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Quantitative determination of fatty acids in the University of Vermont's CREAM herd followed over the course of six months

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Quantitative Determination of fatty acids in the milk of the University of Vermont's CREAM herd followed over the course of six months

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Abstract

Holstein and Jersey cows were used to determine how the fatty acid profile of their milk differed with regard to breed of cow and season over the course of six months. Milk was collected from each cow monthly between November 2011 and April 2012 and analyzed for components and fatty acid composition. Data were analyzed using linear mixed models with a repeated measures design with breed, month, and breed × month as the main effects. Holstein cows produced more milk than Jersey cows (79.98 vs. 50.48 lbs), and significant differences were seen between both breeds (P<0.001) and months (P=0.014) of the study. Jersey cows produced more fat (5.20 vs. 3.91%, P<0.001) and protein in their milk (3.83 vs. 3.12%, P<0.001) when compared to Holstein cows. Jersey cows produced more saturated fatty acids than Holstein cows (72.9% vs. 70.7%, breed P=0.004, month P<0.001). Vaccenic acid was produced in similar levels by both breeds, but Holsteins produced slightly higher levels (0.92%) than Jerseys (0.81%, breed P=0.0016, month P<0.001). Holstein cows produced higher levels of conjugated linoleic acids in their milk (0.49%) when compared to Jersey cows (0.41%, P<0.001). Holstein cows produced higher levels of linoleic acid (1.60% vs. 1.43%, P<0.001, month P=0.005) when compared to Jersey cows. Linolenic acid levels produced by both breeds were similar for Holstein (0.31%) and Jersey cows (0.29%, month P=0.007). From the analysis of concentrations of both selected fatty acids as well as groups of major fatty acids, there is evidence that the fatty acid profile of milk differs with respect to both breed of cow, as well as month of the year.

Introduction

The major components of bovine milk are water, lactose, fat and protein, and are highly variable with respect to animal, management and environmental related factors (Arnould and Soyeurt, 2009). Milk fat is by far the most complex of natural fats, with over 400 individual fatty acids identified (Jensen, 2000). Almost 98% of the fats in bovine milk are made up of triacylglycerides (Bauman and Griinari, 2003) and the remaining approximate 2% is made up of diacylglycerides, cholesterol, phospholipids, and free fatty acids (Lock and Bauman, 2003). The fatty acids in milk fat are derived from four major sources, 1) directly from the ration being fed, 2) the microbial fermentation of feed-derived components in the rumen, 3) the mobilization of the animal's own body fat stores, or 4) from the *de novo* synthesis throughout the body, but mainly in the liver and the lactating mammary gland (Grummer, 1991). A cow's mammary gland synthesizes fatty acids that contain even numbers of carbon atoms. This so-called *de novo* synthesis accounts for fatty acids with 4-14 carbons, such as myristic (14:0) acid as well as about half of the fatty acids with 16 carbons, and are synthesized from acetic and β-hydroxybutyric acid (Bauman *et al*., 2003). Long-chain fatty acids >16 carbon atoms, such as palmitic (16:0) and stearic (18:0) acids, are generally derived from dietary sources and through the mobilization of body fat stores via lipolysis of adipose tissue triacylglycerides (Parodi, 2004.) These three fatty acids are the major fatty acids in bovine milk, and can account for up to 75% of the total fatty acid concentration (Mansbridge and Blake, 1997).

The dietary effect on milk fatty acids comes from three main components in the cow's ration 1) forages, 2) oilseeds, and 3) fat supplements, with each contributing to the overall long-chain fatty acid concentration and profile in a different way. Rumen microbes ferment cellulose

and hemicellulose to create acetate and butyrate, which are precursors for *de novo* synthesis in the mammary gland. Diets that included whole oilseeds and seed oils have shown increased concentrations of linoleic (18:2 n-6) and linolenic acid (18:2 n-3) (Mansbridge and Blake, 1997). Finally, fat supplements are generally fed as energy supplements and are developed to minimize adverse effects of fiber digestion by the rumen microbial population (Mansbridge and Blake, 1997). Cows that are fed ensiled forages generally have higher concentrations of saturated fatty acids in their milk, while feeding fresh pasture showed higher levels of both mono- and polyunsaturated fatty acids in milk (Mansbridge and Blake, 1997).

