Milk from cows grazing on cool-season pastures provides an enhanced profile of bioactive fatty acids compared to those grazed on a monoculture of pearl millet.

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Abstract

The demand for grass-fed dairy products is driven, in part, by their more desirable fatty acid (FA) profile, containing more n-3 FA and conjugated linoleic acids (CLA) than conventionally produced dairy products. This study investigated the effects of pearl millet (PM) vs. cool-season pasture (CSP) on animal performance and milk FA in a grazing system. Eight Holstein dairy cows were used in a repeated measures design with four-week periods. Forage type had no effect on animal performance (estimated dry matter intake, milk production, fat, or protein). The contents of CLA and n-3 FA in a serving of whole milk (3.25% fat) increased when cows grazed a CSP compared to PM. A serving of whole milk from cows grazing PM had a higher content of saturated FA and branched-chain FA. In conclusion, the contents of various bioactive FA were higher in milk fat of cows grazing a CSP compared to PM.

Key words: Conjugated linoleic acids, n-3 fatty acids, organic, summer annuals, warm-season grasses.

1. Introduction

The demand for organic food in the United States (U.S.) is primarily driven by the favorable view of its nutritional content and the ecological benefits of its production (Lee & Yun, 2015). Organic milk, for example, contains higher amounts of the bioactive compounds, n-3 fatty acids (FA) and conjugated linoleic acids (CLA), than conventionally produced milk (Butler, Stergiadis, Seal, Eyre, & Leifert, 2011). This demand for foods with added nutritional value is reflected by the 25% increase in organic whole-milk sales from 2014 to 2015, in comparison to a <1% increase for low-fat and fat-free versions (Maltby, 2016). It is not the effect of the management system (organic vs. conventional) *per se*, but the consequence of the dietary feeding regime associated with the management system that leads to the improved milk FA profile of organic milk (Butler et al., 2008). The feeding regimen on any particular dairy farm is driven by economic, philosophic, and regulatory factors (McBride & Greene, 2009). For example, the U.S. Department of Agriculture (USDA), requires organic dairy producers to have 30% of their cows' dry matter intake (DMI) come from pasture for a 120-day grazing season (U.S. Department of Agriculture, 2010). While the inclusion of fresh forage in the diet of dairy cows leads to an improved milk FA profile, not all forage species affect the milk FA profile in the same way. Turner *et al.* observed a higher content of n-3 FA in milk when cows grazed birdsfoot trefoil (*Lotus corniculatus* L.) in comparison to ryegrass (Turner, Waghorn, Woodward, & Thompson, 2005), and Soder *et al.* demonstrated an increased content of CLA in milk with additional forage species in the pasture (3, 6, and 9 species mixtures vs. 2 species mix) (Soder, Sanderson, Stack,

& Muller, 2006). There has been no evaluation, however, of the influence of summer annual species on the milk FA profile. Summer annuals are C4 plants that grow rapidly and are more productive during the hot-summer months when cool-season (C3) perennial plants experience reduced growth and productivity. Therefore, C4 plants contribute to the dry matter needed to meet the energy demands of the animal and the regulations of the USDA, making them increasingly popular on Northeastern U.S. grass-based dairies. Thus, the objective of this study was to compare the effects of grazing a warm-season annual, pearl millet (*Pennisetum glaucum* L.), on forage quality, animal performance, and bioactive FA in milk with a cool-season diverse pasture.

2. Materials and Methods

2.1 Experimental design

All procedures involving animals were approved under the University of Vermont Institutional Animal Care and Use Committee. Eight multiparous (parity: 2.9 ± 0.6 lactations) mid-lactation (179 ± 20 days in milk) Holstein cows were used in a repeated measures design on a commercial organic dairy farm in northwest Vermont. The treatments consisted of two grazing regimes; a diverse cool-season pasture (**CSP**) and a warm-season monoculture of pearl millet (**PM**) (Table 1). PM was chosen because, it can be grazed directly as soon as it reaches 60 cm, is palatable and produces high yields, is drought tolerant, adapted to low pH soils, and it lacks prussic acid. Cows were grazed on CSP or PM for four-week periods from the months of July to September 2014 in the following sequence: PM, CSP, and PM (the final grazing period was intended to account for the effect of growing month on PM). A 0.5 ha paddock of previously

