Fatty acid and fat soluble antioxidant concentrations in milk from high and low input conventional and organic systems; seasonal variation

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ABSTRACT

BACKGROUND: Previous studies showed differences in fatty acid (FA) and antioxidant profiles between organic and conventional milk. However, they did not **(a)** investigate seasonal differences, **(b)** include non-organic, low-input systems or **(c)** compare individual carotenoids, stereo-isomers of α-tocopherol or isomers of CLA. This survey-based study compares milk from 3 production systems: (i) high-input, conventional (10 farms), (ii) low input, organic (10 farms) and (iii) low input non-organic (5 farms). Samples were taken during the outdoor, grazing (78 samples) and indoor periods (31 samples).

RESULTS: During the outdoor grazing period, on average, milk from the low input systems had lower saturated FAs, but higher mono- and polyunsaturated FAs concentrations compared with milk from the high input system. Milk from both the low input organic and non-organic systems had significantly higher concentrations of nutritionally desirable FAs and antioxidants; conjugated linoleic (60 and 99% respectively) and α-linolenic (39 and 31% respectively) acids, α-tocopherol (33 and 50%) and carotenoids (33 and 80% respectively) compared with milk from the high input system. Milk composition differed significantly between the two low input systems during the second half of the grazing period only with milk from non-organic cows being higher in antioxidants, and conjugated linoleic acid, and that from organic cows in α-linolenic acid. In contrast, few significant differences in composition were detected between high input and low input organic systems when cows were housed.

CONCLUSIONS: Milk composition is affected by production systems by mechanisms likely to be linked to the stage and length of the grazing period, and diet composition, which will influence subsequent processing, sensory and potential nutritional qualities of the milk.

Key words: milk, low input farming, organic farming, fatty acid profiles

INTRODUCTION

The fatty acid (FA) and fat-soluble antioxidant composition in milk fat is known to affect processing and sensory quality of dairy products,[1, 2] and may also affect their nutritional value.[3-5]

The degree of saturation in milk fat has a bearing on the hardness, texture and taste of manufactured dairy products, particularly butter and cheese.[6] The presence of longer chain saturated fatty acids (SFA) increases the hardness of butter, whilst milk with a high proportion of unsaturated FA content (typical range 275-400g/kg fat) tends to give softer products (e.g. more spreadable butter). Unsaturated (especially polyunsaturated) FAs are also more prone to oxidation which results in the development of off-flavour and reduced shelf life in milk and dairy products.[6] However, the sensory quality and shelf life of milk and dairy products is determined by the balance of unsaturated FAs and fat soluble antioxidants, which protect against oxidation and off-flavour development.[6-8]

High dietary intakes of SFA (which account for 60 to 70% of milk fat) is a risk factor for development of obesity, cardiovascular diseases (CVD), impaired insulin sensitivity, and the 'metabolic syndrome'.[4] In contrast, dietary intake of certain unsaturated fatty acids, in particular conjugated linoleic acid (CLA) and omega-3 fatty acids (n-3 FA), and fat soluble antioxidants (e.g. α-tocopherol, carotenoids) has been linked to potential health benefits.[3, 9,10] CLA and n-3 FA have been shown to counteract the negative physiological effects of SFA and CLA has also been linked to anticancer properties, reduced risk of type 2 diabetes, CVD and enhanced immune function.[11-13] However, while CLA isomer C18:2 c9 t11 (CLA9) was only linked to beneficial health impacts, another CLA isomer C18:2 t10 c12 (CLA10) was also associated with some negative health impacts in cell culture and animal models.[13] In studies comparing the impact of different (e.g. organic and conventional) production systems on milk fat

composition, it is therefore important to compare concentrations of both CLA isomers. Most previous comparative studies (for example[14-16]) only reported concentrations of individual isomers or total CLA and also did not report concentrations of VA, the precursor for CLA. Milk contains significant concentrations of VA and since a proportion can be readily converted to CLA9 in the human body, the total potential CLA9 supply can only be estimated if both VA and CLA9 levels are known. [18]

Previous studies showed that the feeding regime has a major effect on the FA profiles of milk, but that other factors (including breed/genotype, stage and number of lactations) may also influence milk composition.[18-20] Dietary unsaturated fatty acids are likely to undergo hydrogenation by rumen microorganisms and long chain fatty acids may be subjected to desaturase activity in the mammary gland.[17-20] The FA profile of milk, therefore, is primarily determined by: (i) the balance of fatty acids in the diet, (ii) the extent of rumen hydrogenation, and (iii) mammary desaturase activity. CLA levels are linked to dietary supply of α-linolenic acid (αLA) and linoleic acid.[18] However, while 70 to 90% of CLA9, (which constitutes >70% of total CLA in milk) is generated from desaturation of VA in the mammary gland, all other CLA isomers (including CLA10) are generated as intermediates of rumen biohydrogenation and are therefore found at much lower concentrations than CLA9 in milk. [18]

Fat soluble antioxidants/vitamins present in milk are derived from dietary sources, either from (i) natural constituents in feed stuff (especially the forage component of the diet)[21] or (ii) synthetic compounds added as supplements to the diet of lactating cows[22]. Carotenoids derived from fresh forage are dominated by β-carotene, but also include lutein, zeaxantin, cryptoxanthin, lycopene and α-carotene.[23] The main vitamin E activity in fresh forage is associated with the RRR-isomer of α-tocopherol (the only isomer synthesised by plants), with some activity being associated with β, γ and δ tocopherol and α, β, γ and δ tocotrianol. [24]

Most high input conventional dairy production systems supplement diets with proprietary mineral and/or vitamin products containing A-vitamins, vitamin D_3 and E vitamins (in particular α-tocopherol); such supplements are prohibited in organic production. [25]

The naturally occurring RRR-isomer of α-tocopherol has a higher Vitamin E activity (1.49 IU/mg) than synthetic vitamin E (1.0 IU/mg), which contains equal proportions of the 8 different stereoisomers of α-tocopherol.[24] Synthetic α-tocopherol products are referred to as 'all rac' α tocopherol and consists mainly of 2R stereo-isomers. Synthetic α tocopherol is absorbed with the same efficiency as the RRR-stereoisomer of α tocopherol, but levels of uptake into key tissues (e.g. the brain) are lower. [24] Also, a recent study with dairy cows found higher α tocopherol concentrations in blood and milk following supplementation of RRR compared with 'all-rac' α-tocopherol and reported preferential transfer of RRR isomers into milk by cows receiving the synthetic isomer mix.[22]

Milk and dairy products from certified organic dairy production systems have been reported to contain higher concentrations of polyunsaturated fatty acids (PUFA), αLA (the main n-3 FA in milk), and/or CLA, and fat soluble antioxidants than those from high input conventional production.[14-16] These studies did not include non-organic, low-input systems in comparisons. However, an increasing number of dairy farms in Europe, New Zealand/Australia and North America, are adapting "lower input' production methods similar to those used in organic farming, but do not comply with all input restrictions prescribed by organic farming standards.[26] Most importantly these systems use mineral NPK fertilisers, but often at reduced levels compared with conventional high input systems. It is unclear whether such non-organic, low-input systems can provide similar benefits in milk composition to certified, organic dairy production systems.

