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Fatty acid composition, fat-soluble vitamin concentrations and oxidative stability in bovine milk produced on two pastures with different botanical composition

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Abstract

Previous research has shown that grazing pastures compared to feeding preserved forages has large impact on milk fatty acid (FA) composition, but differences between grazing red clover (*Trifolium pratense* L.) or white clover (*Trifolium repens* L.) are small, whereas the herbage

proportions of dicotyledon botanical families is positively correlated with the milk-fat proportions of total polyunsaturated FA when grazing pastures in the Alps. The objective of the present study was to investigate the influence of botanically different pastures on bovine milk composition and milk susceptibility to oxidation. Two groups of 8 multiparous Norwegian Red dairy cows [mean (standard deviation); 599 (45.1) kg body weight, 73 (15.0) d in milk, 29.9 (2.90) kg milk/d at experiment start] grazed either a short-term pasture (SP) or a long-term pasture (LP). Both pastures were organically managed, meaning that no artificial fertilizers or herbicides were applied. The SP was representative for pastures, which are frequently, i.e. at least every third year, renewed by soil tillage and seeding, whereas LP was representative for pastures, which are less frequently renewed. The SP contained mainly meadow fescue (*Festuca pratensis* Huds.), timothy (*Phleum pratense* L.) and red clover and LP contained smooth meadow grass (*Poa pratensis* L.), white clover and a variety of unsown species. Sixteen cows were blocked according to milk yield, days in milk and sire, and randomly within block allocated to the 2 dietary treatments with a daily pasture allowance of 15 to 20 kg dry matter per cow, supplemented with 3.0 kg barley (*Hordeum vulgare* L.) concentrate. Milk was sampled during the last week of 3 experimental periods and analysed for FA composition by gas chromatography, concentrations of fat-soluble vitamins by high performance liquid chromatography, and oxidative stability in a light-exposure experiment by measuring the formation of hydroperoxides and by front-face fluorescence spectroscopy. Pasture type had no effect on milk yield, milk gross composition, and only minor effects on milk FA composition. Milk from SP had higher concentration of α-tocopherol than LP. The formation of hydroperoxides in milk was lower for SP than LP after 24 h light exposure, but no differences were found after 48 h. Front-face fluorescence spectroscopy revealed slightly higher formation of components in the area of 409 to 480 nm wavelength for SP than LP, which may be related to milk-lipid oxidation. The experimental pastures differed mainly in

herbage proportions of red clover and white clover and less in proportions of non-legume dicotyledons. This explains small differences in milk FA composition and milk susceptibility to oxidation.

Keywords: grazing, botanical composition, milk composition, fatty acid, fat-soluble vitamin, oxidative stability

1. Introduction

The aim of modulating the fatty acid (FA) composition in bovine milk fat is to decrease the proportions of saturated FA (SFA) and increase the proportions of other FA, e.g. C18:1c9, C18:2c9t11 and C18:3 n-3 in milk to improve its nutritive value for humans (Givens, 2005; Simopoulos, 2008).

Milk FA composition in dairy cows is known to be affected by botanical composition in silages (Lourenço et al., 2005). Compared to grass (*Poaceae*) based silages, silages based on mixed leys with grass and red clover (*Trifolium pratense* L.) or white clover (*Trifoilum repens* L.) increase the milk-fat proportions of C18:3 n-3 (Dewhurst et al., 2003). No or inverse effects on proportions of C18:3 n-3 are reported when cows graze red clover rich pasture compared to white clover rich pastures (Larsen et al., 2012; Wiking et al., 2010). Soder et al. (2006) found increasing milk-fat proportions of C18:2c9t11 for cows grazing more botanical diverse forage mixtures, however, C18:3 n-3 was not affected. It is suggested that the positive effect of red clover silage on C18:3 n-3 is related to the activity of polyphenole oxidase (EC 1.14.18.1) (Lee et al., 2004), however, oxygene is rapidly depleted in the rumen, limiting the activation of polyphenole oxidase during grazing (Lee et al., 2009). The effect of white clover on C18:3 n-3 is most likely related to increased rumen passage rate (Dewhurst et al., 2003). Collomb et al. (2002) found positive correlations between several dicotyledon families in

pastures in the Alps and milk-fat proportions of PUFA, total conjugated linoleic acids and C18:1t-FA. Less is known about the effects of botanical diverse pastures compared to red clover rich pastures on milk FA composition.

Forage concentrations of fat-soluble vitamins are affected by forage botanical composition and stage of maturity in plants (Danielsson et al., 2008). Milk susceptibility to oxidation is decreased by antioxidants like α-tocopherol from forage or supplements (Al-Mabruk et al., 2004). In fresh milk, stored in containers without sufficient light barrier, off-flavours can appear already after 1 d of storage under fluorescent lighting (Moyssiadi et al., 2004). More knowledge is needed on how pasture botanical composition affects milk susceptibility to oxidation.

The objectives of the present experiment were to assess the effects of grazing a newly established grass-red clover pasture or an older pasture with a variety of sown and unsown plant species on FA composition, concentrations of fat-soluble vitamins and oxidative stability in bovine milk.

The hypotheses were (1) that the newly established pasture contains more red clover and less white clover and non-legume dicotyledons than the older pasture and (2) that the higher proportions of non-legume dicotyledons in the older pasture increase milk-fat proportions of C18:1t-FA, C18:2c9t11 and C18:3 n-3, (3) which in turn increase milk susceptibility to oxidation in milk produced on older pastures.

2. Materials and methods

2.1. Cows, feeds, experimental design and feed sampling

Sixteen multiparous Norwegian Red dairy cows in mid-lactation participated in a grazing experiment in Ås, Norway, at the Animal Production Experimental Centre (59.67° N, 10.75° E; 50 m a.s.l.), Norwegian University of Life Sciences. At experiment start the cows weighed

599 (standard deviation 45.1) kg body weight, had 2.7 (0.39) points body condition score, were 73 (15.0) d in milk and milked 29.9 (2.90) kg/d. The cows were blocked on the basis of pre-experimental milk yields, days in milk and sire, and allocated randomly to 2 groups of 8 cows. The grazing experiment was conducted with a continuous design with three 3-week experimental periods with the last week in each period as a sampling week (21 to 27 June, 26 July to 1 August and 30 August to 5 September 2008).