established CSP was chosen based for its uniform composition of forage species and proximate location to the pearl millet on the farm. The CSP consisted of more than 15 species; orchard grass (*Dactylis glomerata* L.), timothy grass (*Phleum pratense* L.), perennial ryegrass (*Lolium perenne* L.), junegrass (*Koeleria macrantha* (Ledeb.) Schult.), meadow fescue (*Festuca pratensis* Huds.), smooth brome grass (*Bromus inermis* L.), creeping bentgrass (*Agrostis palustris* Huds.), quack grass (*Elymus repens* (L.) Gould.), kentucky blue grass (*Poa pratensis* L.), white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), common vetch (*Vicia sativa* L.), dandelion (*Taraxacum officinale* H.F. Wigg), burdock (*Arctium minus* Bernh.), milk weed (*Asclepias syriaca* L.), and bull thistle (*Cirsium vulgare* (savi) Ten.) consistently made up the majority. Two 0.25 ha paddocks were tilled and seeded at 22.4 kg/ha with PM in two-week successions to eliminate the effect of maturity on forage composition. The paddocks were re-seeded in the same manner for the second grazing of PM. The average mean daily temperatures for each treatment period were 20.0 for PM, 18.9 for CSP, and 17.3°C for the second grazing of PM. Cows received grain at 2.42 kg/day (DM basis) during each treatment. The cows were milked twice daily at 0630h and 1730h and after each milking cows were rotated to a new paddock.

2.2 Data and sample collection

Dry matter intake was estimated using an electronic rising plate meter (Jenquip; Feilding, New Zealand) three times per week by taking sixty measurements each in both pre-grazed and post-grazed paddocks. The rising plate meter was calibrated weekly by taking ten 0.2m² quadrats, cut to ground level, in both pre and post-grazed paddocks for a total of twenty measurements per week. Samples were dried at 65°C for 48 hours to determine dry matter content (DM). DM

(kg) of these samples was plotted against the pasture height/density to obtain an equation with an $R^2 > 0.8$. Rising plate meter values were used in the equation derived from the calibration to obtain kg of DM consumed by the eight dairy cattle per ha. Estimated DMI per cow was then calculated from measured pasture areas and the number of cows grazing the pasture. Forage samples for quality measurements were collected weekly from the next paddock in the pasture rotation. Thirty samples, clipped to the height of the post-grazed paddock, were composited then divided into forage classes; grasses, legumes, broad-leaf weeds, and dead matter. Each fraction was dried at 65°C to determine DM and ground through a Wiley Mill (Arthur H. Thomas, Philadelphia, PA) with a 2mm screen and then through an Udy Mill (UDY Corporation; Fort Collins, CO) with 1mm screen. Ground forage samples were analyzed by near-infrared reflectance spectroscopy (NIRS) for forage quality. Milk weights and samples were collected during weeks 3 and 4 of each period from both AM and PM milking. Samples were composited by weight and one aliquot was frozen at -20°C for FA analysis. A second aliquot was preserved with 2-bromo-2-nitropropane-1,3 diol and analyzed by mid-infrared spectroscopy by Lancaster DHIA (Manheim, PA) for total fat, protein, and organic solids.

2.3 Milk and forage FA analysis

Forage and milk FA compositions were determined by the method of Bainbridge *et al.* (Bainbridge, Lock, & Kraft, 2015). A typical chromatograph produced by this method is presented in Supplemental Figure 1. The content (mg) of individual FA in a serving (244g) of whole milk (3.25% milk fat (U.S. Department of Agriculture Agricultural Research Service, 2008)) was calculated as follows, assuming milk fat to be 93.3% of FA (Glasser, Doreau, Ferlay, & Chilliard, 2007):

Total FA per serving (mg) = 7.93g fat/serving x 0.933 x 1000

FA per serving (mg) = Total FA per serving (mg) x [FA proportion (g/100g) / 100]