Milk composition is known to change when switching from outdoor grazing to indoor forage-based diets in winter,[6, 12, 17, 27] however, little is known about whether this dietary change affects the differential in milk quality between organic and conventional systems reported previously.[14-16] There is also limited information on differences in the composition of fat soluble antioxidants in milk from high and low input dairy systems and the few studies available show contradictory results.[14, 28, 29] Such information would, however, be essential to assess (i) the overall nutritional value of milk from low input systems and (ii) whether the higher unsaturated fat content of organic milk (and associated risk of oxidation and off-flavour development) is compensated for by higher concentrations of antioxidants.

The objectives this study were therefore to: **(i)** compare the fatty acid and fat soluble antioxidant composition of milk from 3 UK production systems: certified-organic "low input" (O-LI), non-organic certified "low input" (NO-LI) and standard "high input" (HI) conventional production systems, during the outdoor grazing period, **(ii)** quantify differences in fatty acid and fat soluble antioxidant content of milk between O-LI and HI systems, during the winter indoor (conserved forage-based) feeding period and **(iii)** identify whether there are differences in milk composition between certified-organic "low input" (O-LI) and non-certified "low input" (NO-LI) systems that use spring block calving systems and graze cows outdoors throughout the lactation.

EXPERIMENTAL

Farm details and milk survey design

One hundred and nine milk samples were collected from 25 commercial farms categorised into 3 different production systems. Management and production parameters were recorded for each farm and sampling date using a standard questionnaire (see Table 1 for the most important parameters recorded). The number of

153 cows in early lactation (first 100 days) was also recorded. Live weights (LW) of cows
154 were estimated based on mean weights of breeds (Holstein Friesian = 650 kg; Jersey =
155 450 kg; Ayrshire = 550 kg; Brown Swiss and Scandinavian Red = 575 kg; [30]) and the
156 proportion of each breed in the genetic make-up of the herd. Total dry matter intakes
157 (DMI) were estimated from average milk yields (bulk tank contents divided by the
158 number of milking cows recorded by farmers) and assumed live weight (DMI = 0.025
159 LW+0.125 milk yield). Grazing was calculated at the herd level by difference: DMI
160 (Fresh grass) = total DMI – DMI (conserved forage + concentrate; recorded by
161 producers). Since cow live weight varied between farming systems, recorded levels of
162 dietary components were used to calculate proportions of total intake, to allow a more
163 relevant comparison between systems. Tables 1 and 2 list diet composition for each
164 production system during grazing and the housed periods of this study.
165
166 *Conventional, "high input" (HI) farms*. Ten farms were selected representing common
167 conventional production and feeding systems in the UK. HI farms used predominantly
168 pure ryegrass swards during the grazing period, winter diets based on grass silage and
169 higher concentrate : conserved forage ratio diets during the indoor feeding period than
170 LI-farms (see Tables 1 and 2 for the diets used during the outdoor grazing and indoor
171 feeding periods). The HI group did not include farms with extremely high input/output
172 system (e.g. farms which use more than 50% of the diet coming from concentrates,
173 regularly milk three times per day and/or those that house animals throughout their
174 lactation). All farms were all year round calving and had similar proportions of cows in
175 early lactation at all sampling dates.
176 *Organically-certified "low input" (O-LI) farms:* Ten farms were selected representing
177 two principle organic dairy systems found in the UK: **(a)** an all year round calving
178 system (5 farms) in which lactating cows are grazed when conditions allow (spring to

179 autumn), but fed on conserved forage-based diets during the winter indoor period (see
180 Table 1) and **(b)** a spring block calving system in which cows are grazed throughout
181 lactation (March to October) and were only indoors when not lactating between
182 November to February. All year round calving farms had similar proportions of cows in
183 early lactation at all sampling dates. Diets used in both organic systems were similar
184 during the outdoor grazing period (Table 1); all O-LI farms used mixed grass-clover
185 swards and did not apply mineral N or water-soluble P-fertilisers. Where appropriate,
186 on the basis of soil analyses, finely ground rock phosphate fertilisers were applied.
187 *Non-organically certified "low input" (NO-LI) farms.* Five farms representing the main
188 non-organic, "low input" system found in the UK were selected. All farms used a New
189 Zealand-type production system[26] with spring block calving, in which cows were grazed
190 throughout the lactation and no, or low, levels of concentrate and/or other feed
191 supplements included in the diet (see Table 1). As with the organic spring block
192 calving herds, cows were only housed when not lactating between November and
193 February. NO-LI farms selected used mixed grass-clover swards, but applied up to 120
194 kg N ha^{-1} $year^{-1}$ of mineral N and water-soluble P-fertiliser at levels determined from
195 soil analyses.
196 Samples were taken in August and October in 2004 and in January, March and May
197 in 2005 from all farms. In January 2005 samples could only be collected from O-LI and
198 HI farms that used all year round calving system. Samples of milk were taken from the
199 stirred bulk tank after 2 milkings (representing a 24 hours production period), at each
200 participating farm and frozen immediately after sampling and kept at -20˚C until
201 dispatched for analysis.
202
203 ***Extraction of fat from milk***

The extraction of fat from the milk was carried out as described by Havemose *et al*.,[23] with minor modifications. Milk fat was extracted from milk (2 mL) by adding methanol (2 mL) and chloroform (4 mL). The mixture was shaken vigorously for 1 minute then centrifuged for 10 minutes at 3000 g at 4°C. The lower phase containing the lipid fraction was isolated and evaporated to dryness under nitrogen.

Methylation of fatty acids from milk

The methylation of fatty acids extracted from the milk was carried out as described by Havemose *et al.*,[23] with minor modifications. Fat (approx. 10 mg) was dissolved in sodium methylate solution (2 g L^{-1} methanol) in sealed glass tubes filled with argon, incubated at 60°C for 30 minutes, and then cooled on ice. Saturated sodium chloride solution (4 mL) and pentane (1 mL) were added. The samples were mixed on a Vortex-mixer for 1 minute and centrifuged at 1700 x g for 10 minutes. The upper pentane phase was collected and used for gas chromatography-analysis.