Each group of cows was assigned to one of 2 pasture types, differing in seed mixture and year of establishment. The first pasture, established in August 2007 [seed mixture: 7.5 kg/ha timothy (*Phleum pratense* L., var. 'Grindstad'), 15.0 kg/ha meadow fescue (*Festuca pratensis* Huds., var. 'Fure') and 3.5 kg/ha red clover (var. 'Bjursele')] and fertilised with 29 tonnes/ha cattle manure was defined as short-term pasture (SP). In May 2008, 2.5 kg/ha of red clover seeds was reseeded. The second pasture, established in July 2003 [28 kg/ha seed mixture: timothy, perennial ryegrass (*Lolium perenne* L.), white clover, smooth meadow grass (*Poa pratensis* L.) with a cover crop of 170 kg/ha barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.) and common vetch (*Vicia sativa* L.)] and fertilised with 30 tonnes/ha cattle manure in autumn 2007 was defined as long-term pasture (LP). The 8 cows of each group grazed together and between experimental periods both groups grazed together on a pasture similar to LP. Both feed production and cow management (without certification) followed the standards for organic farming (Council of European Union, 2007).

Both pastures were divided into 4 paddocks, averaging 0.66 ha for SP and 0.92 ha for LP, and rotationally grazed. Pre-grazing herbage mass, measured 5 cm above ground level, was measured with a calibrated rising plate meter (MD, Stjørdal, Norway) before a new paddock was grazed and daily in the last week of each period. After a paddock was grazed post-grazing herbage mass was measured 5 cm above ground level and topped immediately. In experimental period 1 the second growth was grazed, in period 2 the third growth was grazed

and in period 3 the fourth growth was grazed. The cows were grazing day and night and fresh strips of pasture were given twice daily, immediately after milking at 06:00 and 16:00. Both groups were offered a pasture area with a daily herbage allowance, measured 5 cm above ground level, equivalent to 15 to 20 kg dry matter (DM)/cow plus 3.0 kg/d and cow of a barley based concentrate (barley 933 g/kg, molasses 50 g/kg and mineral premix 17 g/kg). The mineral premix in the concentrate contained Ca 110 g/kg, P 65 g/kg, Mg 90 g/kg, Na 95 g/kg, Cl 143 g/kg, S 9 g/kg, Fe 0.494 g/kg, Mn 0.300 g/kg, Zn 0.400 g/kg, Cu 0.900 g/kg, Co 0.021 g/kg, I 0.152 g/kg, mineral-Se 0.030 g/kg and yeast-Se 0.030 g/kg (Vilomix, NORMIN, Hønefoss, Norway).

Herbage botanical composition was estimated using the dry-weight-rank method (Jones and Hargreaves, 1979; Mannetje and Haydock, 1963) before and after grazing with a time span of 4 d in period 1, 5 d in period 2 and 6 d in period 3. Herbage samples were hand-plucked (days 15 to 18 inclusive) to the approximate height at which the cows grazed and cooled immediately with dry ice before storing at -20 °C and pooled for pasture type and period before chemical analysis. Concentrates were sampled (days 15 to 18 inclusive) and pooled for each period before chemical analysis. Herbage samples and concentrate samples were freeze dried (Christ LCM-2, Beta 1-16 and Christ LOC-1m, Alpha 1-4, Martin Christ, Osterode am Harz, Germany; Hetosicc, Birkerød, Denmark) and ground on a cutting mill (1.0 mm pore size except otherwise stated) (Retsch SM 100, Retsch GmbH, Haan, Germany) prior to analysis of DM, ash, Kjeldahl-N, crude fat, NDF, ADF, WSC, in vitro DM digestibility, FA composition, fat-soluble vitamins and starch (0.5 mm pore size) and stored in plastic bags at -20 ºC prior to analysis of chemical constituents.

2.2. Cow measurements and weather conditions

The cows were weighed on 3 consecutive days after morning milking in the beginning of the experiment and in the end of each experimental period. BCS was estimated by a simplified version of the method of (Edmonson et al., 1989) using a 5-point scale (1 = emaciated to 5 = severely over-conditioned) with 0.25-unit increments in the beginning of the experiment and in the end of each period. The mean daily temperature at 2.0 m was 13.0 °C in period 1, 17.5 °C in period 2 and 13.4 °C in period 3 and precipitation for the sum of 21 d was 78 mm in period 1, 64 mm in period 2 and 128 mm in period 3 (Norwegian Meteorological Institute, weather station Ås). The experiment was carried out in agreement with the laws and regulations controlling experiments on live animals in Norway under the surveillance of the Norwegian Animal Research Authority.

2.3. Milk sampling

Aliquot milk samples were collected with fractional sampling milk meters (Tru-Test Industries Ltd, Auckland, New Zealand) from 4 consecutive milkings from each cow, starting evening of day 17, were stored at 4 ºC until the last milk samples were collected in each period. Then the milk was placed in a water bath at 37 ºC, and thereafter the milk from the 4 milkings was gently blended. Milk samples intended for analysis of milk gross composition, urea and free FA were preserved with 2-bromo-2-nitropropane-1,3-diol (Bronopol, D&F Inc., Dublin, CA). Samples intended for analysis of FA composition, concentrations of fat-soluble vitamins and for a light-exposure experiment were stored frozen at -20 ºC until analysis. For the light-exposure experiment, 15 mL of milk was thawed in a water bath at 37 ºC and filled in transparent glass bottles, in 3 replicates, and chilled to 4 ºC before light exposure.

2.4. Chemical analyses and measurements

Freeze dried samples of herbage and concentrates were analysed at the Dairy One, Inc. Forage Testing Laboratory (Ithaca, NY) for ash (AOAC method 942.05.) (AOAC, 1990), N (AOAC method 990.03), ether extract (AOAC method 2003.05), WSC (Hall et al., 1999), starch (YSI 2700 SELECT Biochemistry Analyzer, YSI Incorporated Life Sciences, Yellow Springs, OH), NDF with heat-stable amylase and sodium sulphite (Van Soest et al., 1991), ADF (AOAC method 973.18) and in vitro DM digestibility after incubation for 48 h (ANKOM DaisyII Filter Bag Technique, ANKOM Technology, Macedon, NY).