2.4 Statistical analysis

Data were analyzed by a repeated-measures ANOVA using the PROC MIXED procedure in SAS 9.4 (SAS Institute, Cary, NC). All statistical analyses were performed considering the guidelines presented by Granato *et al.* (Granato, de Araújo Calado, & Jarvis, 2014). The statistical model included the random effect of cow, fixed effect of treatment and residual error. The REPEATED statement included week within period with the SUBJECT=cow option. The effect of treatment x period was originally included in the model and then removed because $P > 0.15$. The Kenward-Roger approximation was used for computing the denominator degrees of freedom for the tests of fixed effects resulting from the model. Least-squares (LS) means were generated for treatments using the LSMEANS/DIFF option to display the results. Significance was declared at $P < 0.05$ and trends at $0.05 \leq P < 0.10$.

3. Results and Discussion

3.1 Diet composition

PM contained a higher amount of neutral-detergent fiber (NDF) and acid-detergent fiber (ADF), while CSP provided more starch, protein, and non-fiber carbohydrates (NFC) (Table 1). These findings are comparable to previous studies demonstrating that warm-season grasses, like PM, have lower digestibility and crude protein levels than cool-season grasses (Ball et al., 2001). Cool-season grasses have been shown to contain greater amounts of unsaturated FA (UFA) to

resist cold temperatures, while warm-season grasses, such as PM, contain less UFA to minimize lipid peroxidation caused by high temperatures (Bell, 2011). Accordingly, the PM in this study, contained less monounsaturated FA (MUFA), polyunsaturated FA (PUFA), and total n-3 FA, and more n-6 FA than CSP (Table 1). The lower PUFA content could also be due to PM being grazed at a mature stage (between boot and milk stages; 37 to 51 days of maturity), as PUFA content has been shown to decrease as forages mature (Glasser, Doreau, Maxin, & Baumont, 2013). The FA profile of CSP was similar to that of cool-season pasture species evaluated by Dewhurst *et al.* (n-3 FA range: 10.33 – 15.28 g/kg DM) (Dewhurst, Scollan, Youell, Tweed, & Humphreys, 2001). This study is the first to characterize the FA profile of PM.

3.2 Animal production parameters

DMI, milk yield, energy-corrected milk, fat-corrected milk, and milk components did not differ between treatments of CSP and PM (Table 2) and thus, producers would not gain any additional production premiums from grazing PM. No studies have compared the effects of cool-season and warm-season forages on dairy cow performance; however, studies have examined the effects of increasing the number of forage species. Muir *et al.* observed no differences in production parameters between cows grazing a monoculture of perennial rye grass and a mixture of perennial ryegrass and chicory (Muir, Ward, & Jacobs, 2014), and Soder *et al.* showed no benefit to production when cows grazed forage mixtures of two-to-nine species (Soder *et al.*, 2006).

3.4 FA content in a serving of whole milk (3.25% fat)

Grazing regime had a significant effect on the FA composition of milk that would be available to the consumer. A serving of whole milk from cows on the CSP treatment provided more MUFA (2289 vs. 2131 mg/serving 3.25% milk; $P<0.01$) and PUFA (433 vs. 319 mg/serving 3.25% milk; $P<0.001$) than a serving of whole milk from cows on PM. The health benefits of increasing these FA classes in the diet have been widely reported, including decreased risk for cardiovascular disease and protection against metabolic disease (Dolecek, 1992; Gillingham, Harris-Janz, & Jones, 2011; Mensink & Katan, 1989), however, the numerically small increases in this study may not impart biological effects. Although no studies have described the effects of warm-season annuals on milk FA composition, Varnold *et al.* evaluated the effects of warm-season annuals on beef FA composition. The results showed a decrease in UFA, MUFA, and PUFA in steaks of cows grazing warm-season grasses in comparison to a cool-season pasture (Varnold *et al.*, 2014). Our study suggests that warm-season grass, PM, show a similar decrease in fatty acids—and that future studies should examine whether this distinction is the result of reduced diversity or warm-season versus cool-season pastures.