Previous research has shown major differences in the fatty acid profile between Jersey and Holstein cows (Beaulieu and Palmquist, 1995). Jersey milk fat generally has a higher concentration of saturated fatty acids (SFAs), with a higher proportion of short and medium chain fatty acids (4:0 - 12:0), while Holsteins have higher concentrations of monounsaturated fatty acids (MUFAs), and long-chain fatty acids (Arnould and Soyeurt, 2009). Most studies that examined the fatty acid profiles of milk between breeds did so in the context of controlled feeding trials, such as pasture versus total mixed ration (TMR) based systems (White *et al*., 2001; Palladino *et al*., 2009). In such a study, White *et al.* (2001) reported that Jersey cows that were fed TMR produced higher concentrations of 6:0, 8:0, 10:0, 12:0, and 14:0 when compared to Holstein cows. The same study reported that Holstein cows managed under a TMR system produced higher levels of conjugated linoleic acids (CLAs) when compared to Jersey cows.

Since the fatty acid profile of bovine milk can be influenced by the ration being fed, most studies regarding the seasonal effects on the fatty acid profile of bovine milk focus on pasture diversity and the availability of forages during the time being investigated, as well as

pasture-based versus silage-based nutritional programs (Frelich *et al*., 2012). Short-chain fatty acids such as 4:0, 6:0, and 8:0 were generally reported to be higher during the winter months when compared to the summer months when cows are typically managed under a TMR-based nutritional program (Frelich et al., 2012). Both oleic acid (18: 9*c*), the major MUFA in bovine milk, as well as CLAs, were generally higher when cows grazed on pasture during the summer months than when they were fed TMR during the winter (Mesnick, 2003).

Previous studies highlighted the differences in the fatty acid profiles between Holstein and Jersey cows when consuming either pasture or TMR. Palladino *et al*. (2009) examined the fatty acid differences of these breeds strictly under grazing conditions. Frelich *et al*. (2012) studied seasonal variation in the fatty acid profiles of Holstein cows on a silage-based diet. White *et al*. (2001) and Palladino *et al*. (2009) demonstrated that there are significant differences in the fatty acid profiles of Holstein and Jersey cows with respect to different nutritional management, and Frelich *et al*. (2012) showed that the fatty acid profile of Holstein milk showed significant variation between summer and winter months.

At the University of Vermont, we have a unique opportunity to examine the differences in the fatty acid profiles of Holstein and Jersey cattle managed under the same husbandry system and nutritional conditions. While the ration being fed certainly has a major impact on the fatty acid profile of bovine milk, potential breed and seasonal variations between the breeds can be examined while maintained under the same nutritional program.

Hypothesis

We tested the hypothesis that the fatty acid profiles of the two breeds of cows (Jersey and Holstein) are different due to factors such as breed and season of the year. The aim of this study was to determine if there are differences in the fatty acids profiles of Jersey and Holstein cows based on these factors.

Materials and Methods

Animals

The animals that were used in this study are owned and managed by the Cooperative for Real Education in Agricultural Management (C.R.E.A.M.) program, a student run dairy herd at the University of Vermont consisting of both Holstein (n=26) and Jersey (n=23) cows. Cows were housed in a tie-stall barn system, with seventeen stalls on each side, and kept inside approximately 22 hours per day. Cows were let out twice a day into a heat lot, once prior to each milking, to check for signs of estrous as well as for exercise. Animals were milked twice daily at 0400 h and at 1600 h. Both Holsteins and Jerseys included in the study were fed the same base TMR as balanced by the herd nutritionist and TMR was top-dressed with grain that varied for each cow based on stage of lactation and peak production. TMR was fed *ad libitum* and water intake was not monitored.

Sample Collection

Milk samples were collected monthly for the duration of 6 months. Two samples were taken from each lactating cow and on the same day (AM and PM) as the Dairy Herd

Improvement Association (D.H.I.A.) test occurred. Milk weights were recorded at each sampling and milk samples were analyzed for contents of fat, and protein at the D.H.I.A. laboratory using mid-infrared techniques.