Fatty acids in feed samples were analysed after a Bligh and Dyer extraction (Jensen, 2008). The samples were acidified by boiling at 80 °C in 3 mol/L hydrochloric acid for 1 h and extracted in a mixture of chloroform and methanol (Bligh and Dyer, 1959). Fatty acids in milk samples were extracted according to Bligh and Dyer (1959) and analysed as FA methyl esters (Jensen and Nielsen, 1996) by gas chromatography (Hewlett Packard 6890series, Agilent Technologies, Palo Alto, CA) equipped with an automatic on-column injector (Hewlett Packard 7673) (split ratio 4.325:1), a capillary column of 30 m x 320 µm inner diameter; 0.25 µm film thickness (Omegawax; Supelco 4-293-415, Sigma-Aldrich, St. Louis, MO), and a flame-ionisation detector with C17:0 as internal standard. Fat-soluble vitamins were analysed by HPLC after saponification and extraction into heptane (Jensen and Nielsen, 1996). A PerkinElmer HS-5-Silica column (4.0 x 125 mm) (Waltham, MA) was used for analyses of α-tocopherol and retinol and a Supelco amino column (4.6 x 250 mm) (Sigma-Aldrich, St. Louis, MO) was used for analysis of β-carotene and lutein. Milk gross composition and concentrations of urea and free FA were analysed by Fourier transformed infrared technology (MilkoScan 6000 FTIR, Foss, Hillerød, Denmark).

In the light-exposure experiment, the unpasteurised and not homogenised milk samples (15 mL) were exposed to light for 0, 24 or 48 h in a specially designed light cabinet at 4 ºC. Standard fluorescent light tubes (L 58 W/830 Lumilux Warm White, Osram, Munich,

199 Germany) were mounted vertically standing in the cabinet, and the transparent glass bottles
200 (50 mL) were positioned 10 cm from the light tube. The light intensity was 1,300 lux
201 (measured by a Lu-Ex 02 Luxmeter, ECOM Instruments GmbH, Assamstadt, Germany).
202 Immediately after light exposure, the samples were warmed in a water bath at 37 ºC in order
203 to take out homogenous samples for the determination of lipid hydroperoxides and for front-
204 face fluorescence spectroscopy.
205 The formation of lipid hydroperoxides was measured by using the method of Shanta and
206 Decker (1994) with the modifications described by Østdal et al. (2000). In brief 2 mL of milk
207 were mixed with 2 mL of methanol and vortexed, mixed with 4 mL of chloroform and
208 vortexed for 30 s. After centrifuging for 10 min at 3,000 g, 1.0 mL of the chloroform phase
209 was transferred to a test tube and mixed with 1 mL of Fe(II)/thiocyanate in
210 methanol:chloroform. After a reaction time of 5 min the absorbance at 500 nm was measured
211 using an Ultraspec 3000 spectrophotometer (Pharmacia Biotech, Cambridge, UK). The
212 absorbance for light exposed milk was calculated as the difference of the light exposed
213 samples and the sample stored in dark.
214 Fluorescence emission spectra were measured on 13 mL milk poured in sample cuvettes
215 with a diameter of 50 mm. The fluorescence emission spectra included 292 wavelengths in the
216 range of 409 to 751 nm were measured with excitation at 382 nm and emission tops were
217 identified by comparison with Wold et al. (2005). This excitation wavelength has earlier been
218 used for measuring lipid oxidation in cheese (Veberg et al., 2007; Wold et al., 2002). The
219 excitation light, generated by a 300 W Xenon light source (Oriel 6258, Oriel Corporation,
220 Stratford, CT) was passed through a 10 nm bandwidth interference filter (Oriel 59920 and
221 Oriel 59295) and directed onto the sample cuvettes at an angle of about 45° with an exposure
222 time of 1.5 s. An imaging spectrograph (Acton SP-150, Acton Research Corporation, Acton,
223 MA) was connected to a sensitive charge coupled device camera (Roper Scientific

224 NTE/CCD-1340/400-EMB, Roper Scientific, Trenton, NJ) to collect the spectra. A cut-off
225 filter at 400 nm (Melles Griot 03FCG049, Melles Griot Inc., Irvine, CA) was placed in front
226 of the spectrograph slit to suppress excitation light reflected from the sample. To ensure stable
227 illumination, the emission intensity at 440 nm at excitation 382 nm was measured from a
228 stable fluorescence standard of washable plastic (Ciba, Basel, Switzerland) before and after
229 the measurements. The spectra were not subjected to any kind of pre-processing before
230 statistical analysis.
231
232 *2.5. Calculations and statistical analyses*
233 For the last week in each period, herbage net energy intake was estimated as: net energy
234 requirement for maintenance and activity [0.0424 * $BW^{0.75}$ (Van Es, 1978) * 0.10 (National
235 Research Council, 2001)], added net energy requirement for milk production [0.44 * ECM +
236 0.0007293 * ECM^2 (Van Der Honing and Alderman, 1988)], added net energy requirement
237 for gestation [((((0.00318 * day of gestations between 190 and 279 – 0.0352) * (40 kg calf
238 birth weight / 45)) / 0.218) * 4.1868) / 6.9 (National Research Council, 2001)], subtracted net
239 energy intake of concentrates. The estimates were not corrected for tissue gain or loss.
240 Herbage DM intake was estimated by dividing the herbage net energy intake by herbage net
241 energy concentration, based on in vitro DM digestibility. Grazing preferences were estimated
242 as the proportions of botanical families in the herbage disappearance, measured with a
243 calibrated raising plate meter 5 cm above ground level divided by the proportions of the
244 botanical families before grazing. For herbage DM intake the experimental unit was the group
245 of 8 cows and thus no statistical analyses were performed. For milk yield and milk
246 composition the cow was considered the experimental unit as herd behaviour has minor
247 effects on milk composition, as discussed by Dumont and Iason (2000).