Bovine milk fat contains many unique rumen-derived FA such as BCFA, CLA, and *trans* 18:1 isomers. Total *trans* 18:1 FA were higher in a serving of whole milk from cows grazing CSP than those grazing on PM (403 vs. 273 mg/serving 3.25% milk; $P<0.001$). Ruminant-derived *trans*-FA do not impart the same deteriorative health effects as industrially produced *trans*-FA (Rice, Kraft, Bauman, & Lock, 2010). In fact, the main *trans*-FA isomer in milk fat, vaccenic acid (VA; *trans*-11 18:1), has been shown to have health benefits, such as reducing tumor growth and the risk for atherosclerosis (Field, Blewett, Proctor, & Vine, 2009; Lock, Horne, Bauman, & Salter, 2005). A serving of whole milk from CSP-fed cows provided an additional 125 mg of VA per

189 serving than milk from cows grazing PM (327 vs. 202 mg/serving 3.25% milk; $P<0.001$). VA is
190 converted to rumenic acid (RA; *cis*-9, *trans*-11 18:2) through the action of delta-9 desaturase at
191 an average rate of 19% in the human body (Turpeinen et al., 2002). The content of RA was
192 higher in cows fed CSP (150 vs. 105 mg/serving 3.25% milk for CSP and PM treatments,
193 respectively; $P<0.01$). A serving of whole milk from cows grazing CSP also had a higher content
194 of total CLA when compared to PM (163 vs. 116 mg/serving 3.25% milk; $P<0.01$). Ritzenthaler *et*
195 *al.* estimated dietary CLA intakes of men and women and found on average men consume 212
196 mg of CLA/d and women to consume 151 mg/day (Ritzenthaler et al., 2001). Three servings of
197 whole milk from CSP-fed cows would provide 489 mg/d of CLA and three servings of milk from
198 cows on PM would provide 348 mg/d of CLA. Both are over the intakes presented by
199 Ritzenthaler *et al.*, but lower than the amount of CLA supplemented to observe decreased
200 tumor proliferation in women with breast cancer (7.5 g/day) and improved blood lipid profile in
201 healthy adults (19.6 g/day)(McGowan et al., 2013; Wanders, Brouwer, Siebelink, & Katan, 2010).
202 However, Aro *et al.* observed a 60% reduction in risk of breast cancer for postmenopausal
203 women who consumed the highest quantile of CLA per day (203 mg CLA/d) (Aro et al., 2001).
204 Branched-chain FA (BCFA) are an emerging class of bioactive FA, unique to dairy products, that
205 have been shown to improve pancreatic β -cell function and have an anti-tumor effect (Kraft,
206 Jetton, Satish, & Gupta, 2015; Wongtangtharn, Oku, Iwasaki, & Toda, 2004). Contents of
207 individual BCFA; *iso*-14:0, *iso*-15:0, and *iso*-16:0, were higher in a serving of whole milk from
208 cows grazing PM when compared to CSP (Table 3). BCFA are derived from the membranes of
209 rumen bacteria, and therefore the increase in the content of these FA in milk from PM-fed cows

210 is likely due to the change or increase in the bacterial populations (Vlaeminck, Fievez, Cabrita,
211 Fonseca, & Dewhurst, 2006).

212 There is a large body of evidence demonstrating the bioactivity of n-3 FA (Fleming & Kris-
213 Etherton, 2014; Pan et al., 2012; Zhao et al., 2004), which has, in turn, caused an increase in
214 consumer demand for products enriched in n-3 FA (*i.e.*, functional foods). Total n-3 FA were
215 approximately 2-fold higher in a serving of whole milk from cows grazing a CSP than cows
216 grazing PM (78.7 vs. 43.0 mg/serving 3.25% milk; $P<0.001$). Consequently, a serving of whole
217 milk from cows on the CSP contained the highest contents of ALA (62.8 vs. 29.6 mg/serving
218 3.25% milk; $P<0.001$) and Eicosapentaenoic acid (EPA) (5.2 vs. 4.5 mg/serving 3.25% milk;
219 $P<0.05$). Recommended intake for n-3 FA is 1.6 g/day for men and 1.1 g/day for women, with
220 10% of the total as EPA and docosahexaenoic acid (DHA) (Institute of Medicine of the National
221 Academies, 2005). Three servings of whole milk from cows grazed on CSP would provide 0.24
222 g/day of n-3 FA. This contributes 15-22% of the recommended n-3 FA intake while three
223 servings of whole milk from cows grazed on PM would contribute 8-9% of the recommended
224 intake. A survey of retail milk samples from conventional and organic systems showed that, on
225 average, conventionally produced milks contain 40.0 mg of n-3 FA per serving, while organically
226 produced milks contain 60.7 mg n-3 FA per serving (O'Donnell, Spatny, Vicini, & Bauman, 2010).
227 Consuming organic milk from cows grazed on PM would provide a n-3 FA content (43.0
228 mg/serving) similar to that of conventional milk, validating that it is not organic dairy
229 management, but the feeding management that can drastically enhance the milk FA profile.

