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Effect of feeding fresh forage and marine algae on the fatty acid composition and oxidation of milk and butter

K. E. Glover,* S. Budge,† M. Rose,‡ H. P. V. Rupasinghe,§ L. MacLaren,# J. Green-Johnson,‖ and A. H. Fredeen*[1]

*Department of Plant and Animal Sciences, Nova Scotia Agricultural College, Truro, Nova Scotia, Canada, B2N 5E3
†Department of Process Engineering and Applied Science, Dalhousie University, Halifax, Nova Scotia, Canada, B3J 2X4
‡Shur-Gain, Nutreco Canada Incorporated, Truro, Nova Scotia, Canada, B2N 6X8
§Department of Environmental Sciences, Nova Scotia Agricultural College, Truro, Nova Scotia, Canada, B2N 5E3
#President's Office, Nova Scotia Agricultural College, Truro, Nova Scotia, Canada, B2N 5E3
‖Faculty of Science, University of Ontario Institute of Technology, Oshawa, Ontario, Canada, L1H 7K4

ABSTRACT

This study evaluated the effects of feeding fresh forage either as pasture plus a concentrate (PAS) or as a silage-based total mixed ration (TMR), combined with either a ruminally inert lipid supplement high in saturated fatty acids ($^-$) or a ruminally protected microalgae containing 22 g of docosahexaenoic acid (DHA)/100 g of fatty acids ($^+$) on the fatty acid (FA) composition and oxidation of milk and butter. For the 8 mid-lactation Holstein cows in this study, milk yield was not significantly affected by treatment, averaging 32.3 ± 1.28 kg/d. Milk fat content was higher for PAS$^-$, averaging 5.05 compared with 4.10 ± 0.17% for the mean of other treatments, and was significantly depressed with microalgae supplementation (3.97 vs. 4.69 ± 0.17%). The saturated fatty acid level in the milk of cows fed TMR$^-$ was significantly higher than that of the other treatments (66.9 vs. 61.2 g/100 g of FA). The level of monounsaturated FA was lowered by feeding TMR$^-$ (27.4 vs. 32.0 g/100 g of FA), whereas levels of polyunsaturated FA were elevated by feeding PAS$^+$ compared with the mean of the other treatments (6.54 vs. 5.07 g/100 g of FA). Feeding the rumen-protected microalgae increased the DHA content of milk more than 4-fold (0.06 to 0.26 g/100 g of FA) with the PAS treatment. The conjugated linoleic acid content of milk was highest for PAS$^+$ compared with the other treatments (4.18 vs. 3.41 g/100 g of FA). In general, the fatty acid composition of butter followed that of milk. Overall, feeding the TMR supplemented with the rumen-protected microalgae increased the levels of volatile products of oxidation in milk and butter. No effect of forage type or microalgae supplementation was observed on the oxidative stability or antioxidant capacity of milk, although the oxidative stability of butter exposed to UV was reduced with microalgae supplementation, particularly with TMR, as assessed by using the ferric reducing ability of plasma assay.

Key words: solid-phase microextraction, thiobarbituric acid reactive substances, ferric reducing ability of plasma (FRAP) assay, docosahexaenoic acid

INTRODUCTION

The ratio of long-chain n-3 fatty acids (**LCn-3FA**), specifically eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**), to n-6 fatty acids (mainly linoleic acid) in North American diets is considered too low (Simopoulous, 2006). Supplemental LCn-3FA lowers the incidence of developmental, chronic, and inflammatory disease conditions and attention deficit-hyperactive disorder (Brookes et al., 2006), affects memory, intelligence, and vision in children (Shiota et al., 1999; Amminger et al., 2007), and may reduce development of childhood obesity (Abete et al., 2011). Consumption of fish containing LCn-3FA is low relative to that of other meats and poultry, and although cow milk fat is widely consumed, it is a poor source of LCn-3FA (Gonzalez et al., 2003). Enriching dairy products with LCn-3FA is a potentially important strategy for improving intake of LCn-3FA because of the wide popularity of dairy products, especially with children. Marine microalgae are an alternative and sustainable source of LCn-3FA, and can contain substantial amounts of EPA and DHA (Barsanti and Gualtieri, 2006). Limited research exists on the effect of using algae as a dietary supplement of beneficial FA in cow milk (Franklin et al., 1999; AbuGhazaleh et al., 2009).

Polyunsaturated fatty acids in the bovine diet are extensively biohydrogenated in the rumen if unprotected; about 70 to 95% of linoleic acid and 85 to 100% of linolenic acid are biohydrogenated before leaving the rumen (Shingfield et al., 2008). Transfer efficiency of

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[1]Corresponding author: afredeen@nsac.ca

LCn-3FA from dietary intake to milk depends on the effectiveness of rumen protection (Chilliard et al., 2001; Jenkins and Bridges, 2007). Research is also needed to determine the susceptibility of milk and dairy products enriched with LCn-3FA to oxidation. Exposure of milk to UV light for just 6 to 24 h may cause LCn-3FA to oxidize (Walstra and Jenness, 1984; Burton et al., 1985; Havemose et al., 2006). Oxidation of LCn-3FA in enriched milk creates volatile compounds (Kataoka et al., 2000; Venkateshwarlu et al., 2004) from the scission of FA (Walstra and Jenness, 1984; Frankel, 2005; Gallagher et al., 2005), which reduces its quality. Antioxidants, including dietary tocopherols and carotenoids, squelch the oxidation process (Olsen, 1996; Noziere et al., 2006). As well as affecting the FA composition of milk (Elgersma et al., 2006), forages are the main natural source of antioxidants in milk. Although the effects on milk FA composition are influenced by the type of FA consumed by the cow and the rate of FA escaping from the biohydrogenating environment of the rumen (Elgersma et al., 2004), the amount of antioxidant available depends on how the forage is handled between its fresh and fed states. Compared with fresh pasture forage, ensiled forages contain fewer antioxidants due to wilting and ensiling. For instance, respective levels of α-tocopherol were 76.5 compared with 225 mg/kg of DM in ensiled and fresh timothy, and 19.5 to 30 compared with 420 mg of β-carotene/kg of DM in dried and fresh timothy forage (Shingfield et al., 2005; Noziere et al., 2006).

Limited research has been conducted on the oxidative stability and volatile compound production of milk naturally enriched with LCn-3FA, particularly using microalgae as the source of LCn-3FA, and even less information is available concerning butter, with its potentially higher concentration of PUFA. Furthermore, consideration of the potential interaction between basal forage ration (TMR versus fresh forage) and microalgae supplementation on the composition and oxidative stability of milk and butter is novel and needs to be investigated. Feeding rumen-protected microalgae to dairy cows could reduce the oxidative stability of milk by increasing LCn-3FA content, and this effect may be counteracted by consumption of naturally occurring antioxidants. Thus, for this study, we hypothesized that feeding a rumen-protected algal source of LCn-3FA to dairy cows would alter the FA composition of milk fat, increase the susceptibility to oxidation of milk and butter manufactured from this milk, and alter volatile compound production. We further hypothesized that the type of forage consumed by cows would influence the antioxidant activity of the enriched milk and butter.

MATERIALS AND METHODS

Animal Management and Experimental Design

Eight Holstein cows in early to mid lactation were assigned to two 4 × 4 Latin squares, one comprising cows of first parity and the other of cows of parity ≥3. The 4 feeding periods were 28 d each, with the first 21 d of each period serving as an adjustment period. The cows were housed in tie-stalls and fed a TMR or they rotationally grazed a perennial pasture (**PAS**) dominant in the following species: bluegrass (>50%), timothy and meadow fescue (~20%), white clover (~10%), and the remainder minor pasture species and forbs. Pasture quality was maintained through rotational grazing (A. Fredeen, unpublished data). Cows were supplemented twice daily with 100 g of a DHA-rich microalgae protected with the inert fat ($^+$) or an inert fat without microalgae ($^-$). Both supplements contained 106.2 g of lipid. The TMR was balanced to meet requirements of a cow producing 35 kg of milk daily (NRC, 2001) and contained (% DM basis): 26.0% first-cut mixed haylage, 26.0% corn silage, 3.61% grass hay, and 44.3% concentrate composed of (% of DM): 22.7% rolled barley grain, 22.7% cracked corn grain, 36.2% soybean meal, 9.95% distillers grain, and 8.67% mineral mix. The TMR was fed at 0700 and 1500 h in ad libitum amounts (10% orts). Grazing cows were fed, at milking, the same concentrate used to balance the silage in the TMR at a daily rate of 25% of milk yield (wt/wt as fed) to a maximum of 8 kg. The nutrient content and FA composition of the TMR and PAS components (pasture forage and concentrate) are shown in Table 1, as well as the FA analysis of the lipid supplements.