248 The variables were analysed statistically using the mixed model procedure by SAS (SAS,
249 2009). A period with indoor silage feeding previous to grazing start (5 May to 11 May) was
250 used as a baseline period (covariate) for milk yield, milk gross composition, and milk
251 concentrations of urea and free FA. The following statistical model was used:
252 $Y_{ijkl} = \mu + T_i + P_j + c(T)_{ik} + b_l + e_{ijkl}$,
253 where Y were the individual dependent variables (n = 1 to 48 inclusive) and μ was the
254 average of all observations, T was the fixed effect of pasture type (i = 1, 2; where 1 = SP and
255 2 = LP), P was the fixed effect of period (j = 1, 2, 3), c was the random effect of cow within T
256 (k = 1 to 16 inclusive), b was the random effect of block (l = 1 to 4 inclusive) and e_{ijkl} were
257 the random residual errors, assumed to be independent and $N(0, \sigma_e^2)$. Repeated measurements
258 taken on the same cow at different time points, i.e. periods, were accounted for in the
259 statistical analysis. A partial least squares regression, with full cross validation, was used to
260 find the correlations between the fluorescence emission spectra and the hydroperoxide values
261 (Statistical software, The Unscrambler, ver. 9.8, Camo AS, Oslo, Norway). A principal
262 component analysis was performed on the 292 measured wavelengths in the area of 409 to
263 751 nm in the fluorescence emission spectra (The Unscrambler).
264
265 **3. Results**
266 *3.1. Botanical composition and chemical composition of the experimental herbages and feed*
267 *intake*
268 The SP was characterised by high proportions of annual and biannual weeds in period 1
269 and high proportions of red clover in period 2 and 3 (Table 1). The LP was characterised by
270 high proportions of species in the grass family and white clover. In contrast to SP, the non-
271 legume dicotyledons on LP were mainly perennials, e.g. northern dock and dandelion. Both
272 groups of cows had a preference for legumes (*Fabaceae*) and avoided grasses except for SP in

period 1 (Table 2). Non-legume dicotyledons were avoided on SP, but not on LP. It was also observed that cows avoided species like tufted hairgrass (*Deschampsia cespitosa* (L.) P. Beauv.), thistle (*Cirsium* spp.) or northern dock as well as stems of grasses. Herbage chemical composition and feed value were similar for the 2 pasture types, however, herbage concentrations of C18:3 n-3 were higher for SP than LP and SP had slightly higher herbage concentrations of fat-soluble vitamins (Table 3).

3.2. Effect of pasture type on cow performance and milk composition

Herbage intakes were similar for both groups of cows (Table 4). Cows grazing SP had a small daily decrease in body weight, whereas cows grazing LP had a small increase, but changes in body condition score were similar. Pasture type had no effect on milk yield (means: SP 24.7 kg/d; LP 25.0 kg/d; SEM 0.70) and milk gross composition (means: 37.4 g/kg fat, 33.4 g/kg protein, 45.8 g/kg lactose, 0.55 mEq/L FFA, 4.41 mmol/L urea). The effect of pasture type on FA composition in milk was small (Table 5). Compared to LP, SP resulted in milk fat with higher proportions of C17:1c9 (P = 0.01), C18:0 (P = 0.02) and C18:1c11 (P = 0.02) and lower proportions of C16:0 (P = 0.02). The proportions of total PUFA and the proportions of individual C18-FA in total C18-FA were not affected by pasture type. Milk produced on SP had higher (P = 0.01) concentration of α-tocopherol, whereas β-carotene and retinol were not affected (Table 6).

3.3. Effect of pasture type on milk oxidative stability

The formation of lipid hydroperoxides increased with duration of light exposure. After 24 h light exposure, milk from SP had lower (P = 0.04) hydroperoxide absorbance than milk from LP, but no differences were found after 48 h light exposure. The fluorescence emission spectra showed a decomposition of riboflavin (emission top at 530 nm) and smaller

differences for the photosensitisers protoporfyrin (635 nm), a tetrapyrrol compound (662 nm), and a compound similar to chlorophyll A (675 nm) from 0 h to 24 h to 48 h light exposure (Figure 1). The first principal component accounted for 0.94 of the spectral variation in the principal component analysis and described the degradation of the photosensitisers by duration of light exposure (Figures 2 and 3; area 480 to 751 nm wavelength). Principal component 2 explained 0.06 of the spectral variance and represented the differences between milk from SP and LP (area 409 to 480 nm). Partial least squares regressions showed positive correlations between hydroperoxide values and fluorescence emission spectra for SP (0.84; 2 components) and LP (0.84; 4 components).

4. Discussion

4.1. Herbage composition, herbage intake and milk production

We hypothesised that more red clover and less white clover and non-legume dicotyledons would be found in the herbage from SP than LP. Regarding the clover species the botanical composition of the 2 pastures were as expected. The appearance of annual and biannual weeds in SP, mainly in period 1, yielded high proportions of non-legume dicotyledons in the herbage of SP. The variation in botanical composition between pasture types and experimental periods within pasture type was likely due to differences in management (annuals and biannuals in SP and perennials in LP) and differences in development of plant species throughout the season. Additionally, differences in botanical composition between paddocks within treatment, especially for LP, have contributed to the variation. The cows' preference of legumes compared to grasses is in accordance with Rutter et al. (2004), who reported a partial preference of white clover compared to ryegrass. Avoiding non-legume dicotyledons from SP may have been caused by low palatability of species like shepherd's-purse (*Capsella bursa-pastoris* (L.) Medik.) or pineappleweed (*Matricaria matricarioides* Porter ex Britton),

whereas the non-legume dicotyledons from LP apparently had higher palatability or could not be sorted out by the cows. Species like tufted hairgrass, thistle or northern dock as well as stems of grasses may have been avoided due to the physical structure and nutrient content (Heady, 1964). Ribeiro Filho et al. (2005) reported higher pasture intake and milk yield for pastures including white clover compared to perennial ryegrass. In the present experiment SP and LP had similar proportions of legumes, which may explain similar herbage intakes and milk yields, in agreement with Wiking et al. (2010). The lower milk yields compared to the initial milk yields before grazing start, for both groups is most likely due to later stage of lactation as milk yields declined from period 1 to 3. As herbage intake was not measured individually and not replicated for pasture type, intake of FA and fat-soluble vitamins could not be tested statistically in this study, however, higher concentrations of C18:3 n-3 and total FA in herbage from SP than LP and similar herbage intakes suggest higher intakes of C18:3 n-3 and total FA.