Laboratory Analyses

Milk samples for fatty acid analysis were composited in proportion to milk yield and skimmed by centrifugation at 12,000 rpm at 10°C for 30 minutes. A modified protocol of the lipid extraction procedure developed by Hara and Radin (1978) was used for extraction of total lipids from the cream. ~320 mg of the isolated cream was dissolved in *n*-hexane/isopropanol (3:2, vol/vol). The extract was mixed and equilibrated with two-thirds its volume of a 6.67% aqueous sodium sulfate solution. The organic phase was dried over anhydrous sodium sulfate and after removal of the solvents under nitrogen flux at 37°C, the lipids were weighed and transferred into 4 mL vials. Fatty acid methyl esters (FAME) were prepared by shaking a mixture of 2.5 mL *n*-hexane containing 25 mg milk lipids and 0.5 mL of 0.5 M sodium methoxide solution in methanol for five minutes. After completion of the methylation reaction, 1 g of sodium bisulfate was added and the vial mixed for 20 seconds. After 5 minutes of centrifugation at 4000 rpm, the supernatant containing the FAME was then transferred into a 2 mL vial and directly used for gas-liquid chromatography (GLC) analysis. The total fatty acid composition covering circa 80 fatty acids in the range 4:0 to 24:0, including branch-chain fatty acids and geometric isomers of octadecenoic acid (18:1) and CLAs, was determined on a GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with split injector (1:100 split ratio) and a flame-ionization detector (FID) using a SP2650 fused silica column (100 m x 0.25mm i.d. x 0.2

μm film thickness.) Integration and quantitation was accomplished with GCsolution software (version 2.30.00) and based on the FID response. FAME were be identified by comparison of retention times with known FAME standards.

Statistical Analysis

Statistical analysis was done to determine if there was a significant difference between the fatty acid profile produced by each animal compared to both breed and month, as well as in terms of milk yield and components in each milk sample. Data were analyzed using a linear mixed model approach with a repeated measure design, and tested at a significance level of P=0.05 by using JMP Statistical Analysis software package (version 11.0). The relationship between vaccenic acid (VA) and CLAs was quantified using the correlation function in Microsoft Excel (2013).

Results

Milk Yield and Composition

Data on milk yield and composition for each breed and each month are presented in Table 1 (see Appendix). Holstein cows produced a significantly higher milk yield than Jersey cows (P<0.001) during every month of the study from November to April, and the amount of milk produced by each breed showed statistically significant differences (P=0.014) in terms of month. There was no significant breed × month interaction. Jersey cows produced a significantly higher percentage of fat in their milk (P<0.001) when compared to Holstein cows. Jersey cows also produced higher levels of protein in their milk (P<0.001) when compared to

Holstein cows, and significant differences were observed between the months of the study (P<0.01). There was no significant interaction between the breeds and the months of the study.

Fatty Acid Composition

Fatty acid composition of milk from each breed and for each month of the study is presented in Table 2 (Appendix 1). Fatty acids are reported as percentage of total fatty acids. In general, Jersey cows produced more short-chain, SFAs (4:0 and 6:0) and medium-chain, SFAs (8:0, 10:0, 12:0) than Holstein cows, except for the month of April, when Holsteins produced more 4:0, 8:0, 10:0, and 12:0. Jersey cows produced more 18:0 in every month of the study when compared to Holsteins, and there were significant differences observed between the breeds (P<0.01). The concentration of 18:1 9*c* was generally higher in Holstein milk than in Jersey milk. VA concentrations were variable, as Jersey cows produced higher levels of VA in November, December and March, and Holstein cows produced more in January, February and April. For VA, there was statistical significance observed between both the breeds (P<0.01) and the months of the study (P<0.001). VA also showed a significant breed × month interaction (P<0.001). Holstein cows produced more linoleic acid when compared to Jerseys for all months of the study except for April, and there were significant differences between breed (P<0.001), month (P<0.01), as well as breed × month interaction (P<0.01). Linolenic acid did not show significant differences between breeds, but there were differences observed December, January, February and March (P<0.01). Jersey cows produced more SFAs for every month of the study except April (P<0.001). Conversely, Holstein cows produced more MUFAs in every month of the study except April (P<0.001). For both SFAs and MUFAs, there were significant

differences between the breeds (SFA $P<0.01$, MUFA $P<0.01$). For every month of the study, Holstein cows produced more CLAs when compared to Jersey cows. There were significant differences observed between breeds ($P<0.001$), as well as a breed × month interaction ($P<0.01$). There were significant differences in PUFA concentrations between the months of the study ($P<0.001$), but not the breeds. Holstein cows generally produced higher levels of PUFAs in their milk when compared to Jerseys, except for the months of March and April, when Holstein PUFA concentrations were higher. Holstein cows produced higher concentrations of n-6 fatty acids when compared to Jersey cows, and there were significant differences between breeds ($P<0.01$) and months ($P<0.01$) of the study. n-3 fatty acids were produced in lower concentrations than n-6 fatty acids for both breeds, but Holsteins produced higher concentrations of n-3 fatty acids when compared to Jersey cows ($P>0.05$). No significant differences were observed between breed and month for n-3 fatty acids.