Analysis of fatty acid composition by gas chromatography

Separation and quantification of the fatty acids isolated from milk was carried out as described by Havemose et al.,[23] with modifications. Samples (1 µL) of the pentane phase containing the fatty acid methyl esters were analysed by gas chromatography (HP6890 GC-system, Hewlett Packard Co., Palo Alto, CA, USA) with a flame-ionisation detector and a Supelco SI 2560 column (100 m x 0.25 mm x 0.20 µm, Supelco, Bellafonte, PA, USA). The inlet temperature was 275°C with a split ratio 40:1, and the carrier gas helium with a constant flow of 1,5 mL per minute. The starting temperature of 140°C was held for 5 minutes and increased by 4°C per minute to an end temperature of 240°C. The detector temperature was 300 °C.

The concentrations of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids and the ratio of n-3 and n-6 isomers of linolenic acid (C18:3) were then calculated as a proportion of total fatty acids recovered, based on the use of external standards To calculate the n-3 FA : n-6 FA ratio, the concentration of the main n-3 fatty acid (α-LA) was divided by the sum of the concentrations of the following n-6 FA isomers: 18:2 t9 t12, 18:2 t10 t12, 18:2 c9 c12, 18:3 c6 c9 c12 and 20:4 c5 c8 c11 c14.

Analysis of fat soluble antioxidant composition

Fat soluble antioxidants (α-tocopherol, β-carotene, lutein and zeaxantin) were analysed using the HPLC method described by Havemose *et al*.[23] Isomers of α-tocopherol were analysed using the methods described by Meglia *et al*.[22]

Statistical analysis

Linear mixed effects models [31] were used to investigate differences in milk quality parameters under the different systems (HI, O-LI and NO-LI). These models use two types of explanatory variables: fixed effects, that affect the mean of the response variable, and random effects, that affect the variance of the response. In these analyses, farm identifier was used as a random effect. Three sets of analyses were undertaken: **(i)** comparison of milk samples from all 3 systems (HI, O-LI and NO-LI) taken during the outdoor grazing period (samples from the spring block and all year calving organic farms were pooled, because no major differences could be detected in preliminary analyses; results not shown); **(ii)** comparison of samples taken from HI and all year calving O-LI farms during the indoor period when cows were on conserved forage-based diets and **(iii)** comparison of samples taken from spring block calving O-LI and NO-LI herds at 4 different sampling dates using a two–factorial model (system and

date), adapted to account for repeated measures from the 4 dates, to identify (a) if at any time during the grazing period, milk quality differed between the two LI systems and (b) interactions between the two factors for any of the milk quality parameters assessed. All proportion data were arcsine transformed prior to statistical analysis, but means presented were calculated from non-transformed data. Pairwise comparisons of means were carried out, where appropriate, using Tukey's Honest Significant difference tests.

All statistical analyses were carried out using the R statistical environment.[32]

RESULTS

Comparison of milk fat composition during the out-door period (fresh forage based diets).

On average the total fat content was higher in milk from low input systems, compared with the high input system, and was significantly higher for the NO-LI system compared with the high input system (Table 3.) When the composition of milk fat was compared, on average, the percentage of SFAs in milk fat was lower, while percentages of both MUFA (of which >80% was oleic acid C18:1 cis9) and PUFA were higher in milk from low input systems, compared with the high input system, and was significantly higher for the NO-LI system compared with the high input system (Table 3).

Percentages of the nutritionally desirable FAs (α-LA and CLA9) were significantly higher, while levels of total n-6 PUFAs were significantly lower in milk from both LI systems, when compared with milk from HI farms (Table 3). As a result, the n3 : n6 ratio was also higher in milk from LI-systems (Table 3). CLA10 was found in low concentrations in milk from all production systems and was not affected by production system (Table 3). Differences between O-LI and NO-LI were generally smaller than those between HI and LI systems, but the percentage of CLA was

significantly higher in milk from NO-LI systems and the percentage of total n-6 FA was significantly higher in milk from O-LI systems (Table 3).

The concentrations of most antioxidants (the RRR-stereoisomer of α-tocopherol, β-carotene, lutein, and zeaxanthin) were highest in milk from NO-LI, at intermediate concentrations in milk from O-LI and lowest in milk from HI systems (Table 3) during the outdoor period. Concentrations of the 2R-stereoisomer of α-tocopherol were not significantly different between systems, but were slightly lower in milk from NO-LI-systems.

Comparison of milk fat composition during the indoor period (conserved forage-based diets)

Since the spring, block-calving NO-LI and O-LI systems did not produce milk during the indoor period only milk from all year calving O-LI and HI systems could be compared.

In contrast to results from the outdoor rearing period, there were few differences in milk composition during the housed period. The percentages of total SFA in milk fat were significantly higher (4%) and MUFA significantly lower (10%) in milk from the O-LI system compared with milk from HI systems (Table 4). There was also a significantly lower (24 %) content of n-6 fatty acids and trends towards a higher content (38%) of α-linolenic acid ($p=0.052$) and a higher (30%) lutein content ($p=0.081$) in O-LI milk compared with HI milk (Table 4).

Comparison of milk fat composition during the grazing period between O-LI and NO-LI spring block calving dairy systems

Apart from CLA9 isomer (which was present in significantly higher percentages in milk from NO-LI farms on the August and May sampling dates), significant differences

in FA-composition between O-LI and NO-LI block calving systems were found only late in the outdoor grazing period (August and October sampling date, Figure 1). The percentages of total SFA and αLA were higher in milk from O-LI systems, while percentages of MUFA, PUFA, VA and CLA9 were higher in milk from NO-LI systems. No significant differences in the percentages of CLA10 and n-6 FAs were detected (data not shown). There were also significant interactions between LI production system and date for PUFA (p=0.020; Fig. 1c), VA (p=0.029; Fig 1e) and CLA (p=0.030; Fig 1f).

The concentration of most antioxidants changed significantly over time and at specific dates significant differences in the concentrations of individual antioxidants between the two LI-systems could be detected. Concentrations of 2R-toc were significantly higher milk from O-LI systems in May, while concentrations of 3R-toc were significantly higher in NO-LI systems in October. Levels of total and all three individual carotenoids were significantly higher in milk from NO-LI-systems in August and May (and for lutein also in October) (Fig. 2). A significant interactions between LI production system and date was only identified for the 2R-stereoisomer of α-tocopherol (p=0.003; Fig. 2a).

