THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF VETERINARY AND BIOMEDICAL SCIENCE

ANALYSIS OF THE EFFECTS OF RUMINATION TIME ON THE MILK FATTY ACID PROFILE IN HOLSTEIN CATTLE

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ABSTRACT

Milk composition is variable among species and influenced by the diet and health status of the animal. The objective of this study to characterize the variation in milk fat concentration and yield between cows and the relationship between these variables prior to determining the relationship with rumination. The biohydrogenation theory is the leading theory for milk fat depression and explains most of the environmental variation in milk fat. Bacteria modify unsaturated fats to saturated fats, and during acidosis the rate of the reaction is decreased in the rumen. The *trans* intermediates escape the rumen and inhibit milk fat synthesis. Milk samples were collected on two separate days from multiparous Holstein cows. Cows were housed in free stalls with sand bedding and fed ad libitum a TMR once daily. A total of 156 milk samples were collected. 91 were collected on the first sampling and 65 samples were taken on the second. Each sample was composited by milk yield based on the proportion of milk produced at the morning and evening milking. Milk samples were methylated prior to fatty acid analysis by gas chromatography. Mid infrared spectrum was used to analyze milk fat, lactose, and protein and predict categories of milk fatty acids. Linear and quadratic regression were used to correlated specific fatty effects on milk fat percent, along with milk yield. Elevated levels of *trans*-10 18:1 was indicative of reduced milk fat in the samples, but explained a limited amount of the variation. The rumination data was not available for analysis due to issue with the manufacturer. However, this work demonstrated that there is considerable variation in milk fat and milk fatty acid profile in cows fed the same diet and managed in a similar way that may be explained by rumination time.

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Chapter 1

INTRODUCTION

Fat is a highly studied component in milk, due to its economic value to the producer and its variability. Milk fat concertation is affected by many physiological, genetic, and environmental factors. The most significant environmental factor is the diet of the animal. One of the major nutritional factors of milk fat composition is the fatty acid composition in the feed. Fat in milk arises from two sources: de novo synthesis in the mammary epithelial and preformed fatty acids from digestive absorption and adipose tissue. The gut health of the animals maximizes the efficiency of the digestion and absorption of these fatty acids.

In ruminants, bacterial modification of unsaturated fatty acids occurs during fermentation in the rumen. Different species of bacteria can lyse esterified lipids and isomerize and hydrogenate unsaturated fatty acids in addition to building some fatty acids from precursors. When the rumen pH is properly maintained around a pH of 6, normal fermentation occurs. However, disease states or changes in diet can alter the feeding behavior of the ruminant. Rumination helps maintain fermentation by introduction of sodium bicarbonate to buffer pH and reduction of particle size. A reduction in rumination and an increase in fermentation in the rumen leads to acidosis. This disease state alters the microbial population in the rumen and can lead to adverse health effects on the animal.

One of the common consequences seen in dairy cattle due to reduced rumen pH is milk fat depression. Milk fat depression has been extensively studied in order to control and manage the problem. Several theories has been present to explain the reduction of milk fat yield and

change in milk fat composition. The Biohydrogenation Theory is currently the most supported theory.

Biohydrogenation is a natural process where certain species of bacteria attempt to alter unsaturated fats to saturated fats. However, during a dietary challenge these microbial processes are altered and specific *trans* isomers of fatty acids escape the rumen and inhibit milk fat synthesis (Bauman and Griinari, 2003). Extensive research has determined the specific *trans* isomers responsible for this inhibition. The isomers with the most scientific evidence is intermediates of the *trans*-10 pathway.

Chapter 2

LITERATURE REVIEW

Importance of Rumination

A unique characteristic in ruminants is their ability to regurgitate and remasticate their food and the digestive physiology of ruminants is dependent on rumination. Rumination is characterized as the rhythmic regurgitation of digesta from the rumen. The process reduces digesta particle size and allows for addition and mixing of saliva with rumen contents. Sodium bicarbonate present in saliva buffers the rumen, which aids proper fermentation (Soriani et al 2007). However, the composition of the diet effects the amount of time spent ruminating and total chewing time is largely affected by fiber content in feed and total fiber intake (Allen, 1997).