Discussion

Results for the components of milk differed from previously published studies, as our results generally showed higher levels of fat and protein for both Jersey and Holstein cows. White *et al*. (2001) reported that Jersey and Holstein cows managed under similar conditions produced on average 4.1% and 3.3% fat respectively. Kelsey *et al.* (2003) showed that when managed under similar conditions, 3.86% and 3.41% of milk was fat for Jerseys and Holsteins respectively. White *et al*. (2001) reported levels of protein to be 2.87% for Holsteins and 3.62% for Jerseys. Kelsey *et al*. (2003) reported similar levels as White *et al.* (2001), with Holsteins producing 3.03% and Jerseys producing 3.46% of their milk as protein. When compared to the

present study, these two studies show lower levels of protein in the milk of both breeds overall. One potential reason for the discrepancies in reported levels of milk fat and protein between previous literature and this study may lie in the management of the CREAM herd. White *et al.* (2001) reported that the average somatic cell count (SCC) for Holstein and Jersey cows managed in similar ways was 233×10^3 cells/mL for a study conducted between January and March of 1998. In the CREAM herd, the highest SCC was observed in November at 191×10^3 cells/mL, and the lowest SCC in January at 74×10^3 cells/mL. The average SCC for all of the cows in the study over the course of six months was 130×10^3 cells/mL, much lower than industry average of American commercial dairy farms of similar size at 286×10^3 cells/mL (USDA-APHIS, 2012). There is a negative correlation between somatic cell count, both at a bulk tank and individual cow level, and component distribution in milk (Verdi *et al.* 1987); thus, by maintaining a bulk tank and cows with very low somatic cell counts could be a contributing factor as to why the CREAM herd's fat and protein percentages are higher than previously published studies.

The current study shows similar concentrations of fatty acids in the both Holstein and Jersey milk when compared to previous research. Kelsey *et al.* (2003) reported similar levels of short and medium-chain fatty acids for Holstein and Jersey cows managed under similar conditions with similar concentrations of 4:0, 6:0, 8:0 and 10:0. White *et al*. (2001) reported that SFA comprised 64.41% of Holstein milk and 67.19% of Jersey milk. Mele *et al*. (2007) reported that MUFA content for Holsteins managed under similar conditions was 21.58%, and PUFA content was 2.58%, of the total fatty acid composition. These concentrations are comparable to those observed in the milk in our study.

The CLA concentrations in the milk of the CREAM herd were also similar to those values reported by previous research. White *et al*. (2001) reported that Jerseys and Holsteins managed under similar conditions produced a CLA content of 0.32% and 0.41% of the total fatty acids respectively, and the 9*c*,11*t* isomer was the only CLA isomer quantified. Kelsey *et al*. (2003) reported similar results, with Holsteins producing 0.44 % of the total fatty acids as 9*c,*11*t* CLA and Jerseys producing 0.41% of the total fatty acids. 9*c,*11*t* CLA is the major geometric isomer of CLA in bovine milk, and while other studies only included this isomer, the present study also included other geometric isomers of CLA, although in concentrations less than 0.01% of total fatty acids. The major source of CLA in milk relates to the conversion of VA into 9*c*,11*t* CLA via the enzymatic activity of the Δ^9-desaturase (Palladino et al. 2010). In the present study, Jersey cows produced the highest levels of VA in November (1.14% of total fatty acids), while Holsteins produced the highest levels of VA in April (also 1.14% of total fatty acids). While the concentrations of VA fluctuated from month to month, the concentrations of CLA in the milk of the CREAM herd did not necessarily correlate to the concentrations of VA during a certain month. For example, when the concentrations of VA were highest for Jerseys during the month of November, there was a moderate correlation between VA and 9*c*,11*t* CLA for that month (R^2=0.5377, Figure 1). When the concentrations of VA were highest for Holsteins during April, there was no correlation between VA and 9*c*,11*t* CLA (R^2=0.0004, Figure 2).