DISCUSSION AND CONCLUSIONS

Effect of feeding regimes on milk fat composition; outdoor grazing period

The finding of lower percentages of SFA and contrasting higher percentages of MUFA, in milk from the NO-LI system and higher PUFA (specifically α-LA and CLA9) and antioxidant content (α-tocopherol and carotenoids) of milk from both LI systems, compared with that from HI farms during the outdoor grazing period, is not surprising in view of the contrasting diets. The two low input systems used a high level of fresh forage (>80% of DMI), with only half that level (<40%) used in HI systems. Increasing the level of fresh forage by similar margins was previously shown to elevate

nutritionally desirable PUFA, CLA, α-LA and antioxidant percentages in milk [17-21, 27] to those found between milk from LI and HI systems here. For example, CLA concentrations were previously shown to increase with the proportion of fresh grass intake, while high proportions of maize silage and/or cereal-based concentrates reduced CLA content.[18, 19, 33] Also cutting and transport of grass to housed animals (a practice used to increase milk yield in zero-grazing systems) was also shown to decrease the CLA and VA content of milk by 50% and that of αLA content by 30% compared to milk from cows grazing pasture.[36] This response may have been due to rapid lipolysis of PUFA after harvest and/or a modification of rumen biohydrogentation.[27]

The finding that concentrations of CLA9 were significantly higher in milk from LI than HI systems, while concentrations of CLA10 were similar in both systems was likely to be caused by contrasting effects of LI and HI diets on the biosynthesis of CLA9 which is mainly (70-90%) generated from VA in the mammary gland, and that of CLA10 which is a minor intermediate of rumen biohydrogenation.[20] Previous studies have shown that VA in the rumen increases with increasing fresh forage and decreasing concentrate levels in dairy diets, while CLA10 generation in the rumen is relatively unaffected by changes in the diet except at very high levels of concentrate feeding. [17, 18]

The greater dietary contribution from fresh forage is also the most likely explanation of elevated levels of RRR tocopherol and carotenoids in milk from the LI herds during the grazing period, compared to the HI milk. Transfer of β carotene and α tocopherol into milk were reported to be directly proportional to dietary supply, being highest in spring grazing. [21]

Effect of feeding regimes on milk fat composition;indoor period

Few significant differences and trends in milk fat composition were found between HI and O-LI production systems during the indoor period when cows were fed conserved

forage-based diets. This may have been due to feeding regimes used by O-LI and HI herds being more similar during the indoor compared with the outdoor feeding period. The higher SFAs and lower MUFA content of organic milk during this feeding period are difficult to explain, since previous studies have shown that fresh forage intake (24% in organic as opposed to none in conventional winter diets) increases dietary PUFA supply[17, 27]. However, some previous studies have reported lower biohydrogenation rates for high concentrate indoor diets,[17, 18] suggesting that the higher proportion of concentrate in the HI diets results in lower biohydrogenation and thereby lower SFA and higher MUFA and that this effect overrides the effect of higher fresh forage intake in the O-LI animals. In order to allow milk from organic or "low input" production systems to be marketed as having "added nutritional value" throughout the year, efforts need to be made to achieve higher concentrations of at least some to the nutritionally desirable compounds during the indoor feeding period, if year round grazing is not an option. This could be achieved by supplementation of conserved forage based winter diet with oil seeds (e.g. rapeseed, linseed, sunflower seed) a practice shown to significantly improve α-LA, VA, CLA9 and/or fat soluble antioxidant concentrations in milk.[12, 18, 33, 35, 37, 38] Changes to the forage conservation methods may also increase the content of desirable FAs. For example, using hay rather then silage was also shown to increase the α-LA content in milk by up to 50%.[33, 34] It is interesting to note that in the UK it is very difficult to find farms feeding hay rather than silage, except among very traditional organic producers that work to biodynamic farming principles (which strongly recommend the use of hay for milking cows).

Effect of vitamin feed supplements on antioxidant concentrations in milk

Results of the study reported here suggest that the addition of synthetic vitamin/antioxidant supplements to feed in HI systems has a relatively minor effect on

385 antioxidant concentrations in milk. For example, milk from HI herds, which received
386 high levels of vitamin E supplements (in our study between 450 and 750 IUs vitamin
387 E/day) contained significantly lower concentrations of total α-tocopherol during grazing
388 than milk from farms working to organic farming standards, which do not permit feed
389 supplementation with synthetic vitamins. It is particularly interesting that the
390 concentration of the 2R-stereoisomer of α-tocopherol was not significantly higher in
391 milk from the HI systems. The 2R stereoisomers account for most of the α-tocopherol
392 in synthetic vitamin E supplements, but are virtually absent from natural sources of α-
393 tocopherol such as forage. This indicates either poor uptake of the 2R-stereoisomers in
394 the gastrointestinal system and/or preferential/selective uptake/transfer of 3R
395 stereoisomers from the blood into milk in the udder, as reported previously.[22]
396
397 ***Potential effects of seasonal forage composition and availability on milk fat***
398 Differences in milk quality (both fatty acid profiles and antioxidant levels) were also
399 detected between spring block calving O-LI and NO-LI systems which appeared to have
400 very similar dietary regimes. These were more likely due to variation in the composition
401 and/or total forage availability between the two systems over the season, since both
402 systems grazed cows throughout the lactation and used very low levels of
403 supplementary feeds such as conserved forage or concentrate. The finding that, in
404 August, milk from O-LI systems had higher percentages of α-LA than milk from NO-LI
405 systems is not surprising, and is likely to be due to a combination of two factors.
406 Firstly, the use of mineral (especially N) fertilisers in the NO-LI system, a practice
407 which has been shown to suppress the relative amounts of white clover in grass clover
408 swards [39, 40] and secondly**,** the impact of higher clover content causing elevation in
409 concentrations of n-3 FAs in milk compared with ryegrass.[27] However, it should be
410 noted that most of the studies reviewed by Dewhurst et al.[27] that compared the effect of

411 clover and rye grass used ensiled forage, where reduced lipolysis in clover would have a
412 greater influence over PUFA supply compared with fresh forage. The significantly
413 higher CLA and antioxidants in milk from NO-LI systems are more difficult to explain,
414 but may be related to differences in the nutritional composition of the herbage resulting
415 from the grazing systems used (e.g. the length of time allowed for pasture re-growth
416 between grazing periods), which has also been shown to affect the fatty acid
417 composition of milk.[27] Milk yields, protein and urea content in this study (data not
418 shown) did not differ at times when differences in milk fat composition were detected
419 between the two LI-systems. This suggests that differences in milk fat composition were
420 unlikely to be linked to contrasting energy or protein supply levels. However, since
421 sward composition and total forage availability was not monitored in the study reported
422 here this will have to be tested in future studies.
423
424 ***Potential effects of dairy genotypes on milk fat composition***
425 The higher proportion of fresh forage in the dairy diet is likely to have been the main
426 reason for the differences in milk composition. However, since contrasing dairy
427 genotypes (breed index) were used in different production systems this may also have
428 contributed to the differences in milk composition recorded between systems.
429 There is relatively little quantification of the effect of breed on fatty acid
430 composition, although breed effects on CLA and antioxidant content have been reported
431 to vary by up to 15-20% between breeds [21, 35]. This differential is considerably lower
432 than the 60-99% for CLA9 and 30-140% for antioxidants measured between Hi and LI
433 systems recorded in this study.
434 The finding of substantial differences in milk fat composition between HI and LI
435 systems during the outdoor grazing period, but similar milk composition during the
436 indoor feeding period also suggests that the differences in feeding regimes (rather than

dairy genotypes), were the main factors responsible for the milk composition differences between systems. However, the exact influence of breed relative to dietary supply and possible interaction, needs to be determined in future studies.