Time spent ruminating is indicative of the health status in ruminants. However, this time is variable amongst different animals and dependent on diet and physiological state. Recently, rumination has been used in animal management. The most common use is to identify cows in estrus as there is a decreases in rumination time during this period. An animal off feed also experiences a decrease in rumination (Albright, 1993).

Changes in rumination also have an impact on the cow. A decrease in rumination leads to a decrease in rumen pH. Rumen pH fluctuates over the day, but an increase in time spent below pH 5 is categorized as acute rumen acidosis and an increase in time spent below pH 5.8 is categorized as subclinical rumen acidosis (Enemark et al., 2004). This acidification damages the epithelial lining of rumen papilla, which compromises the absorptive capacity of the rumen, and also modifies the microbial population in the rumen as some microbes are more susceptible to growth inhibition and death at low pH. Subclinical rumen acidosis normally does not present

clinical signs of rumen acidosis, such as rumenitis or liver abscesses, but decreases performance and health. Specifically, subclinical rumen acidosis is associated with decreases in milk yield and milk fat yield, which have significant economic impact (Stone, 1999).

Rumination Detection

With the increased attention that rumination has received in monitoring health status in cattle, various methods of rumination detection have been implemented to record this behavior. Traditionally rumination was measured by visual observations of real-time observations or recorded video. However, this method is labor and time intensive, so indirect methods of measuring were developed. The technologies that have been developed quantify feeding behaviors based on sound or movement caused by chewing. Schirmann et al. (2009) have validated detection by microphones fitted to collars (Hi-Tag rumination monitoring system, SCR Technology, Israel). The study found that though the method detection may be imperfect, as compared to a control of two manual observers, it is a reasonable method of measuring rumination in dairy cattle. Three trials of observations showed a strong correlation between the ear tag and observer (trial 1: $R^2 = 0.93$, $P < 0.001$; trial 2: $R^2 = 0.86$, $P < 0.001$; trial 3: $r = 0.96$, $P < 0.001$). Head movement during eating and ruminating can also be observed by 3-dimensonal accelerometers, which are fitted as a collar or ear tag. Bikker et al. (2014) conducted a study to validate an ear tag based rumination monitoring system, the Cowmanager SensOor system. The study found that this method of measurement was reliable in detecting ruminating ($r = 0.93$, $P < 0.01$) and resting ($r = 0.98$; $P < 0.01$) for research and management purposes. Eating and

ruminating time detected by the ear tag accelerometer was determined to be reliable for cow management.

Milk Fat Synthesis and Diet Induced Milk Fat Depression

Milk fat has many important physiological and economic properties. Fat is a major energy source in milk and provides many of the physical properties, manufacturing characteristics, and organoleptic qualities of milk and its products (Bauman and Griinari, 2001).

Fat is the most variable component of ruminant milk and arises from two origins, de novo synthesis and preformed fatty acids. De novo synthesis occurs in the mammary gland, specifically within the mammary epithelial cell, and generates fatty acids with chain lengths of four to fourteen carbons with a portion of the sixteen carbon FA (Bauman and Griinari, 2003). Performed fatty acids are taken up from circulating fatty acids originating from dietary lipids or adipose tissue (Bauman and Griinari, 2001). Performed fatty acids are absorbed by the mammary epithelial cell as lipoproteins or non-esterified fatty acids (Bauman and Griinari, 2003). In ruminants, typically only ten percent of the performed fat is from lipolysis of adipose tissue, unless that animal is in a negative energy balance (Bauman and Griinari, 2001). The majority of these circulating fats are from intestinal absorption of dietary and microbial origin (Bauman and Griinari, 2003).

