent review concluded that the predominance of *trans*-10 18:1 in ruminant products is more common than previously thought (Aldai *et al*., 2013), however very few milk-based studies have resulted in concentrations of *trans*-10 18:1 at the level observed in the current study. Roy *et al*. (2006) and Shingfield *et al*. (2005) both reported relatively high

concentrations of *trans*-10 18:1 in milk fat after feeding oilseed supplements (18 and 13 g/100 g total FA, respectively), with suggested possible reasons being increased supply of 18:2 n-6 and low rumen pH. In the current study, 18:2 n-6 content of the oil was low, so the high *trans*-10 18:1 content of modified milk would have been primarily due to isomerisation of *cis*-9 18:1 within the rumen. This was suggested after a similar effect was observed when olive oil (high in *cis*-9 18:1) was fed to dairy sheep (Gomez-Cortes *et al*., 2008). *Trans*-10 18:1 has often been thought partially responsible for milk fat depression, but studies involving abomasally-infused *trans*-10 18:1 have reported inconsistent results (Lock *et al*., 2006; Shingfield *et al*., 2009)

Enriched concentrations of CLA in milk are usually due to an increased supply of 18:2 n-6 or 18:3 n-3 to the rumen, biohydrogenation of which results in increased *trans*-11 18:1 available to mammary Δ^9 desaturase and therefore increased *cis*-9, *trans*-11 CLA, the predominant isomer (Palmquist *et al*., 2005). In the current study modified products had a higher overall CLA concentration than conventional products, but there was no difference in *trans*-11 18:1. Either desaturation of *trans*-11 18:1 was extremely efficient, or the increase in CLA concentration observed was due to increases in other CLA isomers.

The observed difference in SFA and MUFA content between conventional and modified products is comparable to that of previous studies (Livingstone *et al*., 2012). However few previous studies reported a detailed FA profile. The studies of Tholstrup *et al*. (2006) and Lacroix *et al*. (2012) were specifically designed to observe the effects of increased ruminant-derived TFA, and as such the modified products contained enriched concentrations of *trans*-11 18:1 in particular. The current study reported a greater concentration of *trans*-10 18:1 in modified products and the potential for these TFA to impact on CVD risk factors remains to be investigated.

CONCLUSIONS

In conclusion, feeding a specially formulated diet containing a high oleic acid sunflower oil to dairy cows over two production periods resulted in dairy products with the same FA profile, lower in SFA (including 12:0, 14:0 and 16:0), and higher in *cis*- and *trans*-MUFA (particularly *cis*-9 18:1 and *trans*-10 18:1) than conventionally-produced dairy products. Processing the modified milk into UHT milk, butter and Cheddar cheese had minor effects on FA profile. This technique is therefore suitable for the production of modified dairy products, to replicate the FA profile, suitable for use in large-scale human intervention studies, where composition consistency is required over a longer period of time (as is required to assess effects on CVD risk markers). This technique may also suitable for production of modified dairy foods on a commercial scale, although the effect of this dietary strategy on milk fat content should be considered.

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514 **Table 1**. Ingredients and analysed chemical composition of the cow diet used during
515 production period 1 (g/kg dry matter (DM) or as stated)

	g/kg DM
Ingredients	
Maize silage	350
Grass silage	52
Grass hay	33
Straw	33
Wheat by-product[1]	86
Concentrate mix[2]	372
Calcium salts of palm oil distillate[3]	11
Salt[4]	4
Limestone	4
Minerals and vitamins	11
High oleic acid sunflower oil[5]	43
Chemical composition	
DM (g/kg fresh)	515
Organic matter	932
Crude protein	144
Neutral detergent fibre	351
Acid detergent fibre	199
Starch	212
Oil	70.0
ME (MJ/kg DM)[6]	12.6
Fatty acids	
16:0	2.9
18:0	0.9
18:1 *cis*-9	14.6
18:2 n-6	6.1
18:3 n-3	0.05

516 [1]CTraffordgold®; KW Alternative Feeds Ltd., Barrow Hill Barns, Andover, SP11 7RG, UK

517 [2] Containing (g/kg DM): Cracked wheat, 180; Soyabean meal 160; Rapeseed meal, 175;
518 Sugar beet feed, 140; Wheat distillers, 140; Soya hulls, 120; Molasses, 33; Megalac®, 17;
519 Urea, 11; Minerals [KW Alternative Feeds Ltd., Barrow Hill Barns, Andover, SP11 7RG,
520 UK], 22).