Potential nutritional impacts of differences in milk fat composition

Differences in nutritionally desirable FA and antioxidants between HI and LI systems during the grazing period were generally quite large (65 and 45% for α-LA, , 60 and 99% for CLA9, 33 and 50% for α-tocopherol, 30 and 74% for β-carotene, 67 and 148% for lutein and 46 and 82% for zeaxanthin for O-LI and NO-LI systems respectively). This confirms previously published comparisons of conventional and organic, low input production systems carried out in Germany, Italy and the UK.[14-16].

Consumption of milk and milk products from LI-systems produced during this period may therefore contribute significantly to increasing the intake of these compounds in line with nutritional recommendations. Importantly, the higher percentages of nutritionally desirable, PUFA (CLA9 and α- LA) found in milk from LI-systems, did not coincide with a significant increase in nutritionally less desirable PUFA (e.g. CLA10, total n-6 FA). Also, the higher n-3 FA and lower n-6 FA percentages found in milk from LI-systems resulted in a higher n-3:n-6 FA ratio which is also considered nutritionally desirable.[4, 10, 12, 27, 41]

Even if trends elevated α-LA and lutein in organic milk produced during housing were confirmed, it is clear that consumption of organic milk produced during the indoor winter period will not increase the intake of nutritionally desirable compounds to the same extent as low input milks produced during the outdoor grazing period.

While CLA9 and n-3 FA have been linked to a range of beneficial impacts on health[10-13]**,** it should be pointed out, that it is currently uncertain whether the main n-3

463 FAs found in milk, α-linolenic acid **(**αLA**;** C18:3 c9 c12 c15), has similar effects on
464 human health as the long-chain n-3 FAs found mainly in fish oil (C20 or longer) which
465 have been shown to protect against CHD, associated with improved neurological
466 function and linked to reduced risk of type 2 diabetes, hypertension and certain
467 cancers.[10, 12, 41, 42] These long chain n-3 fatty acids are known to be present in low levels
468 in milk fat[27] and were not determined in this study. However, there is now both direct
469 and indirect evidence that significant levels of longer chain n-3 FA especially
470 eicosapentaenoic (EPA; C20:5 n-3) and to a lesser extent docosahexaenoic acid (DHA;
471 C22:6 n-3) are generated from αLA in humans.[42]

472 The impact of fat soluble antioxidants/vitamins on human health has been
473 reviewed extensively.[24, 43-45] Beneficial effects of increased dietary α-tocopherol (a
474 compound belonging to the Vitamin E group) intake on human health have mainly been
475 linked to its ability to reduce oxidative stress, which was shown to be a risk factors for a
476 number of chronic health conditions including cardiovascular diseases, cancer, impaired
477 immunity and premature aging.[45] Carotenoids can act as precursors for vitamin A,
478 although a range of health benefit were linked to their antioxidant properties, and
479 thought to be independent from their contribution to vitamin A generation.[46]

480 With respect to the current availability of milk from LI-systems for consumers,
481 it should be emphasised that milk from organic producers is identifiable and widely
482 available, while milk from the non-organically certified LI farms is currently mixed
483 with milk from HI conventional systems in the supply chain and is not available to
484 consumers. Given the apparently high nutritional quality of milk produced in NO-LI-
485 systems it is important that this practice is reviewed in order to take advantage of the
486 price premiums that can currently be achieved by “nutritionally enhanced” food
487 products.[47]

When data for all sampling dates were pooled the concentrations of α-LA was elevated by 60% and that of CLA9 by 64% in the organic compared to HI milk (α-LA; mean =9.4, SE =0.3 vs mean =5.7, SE =0.3 g/kg fat, $p<0.001$ and CLA9; mean =12.2, SE =0.7 vs mean =7.5, SE 0.4 g/kg fat, $p<0.001$ for O-LI and HI milk respectively).. These data may help explain why consumption of organic dairy produce has been shown to have a significant impact on the CLA content of breast milk in lactating woman[48], and the eczema risk during the first 2 years of life[49]. It is now important to (a) identify exactly those production system components in organic, low input and conventional farming systems that are responsible for differences in milk composition and (b) to allow agronomic strategies in dairy production to be optimised further with respect to compounds that can be linked to positive health impacts.

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653

Table 1. Differences in management and production system parameters between high input conventional (HI), organically certified (O-LI) and non-organic (NO-LI) low input farms (mean values over all samples, with standard deviation in parenthesis)

	Production System		
Parameters recorded	**HI**	**O-LI**	**NO-LI**
Herd characteristics			
Herd size (milking cows)*	252 (125)	160 (93)	322 (141)
Breed Index[a]	0.0 (0)	0.2 (0.3)	0.3 (0.1)
% primiparous cows*	25 (7)	27 (12)	30 (8)
Live weight of cows (kg)[b]	650 (0)	610 (34)	588 (21)
Dry matter intake (kg d^{-1})[c]	19.5 (0.5)	17.6 (1.0)	16.9 (0.7)
Diet composition			
1. Outdoor period			
Fresh forage (proportion DMI)	**0.37 (0.24)**	**0.84 (0.23)**	**0.95 (0.07)**
Conserved forage (proportion DMI)	**0.29 (0.15)**	**0.08 (0.16)**	**0 (0)**
• Grass silage[e]*	0.73 (0.28)	0.72 (0.40)	
• Maize silage[e]*	0.10 (0.20)	0 (0)	
• Other silage[d,e]*	0.13 (0.18)	0 (0)	
• Straw/hay[e]*	0.04 (0.09)	0.28 (0.40)	
Concentrate (proportion of DMI)	**0.34 (0.13)**	**0.08 (0.09)**	**0.05 (0.07)**
• Cereals*	0.31 (0.24)	0.23 (0.40)	0.05 (0.14)
• By-products*[g]	0.30 (0.23)	0.20 (0.40)	0.52 (0.50)
• Other concentrates*[h,i]	0.40 (0.31)	0.57 (0.49)	0.43 (0.53)
Mineral supplements* (g cow^{-1} day^{-1})	142 (75)	8 (17)	3 (13)
Vitamin E supplement* (iu cow^{-1} day^{-1})	450 – 750	0	0
2. Indoor period			
Fresh forage (proportion of DMI)[f]	**0 (0)**	**0.24 (0.38)**	NA
Conserved forage (proportion of DMI)	**0.56 (0.08)**	**0.54 (0.30)**	NA
• Grass silage[e]*	0.69 (0.29)	0.80 (0.19)	NA
• Maize silage[e]*	0.05 (0.12)	0 (0)	NA
• Other silage[d,e]*	0.24 (0.28)	0.20 (0.19)	NA
• Straw/hay[e]*	0.02 (0.04)	0 (0)	NA
Concentrate (proportion of DMI)	**0.44 (0.08)**	**0.23 (0.10)**	NA
• Cereals*	0.31 (0.17)	0.42 (0.16)	NA
• By-products*[g]	0.24 (0.16)	0.07 (0.11)	NA
• Other concentrates*[h,i]	0.45 (0.24)	0.51 (0.23)	NA
Mineral supplements* (g cow^{-1} day^{-1})	150 (53)	22 (31)	NA
Vitamin E supplement* (iu cow^{-1} day^{-1})	250 – 674	0	0