On d 24 to 28 of each period, all cows were confined to tie-stalls and received exercise each morning. During this period, cows on PAS received harvested pasture, similar to that grazed, at 0630 and 1400 h, and intake of pasture forage was determined. Cows were milked twice daily (0500 and 1600 h) by bucket milker. On d 27 and 28, milk was weighed, sampled, and pooled proportionately by milk yield to generate a 2-L composite, which was stored during sampling at 4°C in amber containers. After each morning milking, cows were weighed and condition scored by 2 trained individuals. The experiment followed guidelines established by the Canada Council of Animal Care (2009).

Sample Analysis

Samples of feeds and supplements were collected daily, combined by cow and period, dried (55°C), and ground (0.1 mm) before analysis. Content of CP was

Table 1. Composition of dietary treatments and their components

Analyte[1]	TMR	Pasture forage	Concentrate	Algae	Inert fat
DM (%)	50.41	22.46	90.42	—	—
Ash (% of DM)	6.91	8.14	8.34	—	—
CP (% of DM)	15.18	13.14	23.53	—	—
ADF (% of DM)	24.23	30.4	6.21	—	—
NDF (% of DM)	38.77	57.03	13.97	—	—
Lipid (% of DM)	5.15	6.83	5.92	—	—
Fatty acids (FA; g/100 g of FA)					
SFA	39.01	22.41	47.45	63.25	98.07
MUFA	18.73	11.49	17.04	1.22	1.52
PUFA	42.23	66.05	35.52	35.52	0.41
LA	32.91	20.46	33.06	ND[2]	ND
ALA	8.62	44.39	2.19	ND	0.00
EPA	ND	ND	ND	1.24	0.00
DHA	ND	ND	ND	22.28	0.00

[1]SFA = saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA; LA = linoleic acid; ALA = α-linolenic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

[2]Values <0.1% were considered not detectable (ND).

determined on a FP-528 Nitrogen Determinator (Leco Corp., St. Joseph, MI), and NDF and ADF were determined using an Ankom 200/220 Fiber Analyzer (Ankom Technology, Macedon, NY). The NDF procedure included heat-stable amylase and sodium sulfite; NDF is reported on an ash-free basis, where ash was obtained overnight at 550°C in an Isotemp muffle furnace (Fisher Scientific, Nepean, ON, Canada).

Milk samples were preserved and analyzed for lipid and protein content using infrared techniques. Butter was prepared by centrifugation at 15,000 × *g* for 15 min at 4°C (Goncalves and Collares, 1999) using a Beckman J2-21M/E centrifuge (Beckman, Palo Alto, CA).

Aliquots (15 mL) of pooled milk and butter samples were placed in 15-mL centrifuge tubes and stored at 4°C in the dark (n = 54), or laid horizontally 30 cm beneath a UV light (ExoTerra 12.0, R.C. Hagen Enterprises, Montreal, QC, Canada; UVA 33.3%, UVB 33.3%; n = 54) for 2 or 4 d of continuous UV light exposure. Samples under the light were rotated once daily. Milk and butter samples were then assessed using the thiobarbituric acid reactive substances (**TBARS**) test according to AOCS (1995; method Cd 19–90). Butter samples were first melted for 20 min at 40°C and filtered (Millex, hydrophilic low protein binding durapore, 13 mm × 0.45 μm; Millipore Corp., Billerica, MA) before analysis. In brief, milk and butter samples were mixed with butanol, incubated with TBARS reagent at 95°C for 120 min, and then brought to room temperature (21 to 23°C). Absorbance was determined in duplicate at 532 and 450 nm using a Bausch & Lomb Spectronic 501 spectrophotometer (Rochester, NY).

In addition to TBARS analysis, samples of the milk and butter stored in the dark or exposed to UV radiation for 2 and 4 d were collected, capped with N_2, and stored at −80°C for analysis of FA composition, volatiles, and antioxidant capacity as follows.

For determination of FA composition, lipids were extracted from milk and butter following a modified Folch et al. (1957) method using 2:1 CH_3Cl_3:MeOH (Budge et al., 2006). Fatty acid methyl esters (**FAME**) were prepared from pure lipid using H_2SO_4 in methanol and were dried over anhydrous Na_2SO_4. The FAME were analyzed by GC using a Perkin Elmer Autosystem (Waltham, MA) with flame-ionization detector equipped with a 30-m × 0.25-mm i.d. flexible fused silica column coated with 50% cyanopropyl polysiloxane (0.25 μm film thickness, DB-23, Agilent Technologies, Folsom, CA). The detector and injector were held at 250°C. The oven temperature program followed was initial temperature of 153°C held for 2 min and then increased at 2.3°C/min to 174°C, held for 0.2 min and then increased at 2.5°C/min to 210°C (Budge et al., 2006). The FAME were identified by comparison of retention times with those of standards. Transfer efficiency of DHA from diet to milk was estimated as follows:

$$[\text{g of DHA/d}_{\text{(enriched milk)}} - \text{g of DHA/d}_{\text{(standard milk)}}]/[\text{g of DHA/d}_{\text{(enriched diet)}}].$$

Headspace solid-phase microextraction (**SPME**) was used to determine volatile compounds. Milk and butter samples were thawed overnight and analyzed the next morning using dichlorobenzene (1.0 μg, Sigma Aldrich, St. Louis, MO) as an internal standard. Samples were then heated to 45°C and the SPME fiber (1 cm, 75 μm CAR-PDMS, no. 57318; Supelco, Bellefonte, PA) was exposed to the headspace of the sample for 45 min (Mounchili et al., 2005). The exposed fiber was

retracted and inserted into the injector of a GC-MS (Trace GC Ultra, Polaris Q; Thermo Electron Company, Waltham, MA). The analytical column used a nitroterephthalic acid modified polyethylene glycol phase (30 m × 0.25 mm i.d., 0.25 μm film thickness, FFAP, Agilent Technologies). The initial temperature was 40°C for 5 min. The temperature was then increased at 10°C/min to 230°C and held for 5 min. The injector and transfer line were held at 250 and 230°C, respectively, with a flow rate of 1.2 mL/min. Peak areas of volatile compounds were reported relative to the peak area of the internal standard. Volatiles were tentatively identified by comparing each spectra with the National Institute of Standards and Technology GCMS library; all identifications were then confirmed by comparison to the retention times and spectra of standards.

The ferric reducing ability of plasma (**FRAP**) assay (Benzie and Strain, 1996) was used to estimate antioxidant capacity of milk. Milk samples were thawed overnight at 4°C, and 10 mL of cold acetone was added to 5 mL of milk. The resulting solution was held at 4°C for 30 min and then centrifuged (3,000 × *g*) for 10 min (model Durafuge 300, Precision Scientific, Asheville, NC). After centrifugation, 10 mL of the supernatant was withdrawn into a test tube, the acetone was evaporated using N_2 gas, and the resulting pellet was redissolved in 5 mL of 50% methanol. For the analysis of feed, finely ground samples (0.3 g) were combined with 15 mL of methanol in amber-colored glass vials, sonicated for 30 min, and centrifuged (4,500 × *g*) for 10 min. The supernatant was removed for analysis. Standards from 5 to 900 μ*M* Trolox were prepared from 10 m*M* Trolox stock solution diluted serially in 50% methanol. The working FRAP reagent kept at 37°C was prepared by combining 100 mL of 300 m*M* acetate buffer, and 10 mL each of 10 m*M* 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and 20 m*M* $FeCl_3 \cdot 6H_2O$. Absorbance at 595 nm was determined using a plate reader (model FLUOstar Optima, BMG Labtech, Durham, NC) after a 15-min incubation of the sample and working FRAP reagent at 37°C. Absorbance readings were converted to milligrams of Trolox equivalents (Eq) per liter of milk or milligrams of Trolox equivalents per kilogram of feed.