Figure 1: Correlation between VA and CLA for Jersey cows during the month of November .

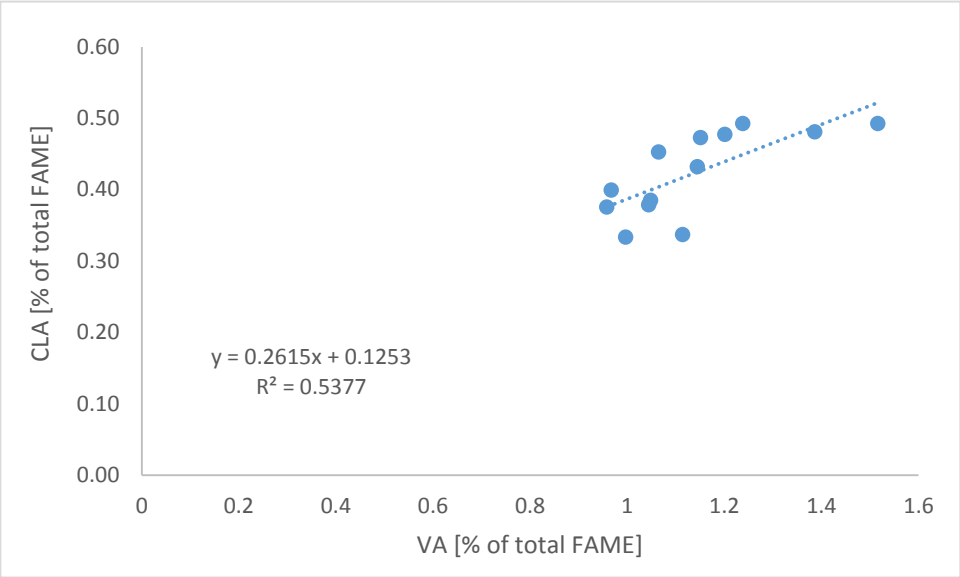
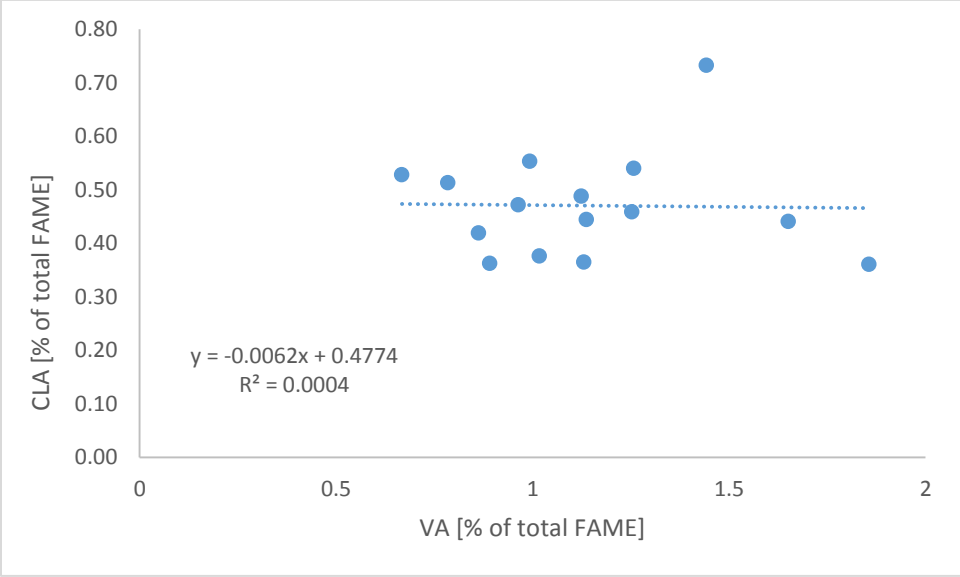


Figure 2: Correlation between VA and CLA for Holstein cows during the month of April.