654 *, based on farm records and collected by questionnaire; [a], Estimated proportion of non-
655 Holstein-Friesian genetics in the herd; [b], estimated based on breed index; [c] estimated
656 based on live weight and milk yield; [d], wholecrop wheat, barley and/or oats, dry matter;
657 [e] proportion of total conserved forage intake; [f], when weather permitted most organic
658 herds were grazed in the day; [g], brewing and distillers waste and/or sugarbeet pulp; [h],
659 bought in or farm produced compound/mixed concentrate feeds; [i] no oil seed or fat
660 supplementation was recorded by farmers; NA, not applicable (NO-LI cows were
661 grazed throughout the lactation)

662

Table 2. Diet composition in organic (O-LI) and non-organic (NO-LI) low input dairy production systems (spring calving herds only), at different sampling dates during the outdoor period (mean values, with standard deviation in parenthesis)

Sampling date	**Dietary components** (proportion of DMI)	**Production system**	
		O-LI	NO-LI
August	Fresh forage	0.96 (0.04)	0.92 (0.08)
	Conserved forage	0 (0)	0 (0)
	Concentrate	0.04 (0.04)	0.08 (0.08)
October	Fresh forage	0.88 (0.11)	0.95 (0.08)
	Conserved forage	0.04 (0.06)	0 (0)
	Concentrate	0.08 (0.08)	0.05 (0.08)
March	Fresh forage	0.86 (0.20)	0.95 (0.07)
	Conserved forage	0.11 (0.15)	0 (0)
	Concentrate	0.03 (0.06)	0.05 (0.07)
May	Fresh forage	0.96 (0.06)	1.00 (0)
	Conserved forage	0 (0)	0 (0)
	Concentrate	0.04 (0.06)	0 (0)

663 DMI = Dry matter intake

664

665

Table 3. Fatty acid composition and fat soluble antioxidant concentrations in milk from conventional high input and, organic and non-organic low input dairy production systems, during the outdoor, fresh forage based feeding period. (mean values, with standard error of means in parenthesis)

	Production system			
	High	**Low Input**		**ANOVA**
Characteristic assessed	**input**	O	NO	(*P*-value)
Number of samples	**24**	**34**	**20**	
Milk yield/cow (kg)	26.2 (0.7) **a**	18.4 (0.8) **b**	17.4 (0.9) **b**	**<0.0001**
Protein content (g/kg)	33.1 (2.3) **c**	34.1 (3.5) **b**	35.9 (3.9) **c**	**0.0006**
Fat content (g/kg)	39.6 (3.1) **b**	42.0 (6.9) **ab**	45.5 (9.0) **a**	**0.0004**
Fatty acid groups				
(g/kg milk fat)				
Total SFA	691 (59) **b**	672 (55) **ab**	660 (64) **a**	**0.042**
Total MUFA *	275 (54) **b**	289 (51) **ab**	305 (57) **a**	**0.017**
Total PUFA	59 (20) **b**	82 (17) **a**	78 (22) **ab**	**0.0017**
Omega 3 and 6 FAs				
(g/kg milk fat)				
α-LA C18:3 c9 c12 c15	6.2 (0.5) **b**	10.2 (0.3) **a**	9.0 (0.3) **a**	**<0.0001**
γLA C18:3 c6 c9 c12	0.26 (0.01)	0.26 (0.06)	0.14 (0.01)	0.242
Total n-6	20.1 (1.3) **a**	15.2 (1.0) **b**	10.6 (0.4) **c**	**<0.0001**
n-3 : n-6 ratio	0.37 (0.13) **b**	0.79 (0.09) **a**	0.88 (0.01) **a**	**<0.0001**
VA and CLA isomers				
(g/kg milk fat)				
VA C18:1 t11	22.5 (1.8) **b**	35.5 (1.6) **a**	41.9 (1.9) **a**	**<0.0001**
CLA C18:2 c9 t11	8.8 (0.7) **c**	14.1 (0.6) **b**	17. 5 (1.4) **a**	**<0.0001**
CLA C18:2 t10 c12	0.31 (0.03)	0.33 (0.03)	0.38 (0.07)	0.589
Fat soluble antioxidants				
(mg/kg milk fat)				
α-tocopherol				
2R α-toc	2.6 (0.1)	2.5 (0.3)	1.8 (0.2)	0.123
RRR α-toc	18.8 (0.8) **c**	26.0 (0.9) **b**	30.2 (1.0) **a**	**<0.0001**
Total α-tocopherol	21.4 (0.8) **b**	28.5 (0.9) **a**	32.0 (1.1) **a**	**<0.0001**
Carotenoids				
β-carotene	5.35 (0.33) **c**	6.95 (0.29) **b**	9.29 (0.48) **a**	**<0.0001**
Lutein	0.46 (0.03) **c**	0.77 (0.04) **b**	1.14 (0.05) **a**	**<0.0001**
Zeaxantin	0.11 (0.01) **c**	0.16 (0.01) **b**	0.20 (0.01) **a**	**<0.0001**
Total carotenoids	5.91 (0.35) **c**	7.88 (0.32) **b**	10.64 (0.52) **a**	**<0.0001**