Statistical Analysis

Data for the two 4 × 4 Latin squares were analyzed in a manner similar to Aquino et al. (2008) to determine effect of square, interactions between square and treatments, and crossover effects, using PROC Mixed (2001 version; SAS Institute Inc., Cary, NC) and the following model:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \tau_k + \delta_l + \tau\delta_{kl} + \varepsilon_{ijklm},$$

where y = the response; μ = population mean; α = cow effect; β = period effect; τ = treatment effect, δ = square effect, ε_{ijklm} = uncontrolled error effect; $i = i$th cow (1 to 8); $j = j$th period (1 to 4); $k = k$th treatment (1 to 4); $l = l$th square (1 or 2). Three contrasts were constructed: $TMR^{\pm}$ (TMR with and without microalgae supplement) versus $PAS^{\pm}$; $TMR^{+} + PAS^{+}$ versus $TMR^{-} + PAS^{-}$; and TMR^{+} versus PAS^{+}. Statistical significance was set at $P < 0.05$.

RESULTS

We observed no differences between the Latin squares, indicating no significant effect of parity on milk characteristics or its oxidative stability; hence, data were combined for analysis.

Feed Intake

Table 2 shows the average DMI of cows on the TMR and PAS treatments with and without the microalgae supplement. Total DMI was significantly different between the TMR and PAS treatments, which averaged 21.3 and 16.6 kg/d (SE = 0.55; $P = 0.01$), respectively. Microalgae supplementation had no significant effect on DMI although intake with the PAS^{+} treatment was at least 5 kg/d (SE = 0.79) lower compared with the other treatments. Concentrate was fed to cows on pasture in relation to their milk production, with a maximum of 8.0 kg/d. The TMR was approximately 44% concentrate and therefore the cows on the TMR consumed, on average, 9.4 kg/d of concentrate compared with the cows on the PAS treatment, in which concentrate intake averaged 5.5 kg/d. Overall, the greatest difference in DMI was observed with the PAS^{+} treatment, where reduced DMI due to pasture and microalgae supplementation was observed.

Cow Performance

Milk fat percentage was reduced by $TMR^{\pm}$ compared with $PAS^{\pm}$ (3.99 vs. 4.69 ± 0.17%; $P = 0.01$); however, yields of milk and FCM were not significantly affected by treatment, averaging 32.33 and 33.33 ± 0.95 kg for $TMR^{\pm}$ and $PAS^{\pm}$, respectively (Table 3). Compared with $PAS^{\pm}$, $TMR^{\pm}$ elevated milk protein content (3.13 vs. 2.89 ± 0.04%; $P = 0.001$), and milk energy content was lower for $TMR^{\pm}$ (3.00 vs. 3.22 ± 0.07 MJ; $P = 0.03$). The rumen-protected microalgae reduced TS (12.57 vs. 13.19 ± 0.17%; $P = 0.02$) and milk fat content (3.99 vs. 4.70 ± 0.17%; $P = 0.007$). Milk urea

Table 2. Intake of pasture plus concentrate (PAS) or silage-based TMR with (+) or without (−) a supplement containing rumen-protected microalgae

						P-value for contrasts[1]		
Intake (kg of DM/d)	TMR$^+$	TMR$^-$	PAS$^+$	PAS$^-$	SE	TMR$^\pm$ vs. PAS$^\pm$	TMA$^+$ + PAS$^+$ vs.TMR$^-$ + PAS$^-$	TMR$^+$ vs. PAS$^+$
TMR	20.49	22.19						
Pasture			8.61	13.69				
Concentrate			5.47	5.44				
Total	20.49	22.19	14.08	19.13	0.79	0.01	NS	0.01

[1]TMR$^\pm$ vs. PAS$^\pm$ compares TMR with and without microalgae supplementation to PAS with and without microalgae supplementation; NS denotes $P > 0.05$.

content tended to be lower for cows fed the rumen-protected microalgae (2.98 vs. 3.22 ± 1.27 mg/dL; $P = 0.01$). The fat content of milk and TS were elevated significantly by the PAS$^-$ treatment compared with the other treatments, averaging 5.05 (vs. mean of 4.10 ± 0.24%; $P = 0.005$) and 13.49 (vs. mean of 12.71 ± 0.24%; $P = 0.03$), respectively. Somatic cell count was not significantly different among treatments, averaging 808 ± 209 × 10^3 cells/mL. The average SCC was high because of subclinical S*taphylococcus aureus* infections in 3 of the 8 cows on trial. We observed no changes in the udder or milk of the infected cows, including gross composition analysis. No significant differences in BW, BCS, or rectal temperature were observed among treatments, averaging 609.8 ± 9.2 kg, 2.7 ± 0.03, and 37.9 ± 0.08°C, respectively.

FA Composition of Milk and Butter

The FA composition of milk and butter was affected by treatment (Table 4). In milk, PAS$^+$ significantly increased the content of PUFA, conjugated linoleic acid (**CLA**), and α-linolenic acid (**ALA**). The SFA level in the milk of cows fed TMR$^-$ was higher than that of the other treatments (66.9 vs. 61.2 ± 0.82 g/100 g of FA; $P = 0.002$), whereas the level of MUFA was lower in TMR$^-$ (27.4 vs. 32.0 ± 0.77 g/100 g of FA; $P = 0.01$). Linoleic acid (**LA**) and EPA were not significantly affected by treatment. The ALA content was higher in milk fat from cows on pasture compared with those fed TMR (0.76 vs. 0.37 ± 0.04 g/100 g of FA; $P < 0.001$). Supplementation with rumen-protected microalgae significantly increased levels of DHA (0.24 vs. 0.08 ± 0.03 g/100 g of FA; $P = 0.001$), and the estimated transfer efficiency of DHA from diet to milk was 2.7 and 5.7% for TMR$^+$ and PAS$^+$, respectively. Microalgae supplementation also significantly increased LA, CLA, and PUFA in milk when comparing TMA$^+$ with TMR$^-$ and PAS$^+$ with PAS$^-$; the highest level of PUFA was with the PAS$^+$ treatment. The FA composition of butter paralleled that of milk in most respects (Table 4). Levels of MUFA were greatest for the PAS$^-$ treatment, whereas levels of PUFA were greatest for the PAS$^+$ treatment. A distinct difference was observed between PAS and

Table 3. Effect of pasture plus concentrate (PAS) or silage-based TMR with (+) or without (−) a supplement containing rumen-protected microalgae on cow performance

						P-value for contrasts[1]		
Analyte	TMR$^+$	TMR$^-$	PAS$^+$	PAS$^-$	SE	TMR$^\pm$ vs. PAS$^\pm$	TMR$^+$ + PAS$^+$ vs. TMR$^-$ + PAS$^-$	TMR$^+$ vs. PAS$^+$
Milk (kg/d)	34.15	33.07	31.89	30.22	1.38	NS	NS	NS
FCM (kg/d)	31.68	33.75	33.44	34.43	1.34	NS	NS	NS
Milk solids (%)	12.35	12.98	12.80	13.49	0.24	NS	0.02	NS
Milk fat (%)	3.61	4.34	4.34	5.05	0.24	0.01	0.007	0.01
Milk lactose (%)	4.45	4.50	4.47	4.46	0.04	NS	NS	NS
Milk protein (%)	3.16	3.10	2.88	2.90	0.05	0.001	NS	0.01
Milk energy (MJ/kg)	2.86	3.14	3.09	3.36	0.09	0.03	0.01	NS
Milk urea (mg/dL)	15.69	24.76	16.76	18.51	1.80	NS	0.01	NS
BW (kg)	619.1	607.7	603.6	608.0	18.3	NS	NS	NS
BCS	2.8	2.7	2.6	2.7	0.05	NS	NS	NS
Rectal temperature (°C)	38.1	37.6	38.0	37.9	0.16	NS	NS	NS
Milk SCC (×10^3 cells/mL)	1,074	567	453	1,138	419	NS	NS	NS

[1]TMR$^\pm$ vs. PAS$^\pm$ compares TMR with and without microalgae supplementation to PAS with and without microalgae supplementation; NS denotes $P > 0.05$.