230 Total n-6 FA were lower in a serving of milk from cows grazing PM than those grazing CSP (76.6
231 vs. 96.6 mg/serving 3.25% milk; $P<0.001$). n-6 FA compete with n-3 FA for the same enzymes in

the bioconversion to eicosanoid products such as prostaglandins, thromboxanes, and leukotrienes. The end products have opposing physiological effects, eicosanoids derived from n-6 FA have potent inflammatory properties, while eicosanoids produced from n-3 FA are anti-inflammatory (Poudyal, Panchal, Diwan, & Brown, 2011). Thus, the ratio of n-6/n-3 FA in the diet should be kept under 4:1 to reduce risk of disease (Simopoulos, 2016). Whereas both grazing regimes produced low ratios, the n-6/n-3 ratio was lower in milk of cows grazing the CSP (1.2) than in those grazing PM (1.8).

4. Conclusion

The link between diet and health has generated increased demand for functional food products that may improve/maintain health without drastic changes to the diet. Demand for organic whole-milk is increasing due to its bioactive FA content, and there is potential for dairy producers to optimize milk FA through the dietary feeding regime. This study is the first to show that planting diverse CSP can be an important management regime for farmers in the northeast U.S. looking to enhance the FA profile in their milk. In contrast, we have demonstrated that inclusion of PM, a summer annual, resulted in lower contents of CLA and n-3 FA in milk fat, which were similar to that in conventionally produced milk. Hence, inclusion of PM in the diet of dairy cows should be limited to maintain levels of bioactive FA in organic dairy products. The effects of PM grazed during the vegetative stage need to be evaluated, as this may produce a less drastic reduction in milk bioactive FA.

Conflict of interest

The Authors' declare no competing interest.

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Table 1. Ingredient and chemical composition (mean $\pm$ standard deviation) of the diet components, cool-season pasture (CSP), pearl millet (PM), and grain.

	Diet component		
	CSP	PM	Grain ^a
%DM	19.3 $\pm$ 2.4	19.9 $\pm$ 2.6	89.6 $\pm$ 0.6
Chemical composition, % DM			
aNDFom ^b	42.5 $\pm$ 3.3	53.7 $\pm$ 5.0	7.4 $\pm$ 1.7
ADF ^c	32.8 $\pm$ 2.1	40.5 $\pm$ 1.3	10.0 $\pm$ 1.4
CP (N $\times$ 6.25) ^d	17.4 $\pm$ 2.3	14.4 $\pm$ 2.3	11.9 $\pm$ 1.0
Starch	2.1 $\pm$ 0.2	1.2 $\pm$ 0.2	30.4 $\pm$ 1.1
NFC ^d	24.9 $\pm$ 0.7	18.4 $\pm$ 1.3	64.1 $\pm$ 3.3
Total fatty acids	2.4 $\pm$ 0.2	1.3 $\pm$ 0.2	2.4 $\pm$ 0.1
Fatty acid composition (mg/g DM)			
16:0	4.13 $\pm$ 0.11	2.93 $\pm$ 0.67	4.26 $\pm$ 0.41
18:0	0.41 $\pm$ 0.01	0.26 $\pm$ 0.05	0.56 $\pm$ 0.06
18:1 c9	0.74 $\pm$ 0.10	0.26 $\pm$ 0.05	6.63 $\pm$ 0.44
18:2 c9,c12	5.08 $\pm$ 0.31	1.83 $\pm$ 0.36	10.72 $\pm$ 0.96
18:3 c9,c12,c15	12.04 $\pm$ 0.96	6.49 $\pm$ 0.93	0.69 $\pm$ 0.10
Σ other ^f	1.32 $\pm$ 0.23	1.16 $\pm$ 0.22	0.70 $\pm$ 0.09
Total SFA ^g	5.57 $\pm$ 0.04	4.10 $\pm$ 0.84	5.19 $\pm$ 0.49
Total MUFA ^h	0.93 $\pm$ 0.13	0.49 $\pm$ 0.18	6.89 $\pm$ 0.43
Total PUFA ⁱ	17.17 $\pm$ 1.91	8.33 $\pm$ 1.26	11.45 $\pm$ 1.04
Total n-3 FA	12.07 $\pm$ 0.96	6.51 $\pm$ 0.94	0.72 $\pm$ 0.08
Total n-6 FA	5.13 $\pm$ 0.35	1.84 $\pm$ 0.36	10.76 $\pm$ 0.95