521 [3] Megalac®; Volac International Ltd., Royston, Hertfordshire, SG8 5QX, UK

522 [4] Dairy Direct, Church Farm, Bury St Edmunds, IP28 6PX, UK.

523 [5]AAK (UK) Ltd., Hull, East Yorkshire, HU9 5PX, UK.

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525 **Table 2**. Fatty acid composition of the high oleic acid sunflower oil used during the two

526 production periods (g/100 g total fatty acids)

Fatty acid	*Production period 1*	*Production period 2*
16:0	3.6	4.1
18:0	3.1	2.7
18:1 *cis*-9	82.1	80.4
18:2 n-6	7.5	10.1
18:3 n-3	0.26	0.27

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541 **Table 3.** Micronutrient composition of the modified and conventional dairy products (average across production periods 1 and 2; units as stated,

542 means ± product s.e.m.)

	UHT milk			Butter			Cheddar cheese		
	Conventional	Modified	s.e.m.	Conventional	Modified	s.e.m.	Conventional	Modified	s.e.m.
Energy (kJ/100 g)	220	232	6.5	3037	3024	12.1	1688	1527	46.5
Energy (kcal/100 g)	52.5	55.4	1.56	739	735	2.9	407	368	11.4
Total carbohydrate (g/100 g)	4.4	4.7	0.09	1.93	0.98	0.332	3.1	2.6	0.22
Ash (g/100 g)	0.72	0.77	0.014	1.8	1.6	0.08	4.0	3.9	0.09
Moisture (g/100 g)	88.8	88.3	0.20	14.9	16.0	0.37	36.2	39.2	0.92
Nitrogen (g/100 g)	0.47	0.51	0.015	0.04	0.07	0.011	3.7	4.2	0.16
Protein (g/100 g)[1]	3.0	3.3	0.09	0.23	0.44	0.070	23.1	26.4	1.00
Fat (g/100 g)	2.5	2.6	0.14	81.1	81.1	0.37	33.6	28.0	1.62

Calcium (mg/100 g dry)	1147	1090	21.9	19.1	20.6	1.60	1242	1428	87.7
(mg/100 g fresh)	126	119	4.5	17.0	17.9	1.33	801	911	47.0
Magnesium (mg/100 g dry)	105	101	1.3	2.1	2.1	0.10	46.6	47.4	0.72
(mg/100 g fresh)	11.5	11.1	0.32	1.9	1.8	0.09	30.1	30.3	0.52
Sodium (mg/100 g dry)	378	417	17.8	795	577	72.6	1123	1187	59.3
(mg/100 g fresh)	41.4	45.5	2.18	707	502	66.9	726	760	43.1
Phosphorus (mg/100 g dry)	896	819	27.5	25.4	27.0	1.73	836	939	51.9
(mg/100 g fresh)	98.1	89.2	3.66	22.6	23.5	1.43	539	599	27.0

[1]Calculated by nitrogen conversion factor 6.38 (Maubois & Lorient, 2016)

545 **Table 4**. Mean fatty acid composition of the lipid from all modified and conventional dairy products over two production periods (g/100 g total

546 fatty acids, least squares means for milk, butter and cheese ± s.e.m.)

	Period 1		Period 2		s.e.m.[1]	P[2]		
	Conventional	Modified	Conventional	Modified		Production period	Treatment	Interaction
4:0	2.9	2.2	2.9	2.1	0.06	0.668	<0.001	0.124
6:0	1.8	1.1	1.9	1.0	0.04	0.732	<0.001	0.057
8:0	1.11	0.62	1.14	0.54	0.030	0.420	<0.001	0.088
10:0	2.7	1.5	2.8	1.2	0.07	0.056	<0.001	0.033
10:1 *cis*-9	0.28	0.13	0.29	0.13	0.011	0.727	<0.001	0.547
11:0	0.053	0.004	0.025	0.002	0.0138	0.329	0.031	0.375
12:0	3.5	2.0	3.5	1.6	0.13	0.194	<0.001	0.133
12:1 *cis*-9	0.10	0.05	0.10	0.05	0.005	0.893	<0.001	0.453
13:0	0.10	0.05	0.09	0.05	0.010	0.601	0.003	0.859
13:0 anteiso	0.09	0.06	0.09	0.05	0.004	0.618	<0.001	0.418
14:0	11.3	8.5	11.4	7.4	0.30	0.188	<0.001	0.077