Table 4. Fatty acid (FA) composition (g/100 g of FA) of milk and butter of cows fed pasture plus concentrate (PAS) or silage-based TMR with (+) or without (−) a supplement containing rumen-protected microalgae

Item[1]	TMR+	TMR−	PAS+	PAS−	SEM	*P*-value
Milk						
SFA	61.90	66.87	61.33	60.21	0.82	0.002
MUFA	31.21	27.39	31.26	33.48	0.77	0.01
PUFA	5.45	4.58	6.54	5.20	0.20	0.01
CLA	3.59	3.12	4.18	3.52	0.12	0.03
LA	2.43	2.26	2.56	2.35	0.05	NS[2]
ALA	0.35	0.38	0.83	0.68	0.05	<0.001
EPA	0.05	0.06	0.08	0.06	0.001	NS
DHA	0.22	0.10	0.26	0.06	0.02	0.01
Butter						
SFA	61.76	66.29	60.96	59.98	0.86	0.02
MUFA	32.65	28.55	32.13	34.59	0.75	0.001
PUFA	5.37	4.65	6.34	5.53	0.20	0.03
CLA	1.15	1.01	1.58	1.27	0.10	NS
LA	2.44	2.21	2.50	2.39	0.05	NS
ALA	0.35	0.43	0.74	0.76	0.05	0.001
EPA	0.05	0.06	0.07	0.12	0.01	NS
DHA	0.25	0.08	0.29	0.09	0.03	0.003

[1]SFA = saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA; CLA = conjugated linoleic acid; LA = linoleic acid; ALA = α-linolenic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

[2]NS denotes $P > 0.05$.

TMR treatments with respect to ALA and PUFA contents, which were significantly higher with PAS. The content of CLA followed the same trend as for milk, except that differences were not statistically significant for butter. Supplementation with rumen-protected microalgae significantly increased DHA levels but did not affect EPA levels in butter.

Analysis of Oxidative Stability

A time effect in TBARS values was observed ($P < 0.05$), with values often higher on d 2 compared with either exposed or unexposed product on d 4 (Table 5). On d 0, absorbance values at 450 nm were highest for TMR− and lowest for PAS− for both UV exposed and unexposed milk. On d 4, values for unexposed milk were higher for TMR+ and, for exposed milk, PAS− had the highest absorbance whereas PAS+ had the lowest. Treatments did not differ in absorbance at 532 nm for milk. In unexposed butter (Table 5), absorbance at 450 nm was highest for PAS+ on both d 0 and d 2 and was also high on d 4 but not significantly different from TMR+, which had the highest absorbance. The lowest absorbance values in the unexposed group were associated with the TMR− and PAS− treatments. For the exposed butter, TMR+ and PAS+ treatments tended to have the highest absorbance values, whereas TMR− and PAS− treatments tended to have lower absorbance values. Similar trends were observed at 532 nm for both exposed and unexposed butter, with TMR− and PAS− treatments having lower absorbance readings than the TMR+ and PAS+ treatments. After 4 d of exposure, the absorbance of TMR+ butter was greater at both 450 and 532 nm, whereas absorbance of TMR− and PAS− were lowest at 532 nm.

Identification of Volatile Compounds

Feeding pasture significantly elevated the concentration of 2-nonenal (22.9 vs. 6.7 mg/mL; $P < 0.01$) in milk and tended to increase several other volatiles, including hexanal (87.7 vs. 50.6 mg/mL), 2- heptanone (77.6 vs. 29.2 mg/mL), 1-pentanol (8.97 vs. 3.08 mg/mL), 2-nonanone (6.62 vs. 3.82 mg/mL), and octanal (2.41 vs. 1.92 mg/mL), although these increases were not significantly different (Table 6). Microalgae had no significant effect on milk volatile formation. Feeding the rumen-protected microalgae tended to reduce the concentration of butanone in milk (5.84 vs. 7.96 mg/mL) and increase the concentration of 2-nonenal (18.17 vs. 11.42 mg/mL). In butter, more statistically significant changes were observed in volatile compound production. The concentrations of 2-nonen-1-ol and 2-octanone were increased ($P < 0.05$) by feeding the rumen-protected microalgae. Significant differences between the TMR and PAS treatments were found for 2-pentanone ($P < 0.01$), heptanal ($P < 0.001$), 2-octanone ($P < 0.01$), 2-decanone ($P < 0.01$), butyric acid ($P < 0.05$), and 2-nonen-1-ol ($P < 0.001$). These volatiles also differed significantly between TMR+ and PAS+ treatments, and with the exception of 2-octanone, concentrations were higher in TMR+ than in PAS+.

Table 5. Mean absorbance units of thiobarbituric acid reactive substances (TBARS) at 450 and 532 nm of milk and butter kept in the dark (unexposed) or exposed to UV light

	Treatment[1]				
Group	TMR$^+$	TMR$^-$	PAS$^+$	PAS$^-$	P-value[2]
Milk, unexposed, 450 nm					
Day 0	0.46	0.49	0.45	0.44	0.25
Day 2	0.41	0.47	0.47	0.48	
Day 4	0.45	0.41	0.42	0.39	
Milk, exposed, 450 nm					
Day 0	0.46	0.49	0.45	0.44	0.02
Day 2	0.44	0.50	0.50	0.51	
Day 4	0.43	0.40	0.38	0.50	
Milk, unexposed, 532 nm					
Day 0	0.13	0.14	0.12	0.10	0.01
Day 2	0.10	0.11	0.12	0.12	
Day 4	0.12	0.12	0.12	0.12	
Milk, exposed, 532 nm					
Day 0	0.13	0.14	0.12	0.10	0.06
Day 2	0.10	0.11	0.13	0.12	
Day 4	0.11	0.08	0.11	0.10	
Butter, unexposed, 450 nm					
Day 0	0.33	0.34	0.35	0.32	<0.001
Day 2	0.39	0.38	0.45	0.39	
Day 4	0.32	0.28	0.31	0.28	
Butter, exposed, 450 nm					
Day 0	0.33	0.34	0.35	0.32	<0.001
Day 2	0.40	0.39	0.42	0.39	
Day 4	0.32	0.30	0.30	0.30	
Butter, unexposed, 532 nm					
Day 0	0.11	0.11	0.11	0.09	0.11
Day 2	0.11	0.08	0.11	0.09	
Day 4	0.10	0.06	0.09	0.08	
Butter, exposed, 532 nm					
Day 0	0.10	0.11	0.11	0.10	0.63
Day 2	0.11	0.08	0.10	0.08	
Day 4	0.14	0.07	0.10	0.08	

[1]Cows fed pasture plus concentrate (PAS) or silage-based TMR with ($^+$) or without ($^-$) a supplement containing rumen-protected microalgae.

[2]P-value indicates effect of day on TBARS absorbance measurement. Dietary treatment had no significant effect on TBARS measurement.

Antioxidant Activity

No significant difference in antioxidant capacity of milk or feed was observed among the treatments, as measured using the FRAP assay (Table 7). The antioxidant capacity of algae was higher than that of the other feedstuffs. Pasture antioxidant capacity appeared to be slightly higher than that of TMR, but type of forage in the ration did not confer any significant effect on the antioxidant capacity of milk as determined using the FRAP assay.