The composition and levels of fat is dependent on genetics, physiological state, and environmental factors. Nutrition is the predominant environmental factor (Bauman et al., 2011). Milk fat depression is generally caused by two main dietary issues. One of these is high concentration of fermentable carbohydrates and low fiber content and the other is a high

concentration of unsaturated oils (Bauman and Griinari, 2001). Both fat yield and fat composition change during milk fat depression. The decrease occurs in many fatty acids, but the greatest decrease occurs in fatty acids from de novo synthesis (Bauman and Griinari, 2001).

Multiple theories have been presented to explain milk fat depression. It was first proposed that milk fat depression could be caused by limited availability of dietary fat or acetate, or by increased propionate, glucose, or insulin (Bauman et al. 2011). Bauman and Griinari (2001) analyzed the changes in milk fatty acid profile during each of these factors compared to diet-induced milk fat depression caused by a traditional high grain, low forage diet. They observed that classical diet-induced MFD decreased the proportion of de novo synthesized fatty acids and increased preformed fatty acids, while insulin and glucose infusion increased de novo synthesis and decreased preformed fatty acids (Figure 2.1). Later it was determined that during diet-induced milk fat depression *trans*-10 intermediates are increased in milk fat. Bauman and Griinari (2001) concluded that the most accurate theory of milk fat depression was due to *trans*-10, *cis*-12 conjugated linoleic acid (CLA) because abomasal infusion of the isomer resulted in a condition similar to diet-induced MFD. This supported the biohydrogenation theory and has become the current dogma of environmental regulation of milk fat synthesis.