14:0 iso	0.08	0.07	0.09	0.07	0.005	0.669	0.008	0.842
14:1 *cis*-9	1.02	0.95	1.04	1.01	0.041	0.366	0.231	0.663
14:1 *trans*-9	0.22	0.16	0.24	0.17	0.010	0.210	<0.001	0.686
15:0	1.10	0.71	1.14	0.71	0.038	0.613	<0.001	0.585
15:0 anteiso	0.43	0.38	0.42	0.35	0.008	0.021	<0.001	0.406
16:0	33.3	20.9	33.8	22.2	0.69	0.220	<0.001	0.602
16:0 iso	0.20	0.19	0.20	0.20	0.010	0.650	0.721	0.629
16:1 *cis*-9 + 17:0 anteiso	1.7	1.6	1.5	1.5	0.07	0.050	0.596	0.340
16:1 *cis*-11	0.21	0.13	0.42	0.40	0.065	0.007	0.516	0.651
16:1 *cis*-13	0.15	0.05	0.17	0.05	0.011	0.412	<0.001	0.417
16:1 *trans*-9	0.03	0.10	0.10	0.05	0.101	0.377	0.001	0.640
17:0 iso	0.33	0.36	0.29	0.31	0.012	0.008	0.073	0.588
17:0	0.48	0.36	0.49	0.33	0.005	0.090	<0.001	0.013
17:1 *cis*-9	0.20	0.16	0.20	0.15	0.003	0.333	<0.001	0.089
18:0	9.6	14.0	9.4	13.0	0.32	0.089	<0.001	0.217
18:0 iso	0.03	0.02	0.06	0.03	0.008	0.026	0.019	0.026

∑ 18:1 *trans*	2.8	9.4	2.6	10.2	0.30	0.348	<0.001	0.130
∑ 18:1 *cis*	20.0	29.6	19.3	30.3	0.74	0.994	<0.001	0.407
∑ CLA[3]	0.57	0.71	0.59	0.98	0.065	0.195	0.001	0.025
∑ NMI[4] 18:2	2.1	2.3	2.1	2.5	0.17	0.605	0.084	0.857
18:3 n-3	0.32	0.23	0.40	0.30	0.032	0.060	0.020	0.872
19:0[5]	0.10	0.10	0.08	0.08	0.016	0.326	0.970	0.966
20:0	0.15	0.15	0.14	0.13	0.003	0.009	0.063	0.118
20:1 *cis*-9	0.10	0.10	0.09	0.08	0.009	0.130	0.604	0.394
20:1 *cis*-11	0.09	0.09	0.02	0.01	0.045	0.108	0.802	0.883
20:2 n-6	0.005	0.008	0.012	0.005	0.0066	0.771	0.719	0.447
20:3 n-6	0.06	0.07	0.07	0.06	0.008	0.634	0.255	0.918
20:4 n-6	0.10	0.07	0.10	0.06	0.007	0.625	0.001	0.300
20:5 n-3	0.05	0.01	0.05	0.02	0.008	0.488	0.002	0.321
22:0	0.05	0.08	0.05	0.08	0.007	0.851	0.003	0.370
22:2 n-6	0.020	0.006	0.034	0.003	0.0119	0.727	0.082	0.554
22:5 n-3	0.08	0.04	0.08	0.04	0.009	0.582	0.002	0.717