4.2. Effect of pasture type on milk composition

As the 2 pastures did not differ in the total proportions of non-legume dicotyledons (hypothesis 1), but mainly in the proportions of red clover and white clover it was not likely that differences in botanical composition would affect milk FA composition as Larsen et al. (2012) and Wiking et al. (2010) found only minor effects of grazing red clover or white clover on Milk FA composition. If this is true, our results suggest that annual and biannual non-legume docotyledons commonly found in herbage from SP have similar effects on milk FA composition as perennial dicotyledons usually found in herbage from LP. Little is known about the effects of annual and biannual weeds on milk FA composition. Generally, high milk-fat proportions of C18:1t-FA, C18:2c9t11 and C18:3 n-3 and low proportions of SFA are typical for milk produced on pasture compared to milk produced on preserved forages

348 (Elgersma et al., 2004; Ribeiro Filho et al., 2005). Compared to these studies, milk-fat
349 proportions of C12:0, C14:0 and C16:0 and total SFA were high.
350 Despite estimated higher intake of C18:3 n-3 and total FA for SP, milk concentrations of
351 C18:3 n-3 were not affected by pasture type, in accordance with Wiking et al. (2010). This
352 may be due to more extensive biohydrogenation for SP than for LP. Less extensive
353 biohydrogenation for LP may be explained by higher rumen passage rate caused by white
354 clover (Dewhurst et al., 2003). Likewise, in the study of Larsen et al. (2012) white clover
355 increased milk-fat proportions of C18:3 n-3, despite lower intake. In contrast to these grazing
356 experiments, feeding of grass-red clover silage vs. grass silage or grass-white clover silage
357 has in most cases decreased rumen biohydrogenation, leading to higher apparent recovery and
358 higher milk-fat proportions of C18:3 n-3 (Dewhurst et al., 2003; Höjer et al., 2012). Based on
359 results from in vitro studies, this effect of red clover has been explained by the activity of
360 polyphenol oxidase (EC 1.10.3.1) that may protect feed PUFA in protein matrices from rumen
361 biohydrogenation (Halmemies-Beauchet-Filleau et al., 2012; Lee et al., 2004) or increased
362 passage rate (Dewhurst et al., 2003). Limited access to oxygen during grazing and mastication
363 of red clover decreases the potential of polyphenol oxidase activation and may explain similar
364 milk-fat proportions of C18:3 n-3 for SP and LP (Lee et al., 2009). The proportions of
365 individual C18-FA in total C18-FA did not differ; however, this suggests that rumen
366 biohydrogenation was not affected by pasture type. Lower milk-fat proportions of C16:0 for
367 SP may be explained by inhibited de novo synthesis due to negative energy balance, leading
368 to mobilisation of FA from the adipose tissue.
369 Generally, the milk concentrations of α-tocopherol and β-carotene were comparable to
370 milk produced during the outdoor feeding period in the study of Butler et al. (2008), but
371 concentrations of α-tocopherol and retinol were higher than reported by Lindmark-Månsson

et al. (2003), however, Ellis et al. (2007) reported higher concentrations of α-tocopherol in summer milk than found in the present experiment. The high concentrations in the present experiment may be explained by high herbage concentrations of α-tocopherol and by the relatively low milk yields (Jensen et al., 1999). Higher herbage concentrations of α-tocopherol for SP than for LP, may explain higher concentrations of α-tocopherol in milk for SP, in accordance with Larsen et al. (2012). It is not clear, however, if differences in herbage α-tocopherol concentration were caused by differences in botanical composition (Danielsson et al., 2008), differences in leaf:stem ratio or environmental factors (Hjarde et al., 1963). In contrast with Petersen et al. (2011), the present experiment showed no effect of pasture type on milk concentrations of retinol, most likely due to small differences in herbage concentrations of β-carotene.

4.3. Effect of pasture type on milk susceptibility to oxidation

Hypothesis 2, that higher proportions of non-legume dicotyledons in LP increase milk-fat proportions of C18:1t-FA, C18:2c9t11 and C18:3 n-3 was not fulfilled and thus the basis for hypothesis 3, suggesting increased susceptibility to oxidation in milk produced on LP disappeared. Small differences in milk susceptibility may therefore have been caused by other factors e.g. the presence antioxidants.

The less extensive formation of lipid hydroperoxides for SP than for LP after 24 h light exposure may be a result of higher α-tocopherol concentrations in milk from SP as PUFA proportions in milk-fat were similar for SP and LP (Al-Mabruk et al., 2004). The differences in fluorescence emission spectra at 409 to 480 nm were found for samples exposed to light and samples stored dark. This indicates that differences were directly related to pasture type, however, Veberg et al. (2007) found fluorescing oxidation products in this area, but further

research is necessary to identify unknown compounds in this area. At 48 h light exposure the response increased for both pasture types, slightly more for SP than for LP, suggesting higher formation of oxidation products for SP. Al-Mabruk et al. (2004) found that milk produced on red clover silage was more susceptible to lipid oxidation during storage than milk produced on grass silage by assessing thiobarbituric acid reactive substances and supplementation with α-tocopherol decreased the milk susceptibility to oxidation for both silages. In the present experiment the PUFA proportions were higher than in the milk produced on the red clover silage of Al-Mabruk et al. (2004). In the latter study, even cows supplemented with α-tocopherol had lower intake of α-tocopherol and they yielded milk with lower concentration of α-tocopherol than in the present experiment. Thus, although milk PUFA proportions in the present experiment were high, the supply of the antioxidant α-tocopherol may have been sufficient to prevent extensive oxidation of milk fat.

5. Conclusions

In contradiction with our hypothesis, the pastures did not differ in herbage proportions of non-legume dicotyledons and thus the basis for the subsequent hypotheses on milk FA composition and milk susceptibility to oxidation disappeared. Differences in pasture proportions of red clover or white clover did not affect milk PUFA proportions and differences in milk susceptibility to oxidation were small.

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Figures

Figure 1. Fluorescence emission spectra of milk produced by cows grazing short-term pasture (SP) or long-term pasture (LP) after 0, 24 or 48 h light exposure (means; n = 24).

Figure 2. Loading plot for principal component (PC) 1 and PC2 for fluorescence emission spectra with 292 measured wavelengths in the area of 409 to 751 nm in milk produced by cows grazing short-term pasture (SP) or long-term pasture (LP) after 0, 24 or 48 h light exposure.

Figure 3. Score plot for principal component (PC) 1 and PC2 for fluorescence emission spectra with 292 measured wavelengths in the area of 409 to 751 nm in milk produced by cows grazing short-term pasture (SP) or long-term pasture (LP) after 0, 24 or 48 h light exposure.