^aThe grain consisted of: 47.5% organic corn meal, 16.9% organic whole grain barley, 15.0% organic field peas, 12.5% organic wheat middings, 3.75% calcium carbonate, 1.5% sodium bicarbonate, 1.5% salt, 0.75% kelpmeal, 0.35% magnesium oxide, and 0.25% concentrated base vitamins consisting of: amino acid chelate, manganese amino acid chelate, copper amino acid chelate, vitamin E supplement, selenium yeast, zinc sulfate, zinc hydroxychloride, vitamin A acetate, vitamin D3 supplement, basic copper chloride, sodium selenite, cobalt carbonate, biotin, calcium iodate. ^baNDFom = Ash-corrected neutral detergent fiber. ^cADF = Acid detergent fiber. ^dCP = Crude protein. ^eNFC = Non-fiber carbohydrate = 100 - (NDF + CP + ether extract + ash). ^f Σ other: 12:0; 14:0; 15:0; 16:1 c9; 17:0; 18:1 c11; 20:0; 18:3 c6,c9,c12; 20:2 c11,c14; 22:0; 22:1 c13; 20:4 c5,c8,c11,c14; 24:0; 24:1 c15. ^gSFA = Saturated fatty acids. ^hMUFA = Monounsaturated fatty acids. ⁱPUFA = Polyunsaturated fatty acids.

Table 2. Dry matter intake, milk yield, and components of dairy cows^a grazing a cool-season pasture (CSP) and pearl millet (PM).

	Treatment		<i>P</i> value
	CSP	PM	
Dry matter intake	17.42 ± 0.27	17.22 ± 0.23	0.35
Milk yield (kg/day)	13.12 ± 1.25	13.76 ± 1.23	0.82
3.5% FCM ^b	12.64 ± 1.14	13.13 ± 1.10	0.54
ECM ^c	12.57 ± 1.08	13.04 ± 1.04	0.48
Milk components (kg/day)			
Fat	0.43 ± 0.04	0.44 ± 0.04	0.39
Crude protein	0.40 ± 0.03	0.41 ± 0.03	0.46
Organic solids	0.73 ± 0.07	0.76 ± 0.06	0.78
Milk components (%)			
Fat	3.38 ± 0.018	3.24 ± 0.016	0.12
Crude protein	3.11 ± 0.10	3.01 ± 0.09	0.17
Organic solids	5.54 ± 0.04	5.54 ± 0.04	0.66

^aLeast-squares (LS) means ± standard error are based on n = 8 dairy cows. ^b3.5% Fat-corrected milk = [0.4324 x milk yield (kg/d)] + [16.216 x fat yield (kg/d)].

^cEnergy-corrected milk = [12.82 x fat yield (kg/d)] + [7.13 x protein yield (kg/d)] + [0.323 x milk yield (kg/d)].

Table 3. Fatty acid contents (mg per serving) in 3.25% milk of dairy cows^a grazing a cool-season pasture (CSP) and pearl millet (PM).