Both linoleic and linolenic acid were observed to be the highest during the months of March and April. As stated previously, long-chain fatty acids are generally derived from dietary sources and from the digestion and absorption of dietary fat (Parodi, 2004). During March and April especially in Vermont, TMR is mixed using ensiled forages from the previous summer. While farmers may have access to better forages in the early spring, it is unlikely that they would be able to incorporate fresh forages into their rations, which could potentially increase linoleic and linolenic acid content (Ward *et al.*, 2003). One potential hypothesis to explain why the concentrations of linoleic and linolenic acid were higher in the early spring has to do with photoperiod. Vincente *et al.* (2008) showed that Holstein cows that were allowed to graze the same pasture during the day showed higher levels of both linoleic and linolenic acid than cows of similar body weight, stage of lactation, and lactation number who were allowed to graze the same pasture at night. Early March is when daylight savings time begins in Vermont, and even though the cows included in this study were kept indoors for most of the day, ample light could permeate into the barn, which could potentially have an effect on the linoleic and linolenic acid levels during these months. When the results of the fatty acid content of the TMR of the current study is analyzed and compared to the fatty acid profile of the milk produced by these animals, this hypothesis can be tested.

Diet can greatly influence the fatty acid profile of bovine milk, especially in the context of long-chain fatty acids. It is important to note that differences in the fatty acid profiles of the TMR that the animals were being fed on the sample date have been collected, and will be analyzed and compared to the fatty acid profiles of the milk obtained throughout the course of this study. Further steps of the study will include comparing the analysis of the fatty acid

profiles of the TMR fed over the course of the study, as well as the concentrate that was top-dressed on the TMR to the fatty acid profiles of the milk reported in this study. Data for the fatty acid profiles of the milk of both breeds from May 2012 to October 2012 will also be included into this study to show long term seasonal and breed effects.

Conclusion

From the present study, we conclude that there are significant differences in the fatty acid profiles of Holstein and Jersey cows with regard to both breed and season of the year. In addition, concentrations of VA did not necessarily correlate with levels of CLA in the milk of the University of Vermont's CREAM herd as shown by other studies.

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Appendix

Table 1: *Milk yield, components, and somatic cell count for Holstein and Jersey cows followed over the course of six months*.

	November		December		January		February		March		April		P-value		
Factor	**Jersey**	**Holstein**	**Jersey**	**Holstein**	**Jersey**	**Holstein**	**Jersey**	**Holstein**	**Jersey**	**Holstein**	**Jersey**	**Holstein**	**Breed**	**Month**	**Interaction**
Milk Yield (lbs)	46.56^{a*}	69.2^{A}	46.92^{a*}	79.73^{B}	47.78^{a*}	86.59^{C}	49.54^{b*}	77.59^{B}	53.91^{c*}	82.63^{D}	58.09^{d*}	84.12^{D}	<0.001	0.0143	NS
Fat (%)	5.2*	3.85	5.36*	3.99	5.48*	3.39	4.74*	4.19	5.26*	4.22	5.16*	3.83	<0.001	NS	NS
Protein (%)	3.84^{a*}	3.24^{A}	4.01^{b*}	3.18^{A}	3.92^{b*}	3.11^{B}	3.77^{a*}	3.15^{B}	3.69^{a*}	3.17^{B}	3.76^{a*}	2.86^{C}	<0.001	0.007	NS
SCC, 10^3 cells/mL	191	191	150	150	74	74	181	181	94	94	93	93	NS	NS	NS

a-c within a row indicates significant differences (P<0.05) between months for Jerseys

A-C within a row indicates significant differences (P<0.05) between months for Holsteins

* indicates differences (P<0.05) between breeds within a given month

Table 2: *Concentrations of selected and majors groups of fatty acids for Holstein and Jersey cows followed over the course of six months.*