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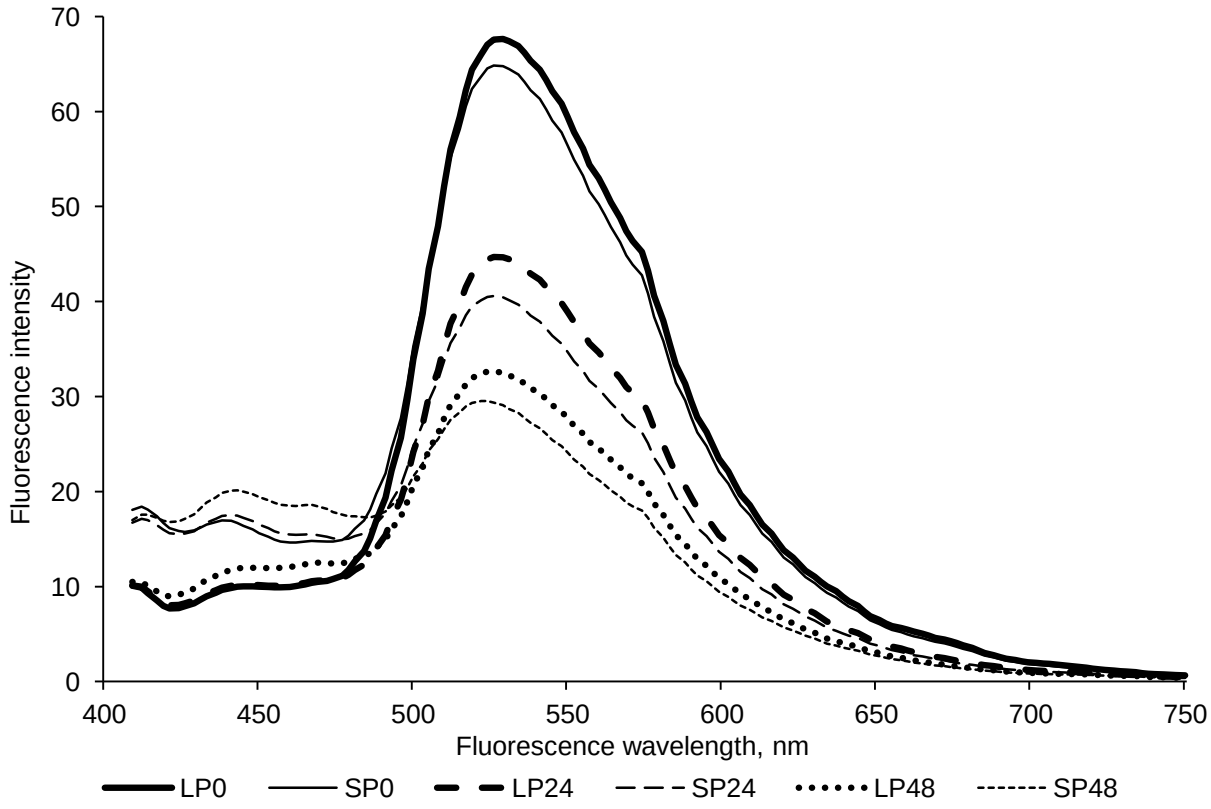

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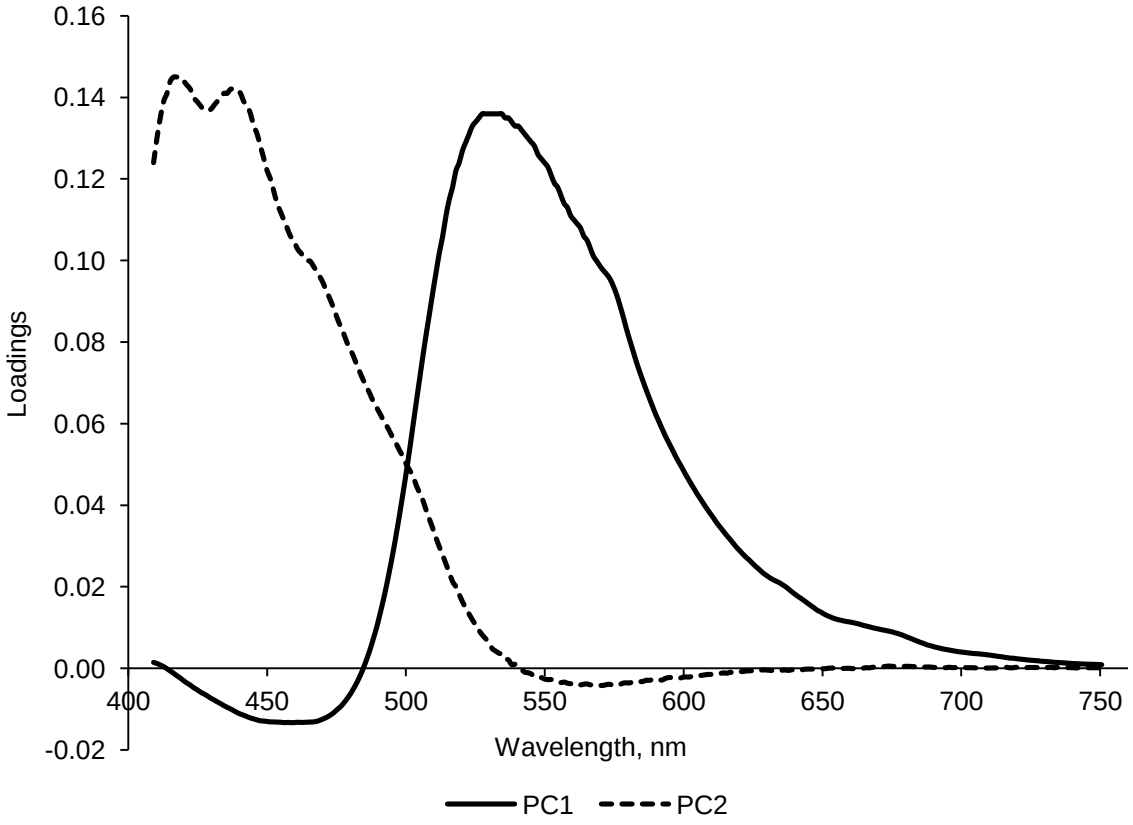