Fatty acid	Treatment		P value
	CSP	PM	
4:0	228.4 ± 7.23	219.8 ± 7.02	0.16
5:0	4.00 ± 0.33	3.76 ± 0.27	0.60
6:0	119.0 ± 3.72	125.0 ± 3.39	0.16
7:0	0.77 ± 0.10	0.99 ± 0.08	0.11
8:0	60.02 ± 2.63	65.03 ± 2.31	0.14
9:0	1.14 ± 0.12	1.01 ± 0.10	0.32
10:0	122.7 ± 4.89	137.3 ± 4.78	0.09
11:0	17.96 ± 1.72	21.28 ± 1.64	0.06
12:0	150.4 ± 6.54	171.5 ± 6.09	0.06
<i>iso</i> 13:0	4.50 ± 0.15	3.81 ± 0.12	0.007
<i>anteiso</i> 13:0	5.86 ± 0.53	6.82 ± 0.50	0.09
13:0	10.04 ± 0.60	11.58 ± 0.53	0.048
<i>iso</i> 14:0	13.03 ± 0.52	15.58 ± 0.42	0.007
14:0	647.2 ± 18.9	726.4 ± 15.5	0.011
<i>iso</i> 15:0	29.99 ± 1.21	34.49 ± 0.97	0.020
<i>anteiso</i> 15:0	50.52 ± 1.46	54.03 ± 1.25	0.08
14:1 <i>c</i> 9	69.96 ± 7.59	83.76 ± 7.37	0.045
15:0	105.3 ± 2.70	109.0 ± 2.13	0.32
<i>iso</i> 16:0	23.24 ± 0.95	28.61 ± 0.74	0.003
16:0	1911 ± 74.4	2254 ± 67.7	0.003
<i>iso</i> 17:0	48.57 ± 1.62	46.41 ± 1.29	0.33
16:1 <i>t</i> 9	2.41 ± 0.60	3.33 ± 0.52	0.23
16:1 <i>c</i> 7	2.36 ± 0.14	1.71 ± 0.10	0.009
16:1 <i>c</i> 8	21.21 ± 0.82	23.40 ± 0.72	0.048
<i>anteiso</i> 17:0	29.00 ± 1.52	35.46 ± 1.14	0.014
16:1 <i>c</i> 9	96.97 ± 9.98	108.0 ± 9.86	0.10
16:1 <i>c</i> 10/ <i>t</i> 13	6.51 ± 0.67	5.82 ± 0.64	0.27
16:1 <i>c</i> 11	11.26 ± 0.57	9.06 ± 0.49	0.017
17:0	58.50 ± 1.21	59.07 ± 1.03	0.70
17:1 <i>t</i> 10	3.66 ± 0.17	4.29 ± 0.13	0.028
17:1 <i>c</i> 8	1.74 ± 0.22	2.49 ± 0.19	0.025
17:1 <i>c</i> 9	20.42 ± 0.50	21.96 ± 0.39	0.047
18:0	893.3 ± 75.1	765.7 ± 74.2	0.023
18:1 <i>t</i> 6-8	22.30 ± 1.14	19.61 ± 0.96	0.09
18:1 <i>t</i> 9	18.06 ± 0.58	15.29 ± 0.45	0.007
18:1 <i>t</i> 10	17.42 ± 1.32	18.65 ± 1.02	0.49
18:1 <i>t</i> 11	326.5 ± 16.4	201.6 ± 14.6	<0.001
18:1 <i>t</i> 12	16.63 ± 1.15	16.65 ± 0.92	0.95
18:1 <i>t</i> 13/ <i>t</i> 14	35.40 ± 2.77	32.97 ± 2.15	0.51
18:1 <i>c</i> 9	1536 ± 36.8	1465 ± 30.2	0.16
18:1 <i>c</i> 11	30.43 ± 1.04	28.06 ± 0.84	0.11
18:1 <i>c</i> 12	7.26 ± 0.41	5.65 ± 0.36	0.011
18:1 <i>c</i> 13	4.20 ± 0.21	3.90 ± 0.17	0.32
18:1 <i>c</i> 14/ <i>t</i> 16	25.63 ± 1.46	21.37 ± 1.23	0.045