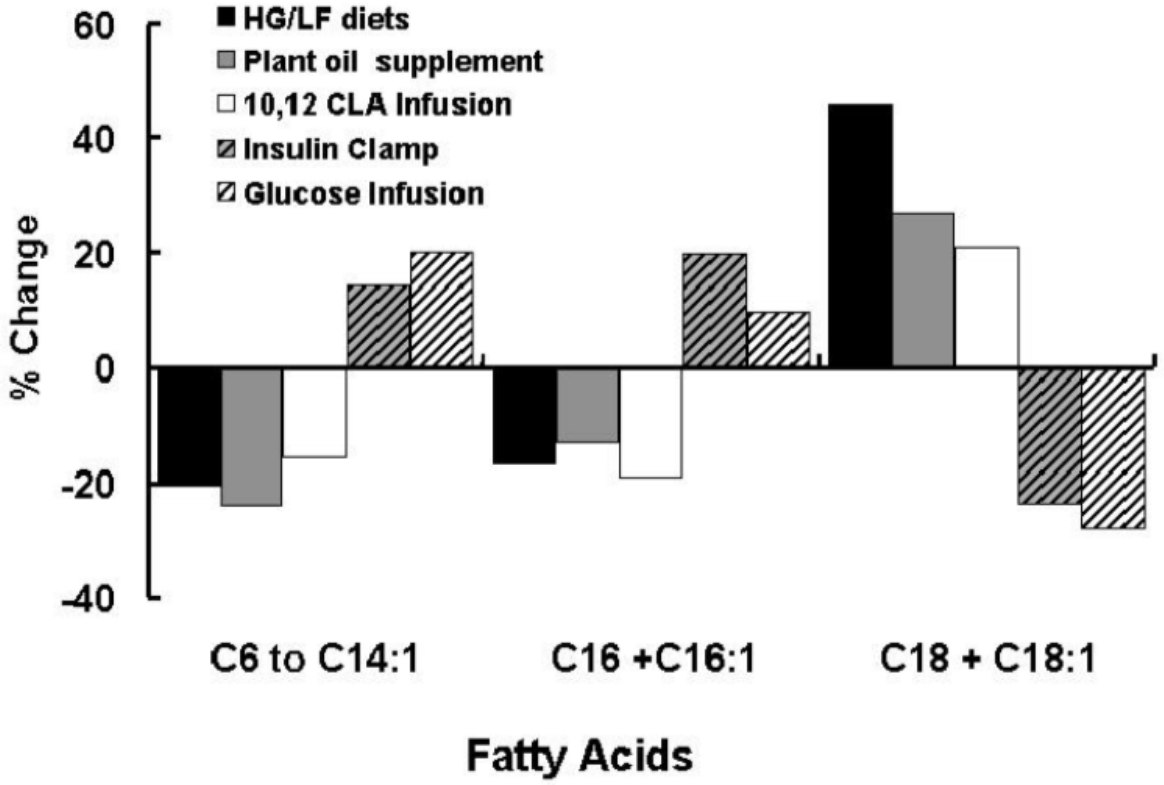

Figure 2.1: Changes in the pattern of milk fatty acids that occur with diet-induced milk fat depression.

The high grain, low forage diet approximately reduced milk fat content by 60% (Storry and Rook, 1965). The plant (soybean) oil supplement reduced milk fat yield by 30% (Steele et al., 1971). Abomasal infusion of *trans*-10, *cis*-12 CLA (10 grams/day) resulted in a 44% reduction in milk fat yield (Baumgard et al., 2000b). Abomasal infusion of glucose (1.5 kg/day) reduced milk fat yield 16% (Hurtaud et al. 1998). The hyperinsulinemic-euglycemic clamp reduced milk fat yield by 7% (Griinari et al., 1997b). (Adapted from Bauman and Griinari, 2001)

Biohydrogenation and the Alternate "trans-10" Pathway

Ruminants consume a plant-based diet that is low in total fat, but predominantly contains unsaturated fatty acids. However, their meat and milk are high in saturated fatty acids. Certain species of bacteria within the rumen modify saturate the unsaturated fatty acids through a process called biohydrogenation (Bauman et al., 2011). The principle role of biohydrogenation is to reduce the toxic effects unsaturated fats have on bacteria growth (Shingfield and Griinari, 2007). Some of the intermediates from these processes escape the rumen and are absorbed by the mammary epithelial cells that then incorporate them into milk.

The primary pathway of biohydrogenation converts linoleic acid to produce *trans*-11 intermediates, including *cis*-9, *trans*-11 CLA and *trans*-11 18:1. However, dietary factors that affect ruminal fermentation result in a microbial population that uses an alternate pathway that produces *trans*-10 intermediates, including *trans*-10, *cis*-12 CLA and *trans*-10 18:1 (Harvatine et al. 2009). Both the classical and alternative pathways are illustrated in Figure 2.2. The reduced milk fat yield with abomasal infusions of *tran*-10, *cis*-12 CLA discussed above supports the theory that the alternate pathway of biohydrogenation of linoleic acid is causatively associated with milk fat depression. Cows treated with the abomasal infusions of *tran*-10, *cis*-12 CLA experienced a significant decrease in milk fat secretion and milk fat yield (de Veth et al., 2004), while infusion of *cis*-9, *trans*-11 CLA that is in the normal pathway did not significantly affect milk fat yield (Baumgard et al., 2000).

There is reasonable variation in milk fat across cows in a herd. The degree of variance in milk fat between cows by subclinical acidosis and milk fat depression due to *trans*-10 intermediates is not known. The ability to assess the extent that rumination time effects the production of *trans*-10 intermediates could explain the variation of milk fat depression.