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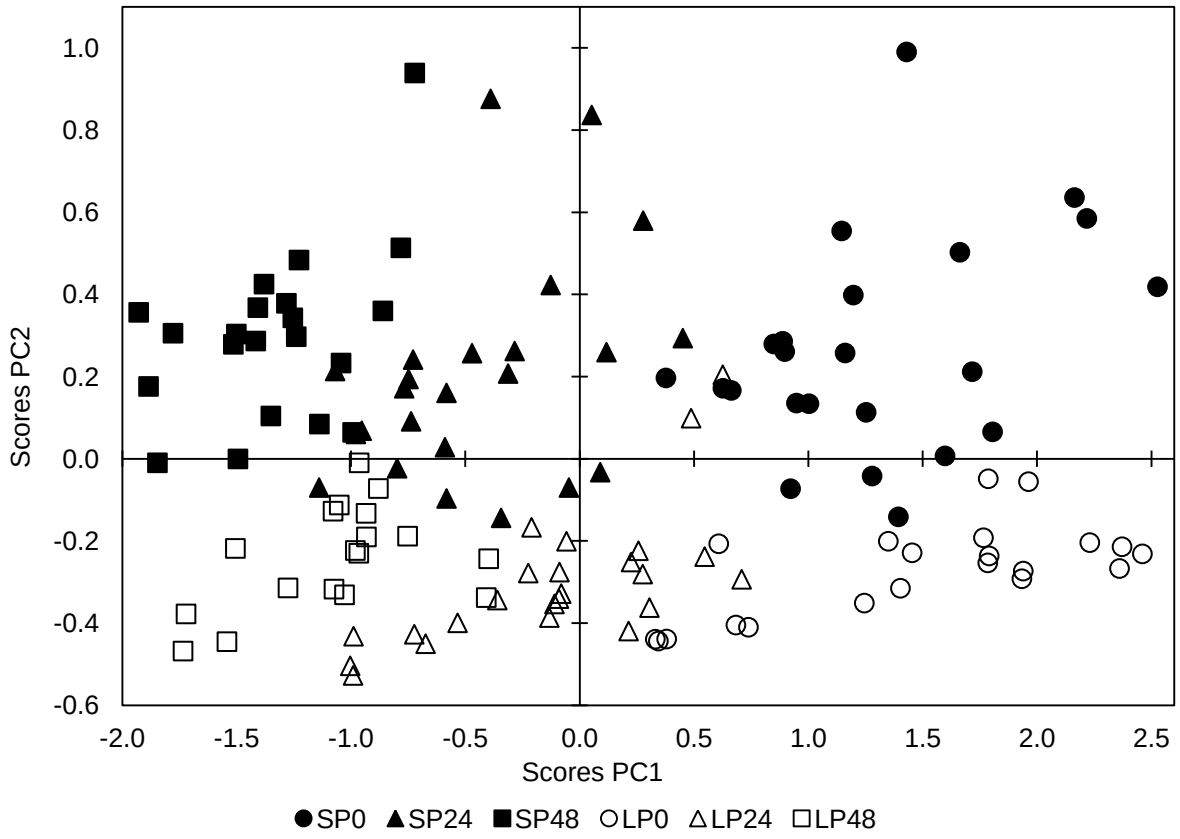

Table 1. Botanical families and prevailing species estimated by the dry-weight-rank method (Mannetje and Haydock, 1963), number of species, pre-grazing herbage mass and herbage height, estimated in herbage from short-term pasture (SP) or long-term pasture (LP) for period 1 to period 3

Botanical families and prevailing species, g DM/kg DM	SP			LP		
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3
Grass family (*Poaceae*)	428	587	593	720	630	625
Meadow fescue (*Festuca pratensis* Huds.)	226	377	411	47	205	189
Timothy (*Phleum pratense* L.)	198	195	177	70	188	251
Smooth meadowgrass (*Poa pratensis* L.)	5	0	0	432	107	40
Perennial ryegrass (*Lolium perenne* L.)	0	0	5	0	98	76
Tufted hairgrass (*Deschampsia cespitosa* (L.) P. Beauv.)	0	14	0	78	0	61
Common couch (*Elytrigia repens* (L.) Desv. Ex Nevski)	0	1	0	81	32	3
Other grass species[1]	0	0	0	14	0	5
Legume family (*Fabaceae*)	139	357	382	175	315	245
Red clover (*Trifolium pratense* L.)	132	350	365	0	77	14
White clover (*Trifolium repens* L.)	7	7	17	175	238	230
Non-legume dicotyledons	432	56	26	105	55	130
Shepherd's-purse (*Capsella bursa-pastoris* (L.) Medik.)	193	0	0	0	0	0
Pineappleweed (*Matricaria matricarioides* Porter ex Britton)	131	10	0	0	0	0
Scentless mayweed (*Tripleurospermum perforatum* (Mérat) Laínz)	78	25	5	0	0	0
Northern dock (*Rumex longifolius* DC.)	0	5	12	42	12	68
Dandelion (*Taraxacum* spp.)	11	0	3	22	3	59
Other species[2]	19	16	6	40	40	3

Number of spp.	20	26	18	35	21	18
Pre-grazing herbage mass, tonnes DM/ha[3]	1.29	1.84	1.53	1.16	2.71	1.68
Herbage height, cm[4]	15	20	17	10	23	15

[1] Marsh foxtail (*Alopecurus geniculatus* L.), cock's foot (*Dactylis glomerata* L.), red fescue (*Festuca rubra* L.), sweet vernal-grass (*Anthoxanthum odoratum* L.), rough meadow-grass (*Poa trivialis* L.).

[2] Creeping buttercup (*Ranunculus repens* L.), meadow buttercup (*Ranunculus acris* L.), spear thistle (*Cirsium vulgare* (Savi) Ten.), yarrow (*Achillea millefolium* L.), greater plantain (*Plantago major* L.), common nettle (*Urtica dioica* L.), marsh thistle (*Cirsium palustre* (L.) Scop.), autumn hawkbit (*Leontodon autumnalis* L.).

[3] Measured 5 cm above ground level with a calibrated rising plate meter.

[4] Measured above ground level with a rising plate meter.