666 O, Organic; NO, non-organically certified; SFA, saturated fatty acids; MUFA,
667 monounsaturated fatty acids (* > 80% oleic acid); PUFA, polyunsaturated fatty acids;
668 α-LA, α-linolenic acid; 2R α-toc, 2R stereo-isomers of α-tocopherol; RRR α-toc, 3R
669 stereo-isomers of α-tocopherol; Means within row with different letters are significantly
670 different ($P<0.05$)
671
672

673

Table 4. Fatty acid composition and fat soluble antioxidant concentrations in milk from conventional high input and, organic and non-organic low input dairy production systems, during the indoor conserved forage based feeding period (mean values, with standard error of means in parenthesis)

Characteristic assessed	High Input	Low Input Organic	ANOVA (*P*-value)
Number of samples	**21**	**10**	
Milk yield/cow (kg)	26.5 (1.0)	19.1 (1.3)	**0.0014**
Protein content (g/kg)	33.0 (0.3)	33.1 (0.6)	0.803
Fat content (g/kg)	40.8 (0.5)	42.1 (0.7)	0.235
Fatty acid groups (g/kg milk fat)			
Total SFA	712 (6)	740 (11)	**0.041**
Total MUFA*	254 (5)	228 (10)	**0.028**
Total PUFA	53 (2)	51 (4)	0.730
Omega 3 and 6 FA (g/kg milk fat)			
α-LA C18:3 c9 c12 c15	5.3 (0.5)	7.3 (0.9)	***0.052***
γLA C18:3 c6 c9 c12	0.2 (0.02)	0.2 (0.03)	0.127
Total n-6	21.7 (1.3)	16.4 (0.7)	**0.018**
n-3 : n-6 ratio	0.30 (0.04)	0.42 (0.06)	0.114
VA and CLA isomers (g/kg milk fat)			
VA C18:1 t11	16.4 (1.0)	17.5 (2.3)	0.636
CLA C18:2 c9 t11	6.2 (0.04)	7.8 (0.21)	0.111
CLA C18:2 t10 c12	0.31 (0.01)	0.34 (0.02)	0.139
Fat soluble antioxidants (mg/kg milk fat)			
α-tocopherol			
2R α-toc	3.5 (0.4)	2.8 (0.4)	0.360
RRR α-toc	20.4 (0.9)	20.3 (1.5)	0.776
Total α-tocopherol	23.9 (1.0)	23.1 (1.6)	0.513
Carotenoids			
B-carotene	5.49 (0.41)	6.29 (0.64)	0.359
Lutein	0.37 (0.03)	0.48 (0.06)	***0.081***
Zeaxantin	0.12 (0.01)	0.14 (0.01)	0.265
Total carotenoids	5.98 (0.44)	6.90 (0.68)	0.314

674 SFA, saturated fatty acids; MUFA, monounsaturated fatty acids (* > 80% oleic acid);
675 PUFA, polyunsaturated fatty acids; α-LA, α-linolenic acid; 2R α-toc, 2R stereo-isomers
676 of α-tocopherol; RRR α-toc, 3R stereo-isomers of α-tocopherol
677

678

Fig. 1. Effect of organic (black bars) and non-organic (white bars) low input production systems on the fatty acid composition of milk fat. **(a)** SFA, saturated fatty acids, **(b)** MUFA, mono-unsaturated fatty acids, **(c)** PUFA, polyunsaturated fatty acids, **(d)** ALA, α-linolenic acid, **(e)** VA, vaccinic acid, **(f)** CLA, conjugated linoleic acid isomer C18:2 c9 t11; * means for organic and non-organic low input systems are significantly different according to Tukey's Honest Significant difference test. Error bars indicate standard error of mean values.

Two-way ANOVA (with production system and date as factors) identified significant differences **(a)** between production systems for VA (P= 0.041) and CLA (P= 0.012) and **(b)** between dates for PUFA (P=0.028), VA (P= 0.005) and CLA (P<0.0001). Significant interactions between system and date were identified for PUFA (P=0.020), VA (P= 0.029) and CLA (P<0.030).

Fig. 2. Effect of organic (black bars) and non-organic (white bars) low input production systems on the levels of fat soluble antioxidants in milk fat. **(a)** 2R α toc, 2R-stereoisomers of α-tocopherol, **(b)** 3R α toc, 3R-stereoisomers of α-tocopherol, **(c)** total carotenoids, **(d)** β carotene, **(e)** lutein, **(f)** zeaxantin; * means for organic and non-organic low input systems are significantly different according to Tukey's Honest Significant difference test. Error bars indicate standard error of mean values.

Two-way ANOVA (with production system and date as factors) identified significant differences **(a)** between production systems for β-carotene (P= 0.003), lutein (P= 0.004), zeaxantin (P=0.027) and total carotenoids (0.002), and **(b)** between dates for 2R αtoc (P=0.0005), 3R αtoc (P= 0.0005), β-carotene (P= 0.005), lutein (P= 0.0008), zeaxantin (P=0.002) and total carotenoids (0.003). A significant interactions between system and date was only identified for 2R αtoc (P=0.003).