DISCUSSION

Feeding rumen-protected microalgae in combination with either TMR or pasture provided a significant dietary source of DHA that elevated levels of DHA in milk and butter. Estimates of the transfer efficiency of supplemental DHA to milk were twice as high for PAS (5.7%) compared with TMR (2.7%). The concentration of DHA was less than that reported by Franklin et al. (1999), who also fed more algae (910 g/d vs. 200 g of microalgae/d in the present study) and observed a level of 0.76 g of DHA/100 g of FA vs. 0.24 /100 g of FA. However, the amount of DHA consumed daily by cows in the study of Franklin et al. (1999) was approximately 30 g, whereas the amount of DHA consumed daily by cows in this experiment was approximately 40 g. Differences in the efficiency of DHA transfer to milk may be explained by differences in the efficiency of protection against rumen degradation of the lipid. Chilliard et al. (2001) reviewed the literature and observed that the transfer efficiency of DHA from fish oil to milk is generally low, averaging 4.1%. High variability in the efficiency of protection processes was also noted in their review.

Elgersma et al. (2004) observed an increase in SFA content of milk fat when cows transitioned from pas-

Table 6. Volatile compounds (mg/mL) in milk or butter that was exposed to UV light for 4 d from cows fed pasture plus concentrate (PAS) or silage-based TMR with (+) or without (−) a supplement containing rumen-protected microalgae

							P-value for contrast[1]		
Retention time (min)	Volatile	TMR^+	TMR^-	PAS^+	PAS^-	SE	$TMR^{\pm}$ vs. $PAS^{\pm}$	$TMR^+ + PAS^+$ vs. $TMR^- + PAS^-$	TMR^+ vs. PAS^+
Milk									
2.15	2-Butanone	5.03	8.63	6.65	7.29	0.26	NS	NS	NS
5.18	Hexanal	56.7	44.50	85.5	89.8	3.89	NS	NS	NS
7.63	2-Heptanone	38.1	20.23	73.3	81.8	5.14	NS	NS	NS
9.14	1-Pentanol	2.98	3.19	8.29	9.66	0.61	NS	NS	NS
9.94	1-Octen-3-one	2.08	1.72	1.85	2.97	0.10	NS	NS	NS
11.34	2-Nonanone	4.09	3.54	6.12	7.12	0.30	NS	NS	NS
11.4	2-Nonen-1-ol	6.21	3.12	3.17	3.14	0.27	NS	NS	NS
11.96	2-Octanal	2.28	1.55	2.18	2.64	0.08	NS	NS	NS
13.4	2-Nonenal[2]	9.34	4.05	27.00	18.80	1.80	**	NS	**
14.77	2-Decenal	0.34	0.56	0.53	0.73	0.03	NS	NS	NS
Butter									
2.98	2-Pentanone	0.59	0.36	0.24	0.23	0.06	**	NS	**
4.61	4-Octen-3-one	0.01	0.00	0.01	0.01	0.00	NS	NS	NS
5.19	Hexanal	1.66	0.83	0.64	0.77	0.16	NS	NS	NS
7.65	2-Heptanone	0.49	0.78	0.79	0.20	0.10	NS	NS	NS
7.74	Heptanal	6.90	3.37	1.81	1.13	0.91	***	NS	***
9.13	1-Pentanol	1.64	0.96	1.12	0.88	0.12	NS	NS	NS
9.66	2-Octanone	0.17	0.08	0.40	0.04	0.06	**	*	*
9.73	Octanal	0.37	0.13	0.08	0.09	0.05	NS	NS	NS
11.42	2-Nonen-1-ol	1.19	0.59	0.21	0.18	0.17	***	*	*
12.38	Acetic acid	0.24	0.21	0.19	0.16	0.01	NS	NS	NS
12.85	2-Decanone	1.86	0.07	0.03	0.05	0.32	**	NS	**
13.42	2-Nonenal[2]	0.70	0.25	0.39	0.27	0.07	NS	NS	NS
14.2	2-Undecanone	0.25	0.06	0.04	0.04	0.04	NS	NS	NS
14.59	Butyric acid	9.51	4.57	3.35	4.03	0.99	*	NS	**

[1]$TMR^{\pm}$ vs. $PAS^{\pm}$ compares TMR with and without microalgae supplementation to PAS with and without microalgae supplementation; NS denotes $P > 0.05$.

[2]Bond position was uncertain. Identity is either 2-nonenal or 6-nonenal.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

ture to silage. Similarly in the present experiment, the SFA level in milk was reduced and MUFA and PUFA levels were elevated by feeding pasture compared with the TMR^- treatment, due in large part to the increased levels of ALA in fresh forage that have been observed previously (Dewhurst et al., 2006).

The LA content of milk fat was not significantly affected by treatment whereas that of ALA was higher in milk fat from cows on pasture. Pasture forage has been noted to have a higher LA concentration compared with that found in hay and ensiled forages, particularly in spring (Chilliard et al., 2001). Forage species, maturity, and processing affect the FA composition of forages (Elgersma et al., 2003). In the current study, the LA content of pasture forage might have been reduced by its late maturity and low quality, as indicated by its high contents of NDF and ADF (McWilliam et al., 2005). Comparatively lower pasture DMI relative to that of TMR was another indication that pasture forage quality was low.

The total CLA content in the milk was significantly higher in the PAS treatments than the TMR treatments (4.18% for PAS^+ vs. 3.59% for TMA^+ and 3.52% for PAS^- vs. 3.12% for TMR^-; SE = 0.12; $P < 0.05$).

Table 7. Antioxidant capacity of milk from cows fed pasture plus concentrate (PAS) or silage-based TMR with (+) or without (−) a supplement containing rumen-protected microalgae and of the TMR and PAS feed components using the ferric reducing ability of plasma (FRAP) assay (mg of Trolox equivalents per L of milk or kg of feed)

Item	TMR^+	TMR^-	PAS^+	PAS^-	SE	*P*-value
Milk	74.1	73.4	73.7	77.6	1.87	0.69
	TMR	Pasture forage	Concentrate	Algae	SE	
Feed	107.3	116.3	87.2	135.4	10.02	

Chilliard et al. (2001) reviewed effects of forage type on milk composition and observed that milk from pasture cows was significantly higher in CLA content compared with cows fed ensiled forages. Levels of PUFA and CLA were also elevated by microalgae supplementation in the current study.

In contrast to the PAS treatment, TMR increased SFA and decreased MUFA and PUFA contents of milk fat. Content of EPA was not affected by treatment. As expected, the fatty acid composition of butter paralleled that of milk.

Dry matter intake was significantly affected by treatment, with lower DMI on the pasture rations. Feeding microalgae also tended to reduce intake, particularly for the pasture diet, perhaps due to effects of PUFA on fiber digestion (Schroeder et al., 2004). Similarly, Franklin et al. (1999) observed lower DMI in cows fed either protected or unprotected algae.

Treatment effects on animal performance were largely nonsignificant. Body weight and condition were not significantly affected by treatment, and milk production was similar for all treatments despite reduced DMI on the pasture ration, particularly when supplemented with the microalgae. Cow health was not compromised by feeding rumen-protected algae or by forage type as reflected by the lack of treatment effect on mammary health (SCC) and body temperature. Franklin et al. (1999) also observed no reduction in milk production or BCS with reduced DMI when feeding microalgae. Other observations on the effects of LCn-3FA supplementation on milk production range from no significant change (AbuGhazaleh et al., 2002; Rego et al., 2005) to increased production (Chilliard, 1993; Wu and Huber, 1994; Schroeder et al., 2004).

The TMR treatment increased milk protein content relative to the PAS treatment, which could be related to dietary effects on postruminal amino acid supply (Wu and Huber, 1994), whereas PAS increased the fat and total solids content of milk. Feeding LCn-3FA has been shown to depress fat percentage in milk (AbuGhazaleh et al., 2002; Gulati et al., 2003; Rego et al., 2005). Consistent with those results, we observed that dietary supplementation of a DHA-rich rumen-protected microalgae reduced fat percentage in milk when cows were fed either type of forage ration. These results are comparable to those of Franklin et al. (1999), who observed values for milk fat content of 3.70% for TMR without rumen-protected microalgae and 2.95% with rumen-protected microalgae. In this study, the fat content of milk was also reduced by feeding TMR compared with PAS.