	November		December		January		February		March		April		*P*-value		
Fatty Acid	**Jersey**	**Holstein**	**Jersey**	**Holstein**	**Jersey**	**Holstein**	**Jersey**	**Holstein**	**Jersey**	**Holstein**	**Jersey**	**Holstein**	**Breed**	**Month**	**Interaction**
C4:0	3.41^{a}	3.02^{A}	3.41^{a}	3.33^{B}	3.39^{a}	3.51^{C}	3.52^{b}	3.35^{B}	3.27^{c}	3.33^{B}	3.44^{a}	3.59^{b}	NS	0.041	NS
C6:0	2.27^{a*}	1.98^{A}	2.26^{a*}	2.07^{B}	2.25^{a}	2.13^{B}	2.15^{b*}	1.98^{A}	2.29^{a*}	2.13^{B}	2.33^{a}	2.26^{C}	<0.001	0.047	0.017
C8:0	1.37*	1.15	1.32*	1.17	1.31*	1.16	1.21*	1.10	1.37*	1.24	1.29	1.30	<0.001	NS	0.019
C10:0	3.07*	2.69	3.11*	2.69	3.07*	2.69	2.69	2.50	3.18*	2.78	2.83	2.86	0.002	NS	NS
C12:0	3.69*	3.28	3.78*	3.25	3.71*	3.19	3.17	3.01	3.77*	3.22	3.21	3.24	0.006	NS	NS
C14:0	11.83	12.14	11.76	11.83	11.85	11.17	10.89	11.01	11.81	11.55	11.29	11.27	NS	NS	NS
C16:0	34.18^{a}	34.24^{A}	36.82^{b}	36.52^{B}	37.29^{c}	36.06^{B}	36.7^{b}	36.65^{B}	36.18^{b}	32.08^{C}	33.88^{a}	31.42^{D}	NS	0.002	NS
C18:0	10.99*	8.56	10.19*	8.88	9.68	9.21	10.48*	9.19	9.69*	9.01	10.25	10.23	0.002	NS	NS
C18:1 *trans*	2.06	2.21	2.13	2.15	2.06	2.21	2.08	2.24	2.34	2.20	2.84	2.59	NS	NS	NS
18:1 9*c*	15.67*	17.25	14.7*	16.52	14.92*	17.20	16.76*	17.70	16.39*	19.76	17.05	16.99	0.006	NS	NS
18:1 11*t*	1.14^{a*}	1.01^{A}	0.78^{b}	0.74^{B}	0.72^{b*}	0.84^{C}	0.85^{c}	0.86^{C}	0.91^{c}	0.88^{C}	1.06^{a*}	1.14^{A}	0.007	<0.001	<0.001
18:2 n-6	1.37^{a}	1.46^{A}	1.40^{a*}	1.62^{B}	1.46^{a*}	1.59^{B}	1.28^{b*}	1.53^{B}	1.34^{a*}	1.79^{C}	1.70^{c}	1.63^{B}	<0.001	0.0048	0.011
18:3 n-3	0.31^{a}	0.30^{A}	0.28^{b}	0.30^{A}	0.25^{b}	0.28^{A}	0.27^{b}	0.29^{A}	0.33^{a}	0.29^{A}	0.32^{a}	0.36^{B}	NS	0.0067	NS
ΣSFA	73.12^{a*}	69.65^{A}	75.04^{b*}	72.22^{B}	74.81^{b*}	71.69^{B}	73.04^{a}	71.17^{B}	72.45^{a*}	68.05^{A}	68.68^{c*}	71.22^{B}	0.004	0.001	NS
ΣMUFA	20.85^{a*}	23.45^{a}	19.22^{a}	21.39^{a}	19.46^{a*}	22.35^{b}	21.61^{a}	22.82^{b}	24.75^{b*}	28.36^{c}	27.95^{c*}	25.57^{d}	0.045	<0.001	NS
ΣPUFA	2.25^{a}	2.43^{A}	2.13^{a}	2.51^{A}	1.67^{b}	2.42^{A}	2.01^{a}	2.38^{A}	4.13^{c}	3.31^{B}	5.08^{d}	5.06^{C}	NS	<0.001	NS
Σn-6	1.58^{a}	1.72^{A}	1.61^{a*}	1.89^{B}	1.31^{b*}	1.83^{B}	1.48^{a*}	1.78^{B}	1.54^{a*}	1.99^{C}	1.85^{c}	1.94^{C}	0.032	0.006	NS
Σn-3	0.40	0.39	0.35	0.39	0.37	0.37	0.35	0.38	0.37	0.43	0.34	0.36	NS	NS	NS
ΣCLA	0.43*	0.56	0.34*	0.43	0.38	0.42	0.36*	0.45	0.46*	0.51	0.47*	0.54	<0.001	NS	0.022
ΣBCFA	1.41^{a}	1.58^{A}	1.39^{a}	1.41^{B}	1.34^{b}	1.29^{C}	1.29^{b}	1.35^{C}	1.72^{c}	1.75^{D}	1.68^{c}	1.83^{D}	NS	<0.001	NS

$^{a-d}$ within a row indicates differences (P<0.05) between months for Jerseys

$^{A-D}$ indicate differences (P<0.05) between months for Holsteins

* indicates differences (P<0.05) between breeds within a given month