18:1 c15	5.29 ± 1.61	14.07 ± 1.40	0.002
18:2 t10,t14	9.17 ± 0.81	5.96 ± 0.77	0.004
18:2 t9,t12	11.16 ± 0.65	7.41 ± 0.49	0.003
18:2 c9,t13/t8,c12	17.55 ± 0.80	15.18 ± 0.62	0.05
cyclohexyl-11 11:0	6.19 ± 0.36	6.33 ± 0.27	0.76
18:2 c9,t14	7.71 ± 0.51	7.09 ± 0.43	0.35
18:1 c16	3.62 ± 0.29	3.49 ± 0.24	0.72
18:2 c12,t16	4.08 ± 0.24	1.78 ± 0.18	<0.001
18:2 t11,c15	54.89 ± 2.07	37.90 ± 1.52	<0.001
18:2 c9,c12	83.12 ± 1.91	65.19 ± 1.62	<0.001
20:0	14.23 ± 0.87	13.40 ± 0.82	0.32
20:1 c9	10.01 ± 0.38	10.35 ± 0.31	0.50
18:3 c9,c12,c15	62.76 ± 1.43	29.57 ± 1.11	<0.001
18:2 c9,t11	150.0 ± 6.50	104.8 ± 5.85	<0.001
18:2 c9,c11	8.33 ± 0.53	7.55 ± 0.39	0.29
18:2 t11,t13	2.18 ± 0.19	1.76 ± 0.15	0.15
18:2 t7,t9/t10,t12	1.70 ± 0.15	1.63 ± 0.12	0.75
20:2 c11,c14	3.50 ± 0.14	2.99 ± 0.11	0.027
22:0	7.31 ± 0.45	6.30 ± 0.44	0.026
20:3 c8,c11,c14	4.44 ± 0.20	3.38 ± 0.16	0.005
20:4 c5,c8,c11,c14	5.25 ± 0.26	5.22 ± 0.20	0.94
23:0	5.24 ± 0.38	3.34 ± 0.33	0.003
20:5 c5,c8,c11,c14,c17	5.17 ± 0.23	4.47 ± 0.19	0.042
24:0	5.19 ± 0.38	5.82 ± 0.36	0.12
22:5 c7,c10,c13,c16,c19	8.30 ± 0.39	7.74 ± 0.31	0.29
Unknown	63.64 ± 1.46	54.13 ± 1.11	0.002
Total SFA ^b	4404 ± 49.1	4663 ± 38.5	0.004
Total MUFA ^c	2289 ± 42.2	2131 ± 33.9	0.021
Total PUFA	432.5 ± 8.79	319.1 ± 6.64	<0.001
Total 18:1 <i>trans</i>	402.9 ± 18.2	273.0 ± 16.0	<0.001
Total n-6 FA ^d	96.55 ± 2.13	76.57 ± 1.83	<0.001
Total n-3 FA ^e	78.70 ± 1.94	42.98 ± 1.57	<0.001
n-6:n-3 ratio	1.23 ± 0.04	1.80 ± 0.04	<0.001
Total CLA ^f	162.7 ± 6.82	116.3 ± 5.94	<0.001
Total OBCFA ^g	442.5 ± 8.13	472.4 ± 6.49	0.022

^aLeast-squares (LS) means ± standard error are based on n = 8 cows.

^bTotal SFA: all saturated fatty acid (4:0 to 26:0). ^cTotal MUFA: all monounsaturated fatty acids (14:1 to 24:1). ^dTotal n-6 FA: all n-6 fatty acids; 18:2 c9,c12; 18:3 c6,c8,c12; 20:2 c11,c14; 20:3 c8,c11,c14; 20:4 c5,c8,c11,c14; and 22:4 c7,c10,c13,c16. ^eTotal n-3 FA: 18:3 c9,c12,c15; 20:3 c11,c14,c17; 20:5 c5,c8,c11,c14,c17; 22:5 c7,c10,c13,c16,c19; and 22:6 c4,c7,c10,c13,c16,c19. ^fTotal CLA: all detected conjugated linoleic acid isomers: 18:2 c9,t11, 18:2 t11,t13, and 18:2 t7,t9/18:2 t10,t12.

^gTotal OBCFA: all odd and branched-chain fatty acids (5:0 to 23:0). ns = non-significant