Oxidation of dairy products reduces their nutritional quality and organoleptic properties (Havemose et al., 2006). The TBARS method is the most common laboratory method for detecting oxidative changes in food products (Schmedes and Holmer, 1989) and was first performed on milk samples by Dunkley and Jennings (1952). Oxidation of MUFA and PUFA can be identified by absorption at 450 and 532 nm wavelengths, respectively (Frankel, 2005). Using a sensory panel, King (1962) confirmed the presence of volatile products of oxidation detected in samples at 532 nm. Although taste is an excellent means of detecting oxidized flavors, Kitessa et al. (2004) observed no flavor defects in milk enriched with FA from rumen-protected tuna, suggesting the need for specific determination of the presence of oxidation products. Ultraviolet light causes spontaneous oxidation of unsaturated FA (Burton et al., 1985; Havemose et al., 2006; Laguerre et al., 2007) and was used in this experiment to enhance potential treatment differences. No significant treatment differences were observed for TBARS in milk that had been unexposed or exposed to UV at either 450 or 532 nm. Although treatment did not significantly affect TBARS in butter at 532 nm, feeding the microalgae supplement tended to increase the level of TBARS compared with treatments without the microalgae. This effect was more pronounced for butter that had been exposed to UV radiation and was produced from milk of cows receiving TMR, providing some evidence that feeding TMR may produce milk with lower antioxidant levels than pasture.

Absorbance values observed in the present experiment were generally higher than those of Hedegaard et al. (2006), who compared the oxidative stability of milk from cows fed oilseed. The TBARS values in milk were higher than those in butter despite the higher lipid content of butter and marginally higher LCn-3FA concentration. The anhydrous medium of butter may impede oxidation compared with the aqueous medium of milk. Lipid oxidation in emulsions is initiated at or near the oil–water interface, where the concentration of metal ions is higher (Venkateshwarlu et al., 2004), and therefore production of volatile compounds may be faster in emulsions than in oils. Parameters other than oxidation products (pigments or proteins) that interact with malonaldehyde may also produce a color change (Frankel, 2005), and because the TMR treatment produced higher levels of milk protein, it is possible that this contributed to the TMR effect. However, the milk protein content was not significantly different between TMR^+ and TMR^-. Thus, the higher level of TBARS in butter produced from the TMR^+ treatment was likely the result of the increased PUFA content of this butter.

Solid-phase microextraction analysis has been used to examine the types and amounts of volatiles produced from the oxidation of milk (Bassette et al., 1986; Kataoka et al., 2000) and was used in this study to

determine the effects of contrasting forage source and microalgae supplementation on the profile of primary volatile compounds in milk and the butter generated from that milk. Using SPME analysis, Venkateshwarlu et al. (2004) identified 14 volatiles arising from oxidation of milk, which could be classified as ketones, primarily methyl ketones, straight-chain aldehydes, and n-alcohols. Methyl ketones are considered characteristic of volatile compounds found in pasteurized milk, and 3 of the 4 methyl ketones identified by Venkateshwarlu et al. (2004) were also found in milk from the 4 dietary treatments of this study; namely, 2-butanone, 2-heptanone, and 2-nonanone.

Ketones and alcohol volatiles (e.g., 2-nonen-1-ol) play a role in the flavor quality of dairy products. They have been said to be the chief factors in the development of off-flavors in oxidized milk (Barrefors et al., 1995). These volatiles are secondary oxidation products and although some research has shown that ketone volatiles can be produced by microbial oxidation of free fatty acids (not measured in this trial) and concentrated in cold-stored raw milk (Urbach, 1990; Havemose et al., 2006), others (Barrefors et al., 1995) attribute the amount of ketones present to the amount of PUFA in the milk. Feeding microalgae increased the levels of several methyl ketones when cows were fed either TMR or pasture, although types of volatiles differed between forage types. Feeding TMR elevated the levels of 2-pentanone and 2-decanone compared with feeding PAS, and the addition of microalgae enhanced the levels further. Levels of the alcohol 2-nonen-1-ol were elevated in butter in the TMR treatments compared with the PAS treatments, and again the increase was enhanced with the feeding of microalgae. In contrast, levels of 2-octanone were higher in butter made from milk of cows receiving the PAS treatment and microalgae enhanced this effect. This effect of feeding microalgae concurs with the observation of Contarini et al. (1997), who hypothesized that it may be due in part to high concentrations of PUFA. They also reported significantly higher 2-heptanone production, whereas production of this volatile was not significantly different among the treatments evaluated in this study.

Hexanal was the primary aldehyde found in milk samples of this study, with values ranging from 44.50 to 89.80 mg/mL for the 4 diets. Hexanal was also the main volatile found in milk to which fish oil had been added (Iglesias and Medina, 2007) although in the current study no relationships were observed between the amount of hexanal in the milk and either dietary forage type or microalgae supplementation. However, the amount of hexanal in butter from the TMR^+ diet was more than double that of either the PAS^+ or PAS^- treatment. Following exposure to fluorescent light, increased levels of hexanal, heptanal, and pentanal were found in milk produced from cows fed grass/clover silage compared with hay (Havemose et al., 2006). Milk produced from the cows fed the grass/clover silage was higher in linolenic acid content, which was thought to influence the degree of lipid oxidation and differences in the antioxidant content of milk. Feeding pasture significantly elevated the level of the aldehyde 2-nonenal in milk, which tended to be increased with microalgae supplementation. Aldehydes are indicative of deterioration of n-3 to n-9 fatty acids (Venkateshwarlu et al., 2004). However, aldehydes are been reported to have flavor thresholds typically lower than that of ketones and alcohols (Havemose et al., 2006), meaning the latter will have a greater organoleptic effect.

Butyric acid levels were higher in butter produced from milk of cows fed the TMR compared with PAS. As with other volatile compounds, the combination of the TMR and microalgae substantially increased the level of butyric acid in butter. The presence of butyric acid produces a sharp taste in cheese, which may be desirable. In contrast, many volatile compounds are known to impair flavor quality and are the chief factors in the development of off-flavors in oxidized milk (Barrefors et al., 1995).

In contrast to the 14 volatile compounds found by Venkateshwarlu et al. (2004) in pure milk, in the same study 60 volatile compounds were identified in milk to which fish oil had been added. The volatiles identified in the fish oil–enriched milk included alkenals, alkadienals, alkatrienals, and vinyl ketones. The number of volatiles identified in the milk and butter of the current study did not approach this complexity, even though the PUFA content of the milk was enhanced with the feeding of microalgae. Exogenous addition of PUFA to milk may cause a different degree of oxidation compared with that of milk naturally enriched with PUFA because the form and location of the PUFA within the milk fat globule or milk fat globule membrane may confer properties that change its oxidative potential.

In this study, volatiles were generally elevated in milk and butter from cows receiving the TMR^+ diet and lowest from cows receiving the PAS^- diet, although there were exceptions to this, as discussed above. The observed trend of higher volatile compound levels for TMR^+ concurs with Barrefors et al. (1995), who concluded that low concentrations of antioxidants of ensiled forages increased the potential occurrence of oxidation and production of off-flavors from volatiles. Havemose et al. (2006) found increased accumulation of lipid hydroperoxides and several volatile compounds in milk from cows fed grass-clover silage compared with milk from cows fed hay, which might not be expected with the increased antioxidant levels found in the milk

of the grass-clover fed cows. They conclude that the increased level of linolenic acid in the milk of the grass-clover fed cows was a significant factor in the development of lipid hydroperoxides and volatile compounds. Our results suggest that both the level of PUFA and potential antioxidant capacity (TMR versus pasture) are important determinants of the amount and type of volatile compounds produced.