24:0	0.02	0.03	0.04	0.04	0.008	0.189	0.478	0.531
∑ SFA[6]	70.3	54.3	70.9	52.2	0.97	0.479	<0.001	0.204
∑ *cis*-MUFA[7]	23.0	32.2	22.5	32.9	0.67	0.932	<0.001	0.372
∑ *trans*-MUFA[7]	3.3	10.0	3.2	10.8	0.29	0.268	<0.001	0.125
∑ *trans* fatty acids	4.0	10.7	3.8	11.8	0.31	0.208	<0.001	0.082
∑ n-3 PUFA[8]	0.63	0.48	0.72	0.59	0.054	0.096	0.033	0.791
∑ n-6 PUFA[8]	1.7	1.9	1.9	2.0	0.18	0.547	0.578	0.865

[1] Standard error of the mean for n=12 measurements

[2] Refers to the significance of overall effect of period, treatment and their interaction

[3] CLA – conjugated linoleic acid

[4] NMI - non methylene-interrupted

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

[6] SFA – saturated fatty acids

[7] MUFA – monounsaturated fatty acids

[8] PUFA – polyunsaturated fatty acids

556 **Table 5**. Mean 18:1 isomer composition of the lipid from all modified and conventional dairy products over two production periods (g/100 g

557 total fatty acids, least squares means for milk, butter and cheese ± s.e.m.)

	Period 1		Period 2		s.e.m.[1]	P[2]		
	Conventional	Modified	Conventional	Modified		Production period	Treatment	Interaction
cis-9 18:1[3]	18.8	28.2	18.4	28.9	0.72	0.859	<0.001	0.439
cis-11 18:1	0.61	0.81	0.44	0.70	0.048	0.023	0.001	0.522
cis-12 18:1	0.22	0.16	0.21	0.19	0.016	0.467	0.042	0.318
cis-13 18:1	0.09	0.16	0.08	0.11	0.017	0.103	0.017	0.364
cis-16 18:1	0.06	0.06	0.05	0.04	0.005	0.091	0.454	0.294
trans-6, -7, -8 18:1	0.24	1.28	0.24	1.25	0.290	0.955	0.008	0.961
trans-9 18:1	0.20	0.94	0.18	1.39	0.119	0.102	<0.001	0.082
trans-10 18:1	0.36	4.01	0.41	2.49	0.413	0.113	<0.001	0.095
trans-11 18:1	1.10	0.94	0.85	1.81	0.428	0.490	0.375	0.227
trans-12 18:1	0.38	1.01	0.32	1.09	0.065	0.816	<0.001	0.310
trans-15 18:1	0.40	0.94	0.47	1.82	0.231	0.074	0.004	0.119

trans-16 18:1[4]	0.31	0.35	0.31	0.48	0.024	0.023	0.003	0.026

[1] Standard error of the mean for n=12 measurements

[2] Refers to the significance of overall effect of period, treatment and their interaction

[3] Co-elutes with 18:1 *trans*-13/14

[4] Co-elutes with 18:1 *cis*-14.

572 **Table 6**. Mean non-methylene-interrupted 18:2 isomer composition of the lipid from all modified and conventional dairy products over two
573 production periods (all values as mg/100 g total fatty acids, least square means for milk, butter and cheese ± s.e.m.)

	Period 1		Period 2		s.e.m.[1]	P[2]		
	Conventional	Modified	Conventional	Modified		Production period	Treatment	Interaction
cis-9, *trans*-13 18:2	182	238	177	334	11.4	0.004	<0.001	0.002
cis-10, *trans*-14 18:2	122.6	122.6	100.0	92.1	9.12	0.020	0.679	0.680
cis-9, *trans*-14 18:2	80.4	100.8	75.4	107.9	5.83	0.861	0.002	0.330
cis-9, *trans*-12 18:2	33.7	50.4	26.9	54.0	3.78	0.697	<0.001	0.206
trans-9, *cis*-12 18:2	7.5	8.2	46.0	15.5	10.80	0.067	0.205	0.187
trans-11, *cis*-15 18:2	122.5	105.5	66.7	134.4	23.56	0.585	0.312	0.110
cis-9, *cis*-12 18:2	1449	1629	1563	1686	141.3	0.561	0.316	0.845

574 [1] Standard error of the mean for n=12 measurements

575 [2] Refers to the significance of overall effect of period, treatment and their interaction

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