Fig 1

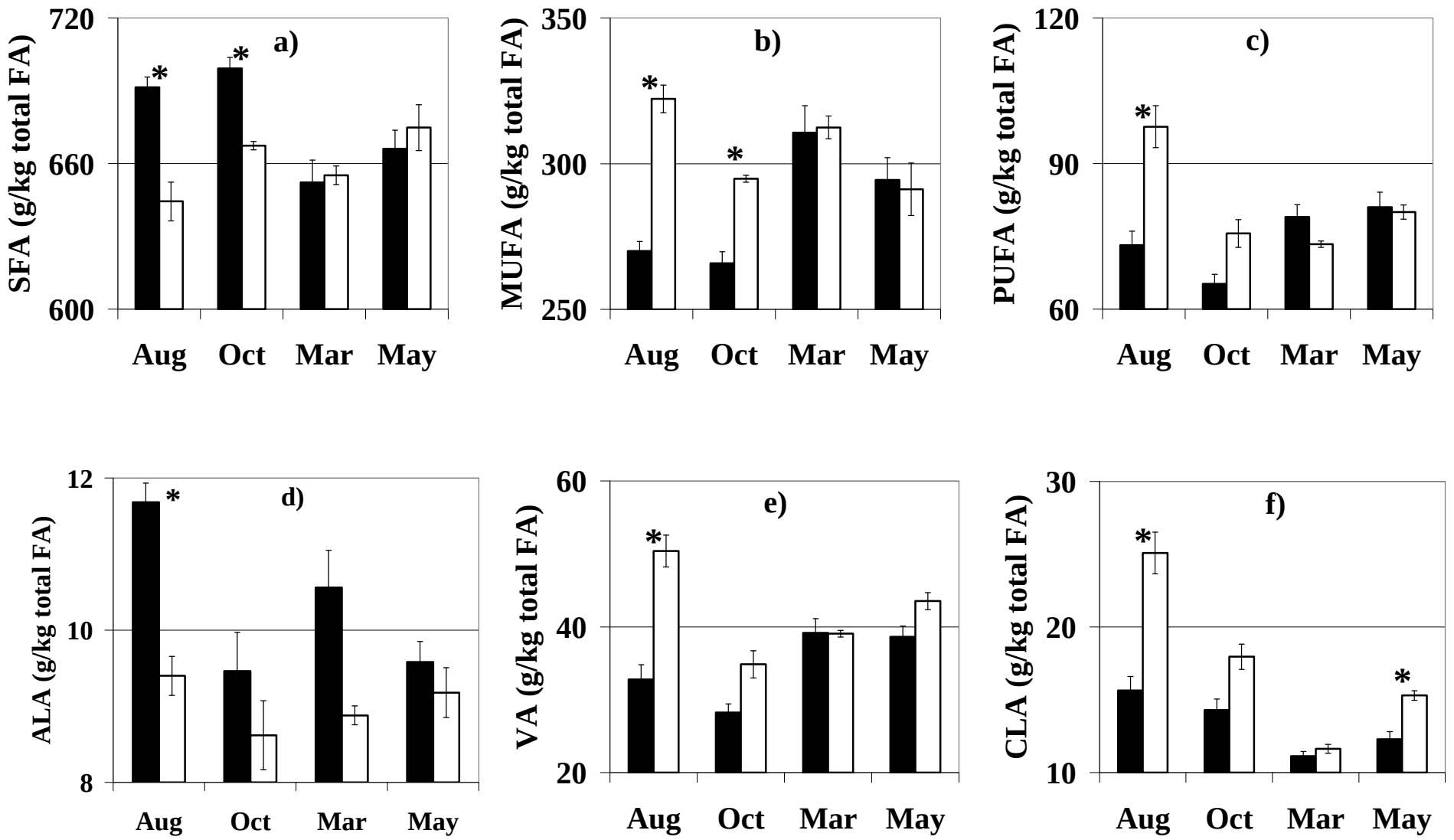
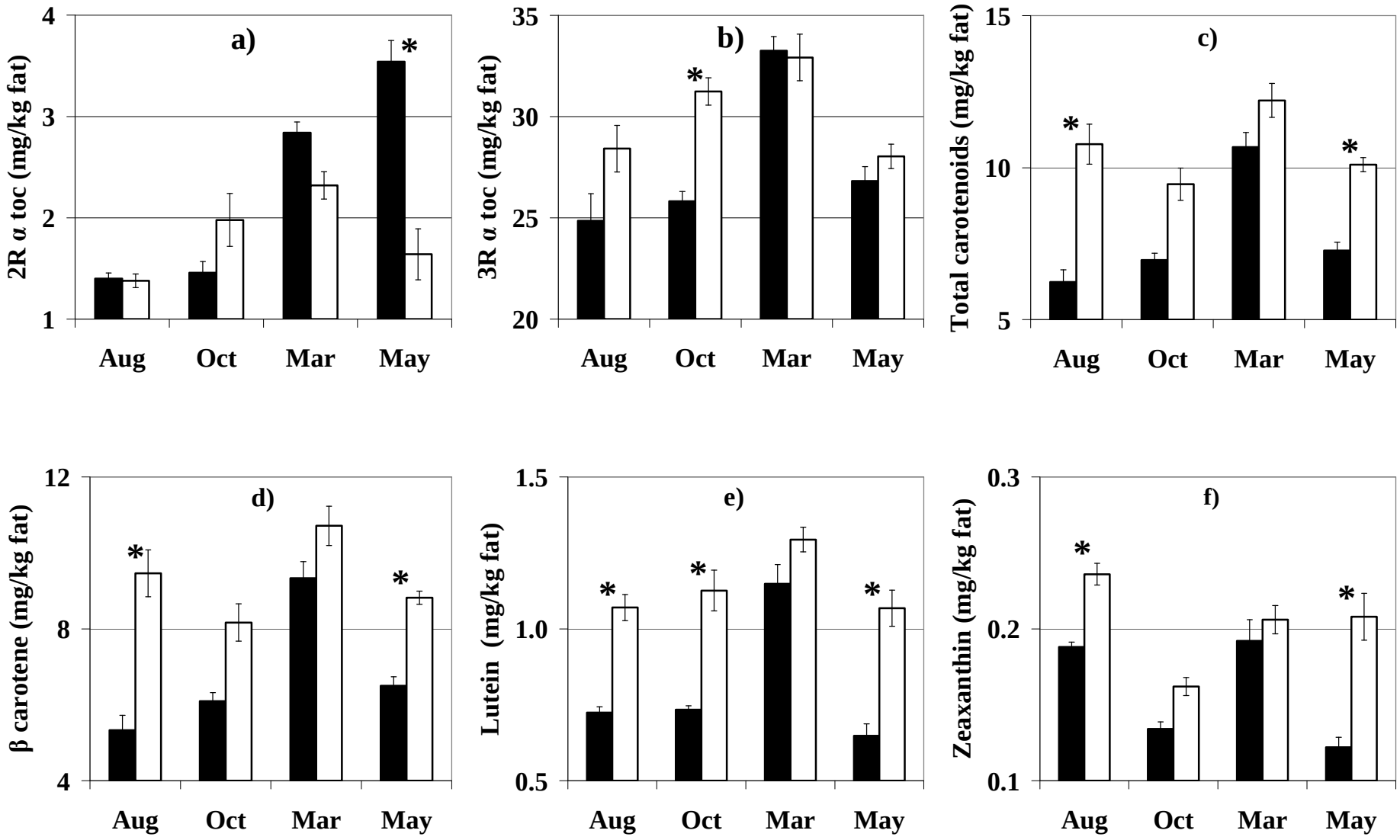

a)
SFA (g/kg total FA)
720
660
600
b)
MUFA (g/kg total FA)
350
300
250
c)
PUFA (g/kg total FA)
120
90
60
d)
ALA (g/kg total FA)
12
10
8
e)
VA (g/kg total FA)
60
40
20
f)
CLA (g/kg total FA)
30
20
10
Aug
Oct
Mar
May
*

Fig 2.


a)
2R α toc (mg/kg fat)
4
3
2
1
Aug
Oct
Mar
May
b)
3R α toc (mg/kg fat)
35
30
25
20
c)
Total carotenoids (mg/kg fat)
15
10
5
d)
β carotene (mg/kg fat)
12
8
4
e)
Lutein (mg/kg fat)
1.5
1.0
0.5
f)
Zeaxanthin (mg/kg fat)
0.3
0.2
0.1
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