The natural antioxidants most commonly present in milk from the diet include tocopherols and carotenoids (Olsen, 1996). Vitamin E (α-, β-, γ-, or δ-tocopherol) is a widely researched antioxidant in dairy nutrition, and feeding diets rich in α-tocopherol has been shown to significantly improve the oxidative stability of milk (Charmley and Nicholson, 1994). Forages are an important natural source of tocopherols, particularly fresh pasture forage. Even though substantial variation exists in the antioxidant content of milk, the FRAP assay has not been used extensively to measure milk antioxidant capacity. Wegrzyn et al. (2008) observed increased antioxidant capacity of milk with increasing addition of polyphenol antioxidants derived from apple. Significant differences in the antioxidant capacity of the milk produced in this study were not detected using the FRAP assay. The PAS^- treatment had the highest FRAP value (77.61 mg of Trolox Eq/L of milk), whereas the TMR^- treatment had the lowest FRAP value (73.40 mg of Trolox Eq/L of milk). This result is consistent with the predicted loss in antioxidant content of silage during wilting and ensiling (Shingfield et al., 2005; Noziere et al., 2006; Muller et al., 2007) and observed differences in the oxidation of milk and butter produced from cows fed TMR or pasture. Smet et al. (2008), measuring the antioxidant capacity of pasteurized milk, found a decrease in antioxidant capacity after only 1 d of storage. It is not known whether differences in the milk analyzed in this study would have been observed had we conducted the analysis earlier than after 4 d of storage.

Descalzo et al. (2007) used the FRAP assay to determine the effect of antioxidant capacity on the meat of beef cows on pasture or grain-based diets and found significant differences in the antioxidant capacity of the meat that correlated with an odor profile analysis. Pasture-based meat and milk have an increased natural content of CLA, which may also act as an antioxidant. Research on beef carcasses shows that CLA reduces TBARS (Hur et al., 2007); higher CLA levels in the pasture treatment of this study may have had a greater effect on lowering levels of TBARS in milk than the antioxidants in the forage.

The FRAP analysis of the feed and feed components revealed a high antioxidant content for the microalgae, perhaps due to astaxanthin, which is often found in microalgae (Odeberg et al., 2003). Milk from grazing cows fed rumen-protected microalgae had not only an increased DHA content but also a greater CLA content and higher levels of antioxidants.

CONCLUSIONS

Feeding rumen-protected marine algae to dairy cows enriched milk and butter with DHA. The oxidative stability of these products was confirmed after exposure to UV light. Antioxidant capacity of the forages was not significantly different, as determined using ferric oxide reduction. However, forage type appeared to affect the oxidative stability of enriched milk and butter. Feeding TMR in combination with rumen-protected marine algae tended to increase the susceptibility of butter to oxidation and significantly elevated the levels of volatiles produced compared with milk from cows fed pasture forage. Feeding pasture significantly increased milk PUFA content, milk fat percentage, and DHA level (in cows fed rumen-protected marine algae), but few instances were observed of increased oxidative volatile production compared with TMR. Feeding the TMR treatments decreased PUFA content and milk fat percentage, and increased levels of oxidative volatiles, especially when microalgae was fed, even though the DHA content was similar to that of pasture-fed cows supplemented with algae. Thus, it seems likely that the antioxidant capacity of the pasture treatments was higher compared with that of the TMR. Limited information is available on the effects of naturally increasing the level of PUFA in cow milk with respect to increasing the oxidative potential of milk and producing volatile compounds that may alter the flavor and quality of milk and its associated products. Further work is needed to characterize the differences in antioxidant capacity of milk enriched naturally with DHA produced from pasture compared with TMR. The significance of the differences in volatile compound production from these contrasting feeding regimens is not known and studies determining the organoleptic effect are warranted. Finally, further research is needed to determine how oxidative stability is affected by incorporating DHA into milk fat by feeding it to cows as opposed to adding it to milk.

REFERENCES

Abete, I., E. Goyenechea, M. A. Zulet, and J. A. Martinez. 2011. Obesity and metabolic syndrome: Potential benefit from specific nutritional components. Nutr. Metab. Cardiovasc. Dis. 21:B1–B15.

AbuGhazaleh, A. A., R. B. Potu, and S. Ibrahim. 2009. The effect of substituting fish oil in dairy cow diets with docosahexaenoic acid-micro algae on milk composition and fatty acids profile. J. Dairy Sci. 92:6156–6159.

AbuGhazaleh, A. A., D. J. Schingoethe, A. R. Hippen, K. F. Kalscheur, and L. A. Whitlock. 2002. Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. J. Dairy Sci. 85:2266–2276.

Amminger, G. P., G. E. Berger, M. R. Schafer, C. Kiler, M. H. Friedrich, and M. Feucht. 2007. Omega-3 fatty acids supplementation in children with autism: A double-blind randomized placebo-controlled pilot study. Biol. Psychiatry 61:551–553.

AOCS. 1995. Official Methods and Recommended Practices of the American Oil Chemists' Society. AOCS Press, Champaign, IL.

Aquino, A. A., Y. V. R. Lima, B. G. Botaro, C. S. S. Alberto, K. C. Peixoto Jr., and M. C. Santos. 2008. Effects of dietary urea levels on milk protein fractions of Holstein cows. Anim. Feed Sci. Technol. 140:191–198.

Barrefors, P., K. Granelli, L. A. Appelqvist, and L. Bjoerck. 1995. Chemical characterization of raw milk samples with and without oxidative off-flavor. J. Dairy Sci. 78:2691–2699.

Barsanti, L., and P. Gualtieri. 2006. Algae: Anatomy, Biochemistry, and Biotechnology. CRC Press, Boca Raton, FL.

Bassette, R., D. Y. C. Fung, and V. R. Mantha. 1986. Off-flavors in milk. Crit. Rev. Food Sci. Nutr. 24:1–52.

Benzie, I. F. F., and J. J. Strain. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem. 239:70–76.

Brookes, K. J., W. Chen, X. Xu, E. Taylor, and P. Asherson. 2006. Association of fatty acid desaturase genes with attention-deficit/hyperactive disorder. Biol. Psychiatry 60:1053–1061.

Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. Mar. Mamm. Sci. 22:759–801.

Burton, G. W., D. O. Foster, B. Perly, T. F. Slater, I. C. P. Smith, and K. U. Ingold. 1985. Biological antioxidants. Philos. Trans. R. Soc. Lond. B Biol. Sci. 311:565–578.

Canadian Council on Animal Care. 2009. CCAC guidelines on: The care and use of farm animals in research, teaching and testing. CCAC, Ottawa, ON, Canada.

Charmley, E., and J. W. G. Nicholson. 1994. Influence of dietary fat source on oxidative stability and fatty acid composition of milk from cows receiving a low or high level of vitamin E. Can. J. Anim. Sci. 74:657–664.

Chilliard, Y. 1993. Dietary fat and adipose tissue metabolism in ruminants, pigs, and rodents: A review. J. Dairy Sci. 76:3897–3931.

Chilliard, Y., A. Ferlay, and M. Doreau. 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livest. Prod. Sci. 70:31–48.

Contarini, G., M. Povolo, R. Leardi, and P. M. Toppino. 1997. Influence of heat treatment on the volatile fraction of milk. J. Agric. Food Chem. 45:3171–3177.

Descalzo, A. M., L. Rossetti, G. Grigioni, M. Irurueta, A. M. Sancho, J. Carrete, and N. A. Pensel. 2007. Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle. Meat Sci. 75:299–307.

Dewhurst, R. J., K. J. Shingfield, M. R. F. Lee, and N. D. Scollan. 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Anim. Feed Sci. Technol. 131:168–206.

Dunkley, W. L., and W. G. Jennings. 1952. A procedure for application of the thiobarbituric acid test to milk. J. Dairy Sci. 34:1064–1069.

Elgersma, A., G. Ellen, H. van der Horst, H. Boer, P. R. Dekker, and S. Tamminga. 2004. Quick changes in milk fat composition from cows after transition from fresh grass to a silage diet. Anim. Feed Sci. Technol. 117:13–27.

Elgersma, A., G. Ellen, H. van der Horst, B. G. Muuse, H. Boer, and S. Tamminga. 2003. Comparison of fatty acid composition of fresh and ensiled perennial ryegrass (*Lolium perenne* L.) affected by cultivar and regrowth interval. Anim. Feed Sci. Technol. 108:191–205.