Table 2. Estimated grazing preference[1] for botanical families of cows grazing short-term pasture (SP) or long-term pasture (LP) in period 1 to period 3

Item	SP			LP		
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3
Grass family (*Poaceae*)	1.03	0.88	0.76	0.59	0.84	0.72
Legume family (*Fabaceae*)[1]	1.76	1.33	1.38	2.65	1.27	1.53
Non-legume dicotyledons[1]	0.72	0.14	0.91	1.03	1.31	1.36

[1] Calculated as proportion of plant family in herbage disappearance, measurements with a calibrated raising plate meter 5 cm above ground level divided by proportion of plant family prior to grazing. Botanical composition before and after grazing was estimated by the dry-weight-rank method (Mannetje and Haydock, 1963).

Table 3. Dry matter (DM) concentrations, chemical composition, feed values, fatty acid (FA) concentrations and fat-soluble vitamin concentrations in herbage from short-term pasture (SP) or long-term pasture (LP) and barley concentrate (means of 3 periods)

Item	SP		LP		Concentrate
	Mean	SD	Mean	SD	Mean
DM, g/kg	185		213		889
Chemical composition					
CP, g/kg DM	190	41.4	180	20.0	119
Soluble protein, g/kg CP	290	36.1	287	51.3	240
Crude fat, g/kg DM	43	24.4	29	2.6	26
NDF, g/kg DM	474	43.1	472	10.7	167
ADF, g/kg DM	267	25.0	291	25.5	53
NFC, g/kg DM	243	42.8	274	21.4	650
Starch, g/kg DM	22	2.6	22	6.0	505
WSC, g/kg DM	128	37.8	137	28.6	-
Ash, g/kg DM	93	14.1	83	10.9	56
Feed value					
NE_L, MJ/kg DM	6.19	0.180	6.33	0.181	7.02
IVD DM, g/kg DM[1]	880	30.0	887	20.8	930
Digestible OM, g/kg DM	704	16.7	717	16.6	780
FA, g/kg DM					
C16:0	3.4	0.52	2.9	0.54	6.4
C18:0	0.4	0.07	0.3	0.06	0.3
C18:1c9	0.7	0.04	0.6	0.12	3.1
C18:2 n-6	4.2	0.75	3.4	0.75	14.0
C18:3 n-3	14.5	4.32	9.2	2.05	1.5
Total FA	24.8	5.88	17.7	3.54	26.3
Vitamin, mg/kg DM					
α-Tocopherol	82.9	5.98	75.6	8.20	59.7
β-Carotene	47.6	17.01	45.3	15.25	0.0
Lutein	244	77.1	193	49.8	1.0

[1] In vitro digestibility of DM after incubation for 48 h.

Table 4. Body weights (BW), body condition scores (BCS) and feed intakes for two groups of 8 cows grazing either short-term pasture (SP) or long-term pasture (LP) (changes during the whole experiment and means of 3 periods) (n = 24)

Item	SP		LP	
	Mean	SD	Mean	SD
Initial BW, kg	597	34.5	598	56.3
Initial BCS, points[1]	2.6	0.60	2.7	0.73
BW change, g/d	-74	116.3	50	155.4
BCS change, points/100 d	0.09	0.202	0.11	0.321
Feed intake, kg DM/d				
Herbage[2]	15.3	1.22	15.3	1.64
Concentrate	2.7		2.7	

[1] Estimated using a 5-point scale (1 = emaciated to 5 = severely over-conditioned) with 0.25-unit increments.

[2] Estimation based on the net energy requirements of the cows.

Table 5. Fatty acid (FA) composition in milk from cows grazing short-term pasture (SP) or long-term pasture (LP) (n = 24)

Item	Treatment		SEM[1]	*P*-value[2]
	SP	LP		
FA, g/kg FA methyl esters (FAME)				
C4:0	30.3	28.2	0.99	NS
C6:0	21.6	20.5	0.60	NS
C8:0	14.6	13.9	0.36	NS
C10:0	32.7	31.9	0.93	NS
C12:0	37.3	38.1	1.13	NS
C13:0	1.0	1.0	0.07	NS
C14:0	122.5	125.5	2.00	NS
C14:1c9	10.0	12.2	0.92	NS
C15:0	11.4	12.2	0.73	NS
C16:0	278.3	309.2	9.16	0.02
C16:1c7	0.9	0.9	0.05	NS
C16:1c9	13.4	15.2	0.87	NS
C17:1c9	3.6	3.4	0.09	0.01
C18:0	114.1	99.6	4.03	0.02
C18:1c9	197.3	187.5	6.07	NS
C18:1c11	5.8	4.4	0.36	0.02
C18:1t-FA	46.7	40.4	2.40	0.09
C18:2 n-6	20.1	18.5	0.88	NS
C18:2c9t11	10.8	10.0	0.86	NS
C18:2t10c12	1.1	1.2	0.32	NS
C18:3 n-6	1.6	1.6	0.09	NS
C18:3 n-3	9.7	9.9	0.55	NS
C18:4 n-3	0.7	0.6	0.08	NS
C20:0	2.3	2.2	0.32	NS
C20:1c9	5.4	4.8	0.59	NS
C20:2 n-6	0.9	0.8	0.39	NS
C20:3 n-6	0.6	0.6	0.05	NS
C20:3 n-3	0.0	0.0	0.01	NS
C20:4 n-6	1.3	1.2	0.04	NS
C20:5 n-3	1.2	1.2	0.37	NS
C22:0	0.9	0.8	0.03	NS
C22:1c11	0.2	0.2	0.02	NS
C22:1c13	0.1	0.0	0.01	0.06
C22:5 n-6	0.1	0.3	0.16	NS
C22:5 n-3	1.3	1.1	0.21	NS
C22:6 n-3	0.0	0.0	0.01	NS
C24:0	0.5	0.6	0.03	0.09
C24:1c15	0.1	0.1	0.03	NS

[1] Standard error of means.

[2] NS: $P > 0.10$.

Table 6. Effect of short-term pasture (SP) or long-term pasture (LP) on milk concentrations of fat-soluble vitamins and formation of lipid hydroperoxides in a light-exposure experiment (n = 24)

Item	Treatment		SEM[1]	*P*-value[2]
	SP	LP		
Vitamin concentration in milk, mg/kg				
α-Tocopherol	1.46	1.28	0.074	0.01
β-Carotene	0.24	0.23	0.015	NS
Retinol	0.50	0.43	0.028	0.09
Hydroperoxides, absorbance 500 nm				
24 h light exposure	0.24	0.32	0.024	0.04
48 h light exposure	0.46	0.45	0.034	NS

[1] Standard error of the means.

[2] NS: $P > 0.10$.