Elgersma, A., S. Tamminga, and G. Ellen. 2006. Modifying milk composition through forage. Anim. Feed Sci. Technol. 131:207–225.

Folch, J., M. Lees, and G. H. Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226:497–509.

Frankel, E. N. 2005. Lipid Oxidation. 2nd ed. The Oily Press, Bridgewater, UK.

Franklin, S. T., K. R. Martin, R. J. Baer, D. J. Schingoethe, and A. R. Hippen. 1999. Dietary marine algae (*Schizochytrium* sp.) increase concentrations of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. J. Nutr. 129:2048–2054.

Gallagher, J. J., R. Hollender, D. G. Peterson, R. F. Roberts, and J. N. Coupland. 2005. Effect of composition and antioxidants on the oxidative stability of fluid milk supplemented with an algae oil emulsion. Int. Dairy J. 15:333–341.

Goncalves, C. V., and F. P. Collares. 1999. Industrial application of the Creamatocrit method to estimate the total contents of lipids in natural cow milk. Food Chem. 64:567–570.

Gonzalez, S., S. E. Duncan, S. F. O'Keefe, S. S. Sumner, and J. H. Herbein. 2003. Oxidation and textural characteristics of butter and ice cream with modified fatty acid profiles. J. Dairy Sci. 86:70–77.

Gulati, S. K., S. McGrath, P. C. Wynn, and T. W. Scott. 2003. Preliminary results on the relative incorporation of docosahexaenoic and eicosapentaenoic acids into cow's milk from two types of rumen protected fish oil. Int. Dairy J. 13:339–343.

Havemose, M. S., M. R. Weisbjerg, W. L. P. Bredie, H. D. Poulsen, and J. H. Nielsen. 2006. Oxidative stability of milk influenced by fatty acids, antioxidants, and copper derived from feed. J. Dairy Sci. 89:1970–1980.

Hedegaard, R. V., D. Kristensen, J. H. Nielson, M. B. Frost, H. Ostdal, J. E. Hermansen, M. Kroger-Ohlsen, and L. H. Skibsted. 2006. Comparison of descriptive sensory analysis and chemical analysis for oxidative changes in milk. J. Dairy Sci. 89:495–504.

Hur, S. J., G. B. Park, and S. T. Joo. 2007. Biological activities of conjugated linoleic acid (CLA) and effects of CLA on animal products. Livest. Sci. 110:221–229.

Iglesias, J., and S. L. I. Medina. 2007. Development of a solid-phase microextraction method for determination of volatile oxidation compounds in fish oil emulsions. J. Chromatogr. A 1163:277–287.

Jenkins, T. J., and W. C. Bridges. 2007. Protection of fatty acids against ruminal biohydrogenation in cattle. Eur. J. Lipid Sci. 109:778–789.

Kataoka, H., H. L. Lord, and J. Pawliszyn. 2000. Applications of solid-phase microextraction in food analysis. J. Chromatogr. A 880:35–62.

King, R. L. 1962. Oxidation of milk fat globule membrane material. I. thiobarbituric acid reaction as a measure of oxidized flavor in milk and model systems. J. Dairy Sci. 45:1165–1171.

Kitessa, S. M., S. K. Gulati, G. C. Simos, J. R. Ashes, T. W. Scott, E. Fleck, and P. C. Wynn. 2004. Supplementation of grazing cows with rumen-protected tuna oil enriches milk fat with n-3 fatty acids without affecting milk production or sensory characteristics. Br. J. Nutr. 91:271–278.

Laguerre, M., J. Lecomte, and P. Villeneuve. 2007. Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. Prog. Lipid Res. 46:244–282.

McWilliam, E. L., T. N. Barry, N. Lopez-Villaobos, P. N. Cameron, and P. D. Kemp. 2005. Effects of willow (*Salix*) supplementation for 31 and 63 d on the reproductive performance of ewes grazing low quality drought pasture during mating. Anim. Feed Sci. Technol. 119:87–106.

Mounchili, A., J. J. Wichtel, J. O. Bosset, I. R. Dohoo, M. Imhof, D. Altieri, S. Mallia, and H. Stryhn. 2005. HS-SPME gas chromatographic characterization of volatile compounds in milk tainted with off-flavour. Int. Dairy J. 15:1203–1215.

Muller, C. E., J. Moller, S. Krogh Jensen, and P. Uden. 2007. Tocoperol and carotinoid levels in baled silage and haylage in relation to horse requirements. Anim. Feed Sci. Technol. 137:182–197.

Noziere, P., B. Graulet, A. Lucas, B. Martin, P. Grolier, and M. Doreau. 2006. Carotenoids for ruminants: From forages to dairy products. Anim. Feed Sci. Technol. 131:418–450.

NRC. 2001. Nutrient Requirements of Dairy Cattle. National Academy Press, Washington, DC.

Odeberg, J. M., A. Lignell, A. Pettersson, and P. Hoglund. 2003. Oral bioavailability of the antioxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations. Euro. J. Pharm. Serv. 19:299–304.

Olsen, J. A. 1996. Benefits and liabilities of vitamin A and carotenoids. Am. Inst. Nutr. Suppl. 126:1208S–1212S.

Rego, O. A., H. J. D. Rosa, P. Portugal, R. Cordeiro, A. E. S. Borba, C. M. Vouzela, and R. J. B. Bessa. 2005. Influence of dietary fish oil on conjugated linoleic acid, omega-3 and other fatty acids in milk fat from grazing dairy cows. Livest. Prod. Sci. 95:27–33.

Schmedes, A., and G. Holmer. 1989. A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. J. Am. Oil Chem. Soc. 66:813–817.

Schroeder, G. F., G. A. Gagliostro, F. Bargo, J. E. Delahoy, and L. D. Muller. 2004. Effects of fat supplementation on milk production and composition by dairy cows on pasture: A review. Livest. Prod. Sci. 86:1–18.

Shingfield, K. J., S. Ahvenjärvi, V. Toivonen, A. Vanhatalo, P. Huhtanen, and J. M. Griinari. 2008. Effect of incremental levels of sunflower-seed oil in the diet on ruminal lipid metabolism in lactating cows. Br. J. Nutr. 99:971–983.

Shingfield, K. J., P. Salo-Vaananen, E. Pahkala, V. Toivonen, S. Jaakkola, V. Piironen, and P. Huhtanen. 2005. Effect of forage conservation method, concentrate level and propylene glycol on the fatty acid composition and vitamin content of cows' milk. J. Dairy Res. 72:349–361.

Shiota, M., H. Konishi, and K. Tatsumi. 1999. Oxidative stability of fish oil blended with butter. J. Dairy Sci. 82:1877–1881.

Simopoulous, A. P. 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: Nutrient implications for chronic diseases. Biomed. Pharm. 60:502–507.

Smet, K., K. Raes, J. De Block, L. Herman, K. Dewettinck, and K. Coudijzer. 2008. A change in antioxidant capacity as a measure of onset of oxidation in pasteurized milk. Int. Dairy J. 18:520–530.

Urbach, G. 1990. Headspace volatiles from cold-stored raw milk. Aust. J. Dairy Technol. 45:80–85.

Venkateshwarlu, G., M. B. Let, A. S. Meyer, and C. Jacobsen. 2004. Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. J. Agric. Food Chem. 52:311–317.

Walstra, P., and R. Jenness. 1984. Dairy Chemistry and Physics. John Wiley & Sons, New York, NY.

Wegrzyn, T. F., J. M. Farr, D. C. Hunter, J. Au, M. W. Wohlers, M. A. Skinner, R. A. Stanley, and D. Sun-Waterhouse. 2008. Stability of antioxidants in an apple polyphenol-milk model system. Food Chem. 109:310–318.

Wu, Z., and J. T. Huber. 1994. Relationship between dietary fat supplementation and milk protein concentration in lactating cows: A review. Livest. Prod. Sci. 39:141–227.