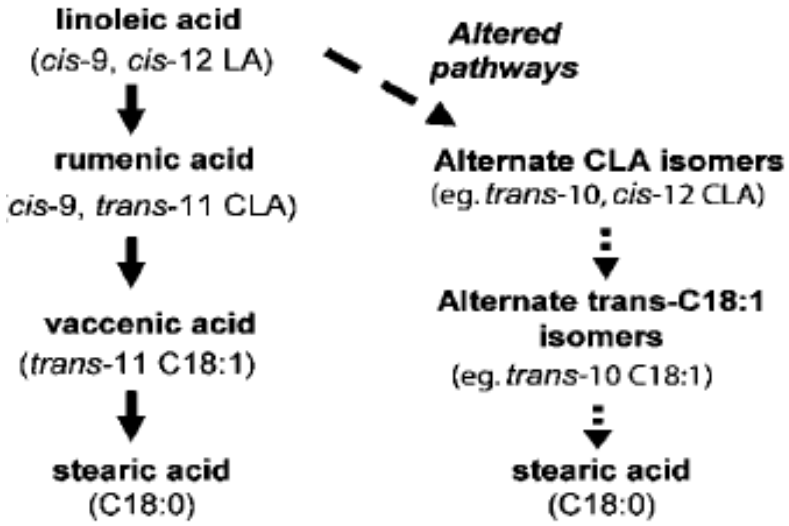

Figure 2.2: Classical pathways of rumen biohydrogenation of linoleic acid under normal rumen conditions (solid lines) and alternative pathway during milk fat depression (dotted line). (Adapted from Harvatine et al., 2009)

Chapter 3

MATERIALS AND METHODS

Animals and Experimental Design

All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. A total of 156 milk samples were collected over two separate days. In the first collection 91 multiparous Holstein cows from the Pennsylvania State University dairy farm on April 19, 2016. The second collection used 65 cows from the same population on October 17, 2016. The cows were held in free stalls with sand bedding and fed a total mixed ration, described in Table 3.1, once daily with free access. The cows were milked twice at 0500 and 1700 hours on the day of collection and milk yield was determined using an integrated milk meter. Distributions expressing the days in milk and lactation periods of all samples are shown in Figure 3.1. The statistical parameters of the mean, standard error, 10^{th} percentile, and 90^{th} percentile for these distributions are summarized in Table 4.1. Milk samples were composited within cow based on milk yield from each milking from the same day. Milk fatty acid profile was analyzed by gas chromatography according to procedures from Rico and Harvatine (2013). Protein, lactose, and milk fat concentration were determined by mid infrared spectroscopy completed by Lancaster DHIA. The mid-infrared spectroscopy also predicted the concentration of groups of fatty acids.

Table 3.1: Ingredients of the total mixed ration (TMR) during the experiment.

Ingredient	Grams per 100g of DM
Roasted beans	7.02
Vitamin and Mineral Mix[1]	2.07
Grass hay/straw	1.65
Canola meal	6.61
Cookie meal	6.61
Ground corn	13.22
Whole cotton seed	3.31
Sorghum/sudan	2.48
Corn silage	39.67
Alfalfa silage	13.22
Sugar (no glycerine)	4.13

[1]Contained (as fed basis): 29.9% dried corn distillers grains with solubles; 37.03% calcium carbonate; 4.2% magnesium oxide; 24.5% salt; 2.42% monocalcium phosphate; 0.47% zinc sulfate; 0.33% magnesium sulfate; 0.26% copper sulfate; 0.20% mineral oil; 0.13% ferrous sulfate; 0.04% selenium selenite; 0.02% vitamin AD_3 mix (retinyl acetate 1,000,000 IU/g, cholecalciferol 200,000 IU/g); 0.01% calcium iodate; 0.37% vitamin E mix (dl-α tocopheryl acetate 500 IU/g), [composition (DM basis): 7.9% CP; 7.1% NDF; 3.65% ADF; 15% Ca; 0.75% P; 2.6% Mg; 0.33% K; 0.52% S; 652 mg/kg of Cu; 1,718 mg/kg of Zn; 770 mg/kg of Fe; 12.75 mg/kg of Se; 195,374 IU/kg of vitamin A (retinyl acetate); 62,156 IU/kg of vitamin D_3 (cholecalciferol); and 1,872 IU/kg of vitamin E (dl-α tocopheryl acetate)].

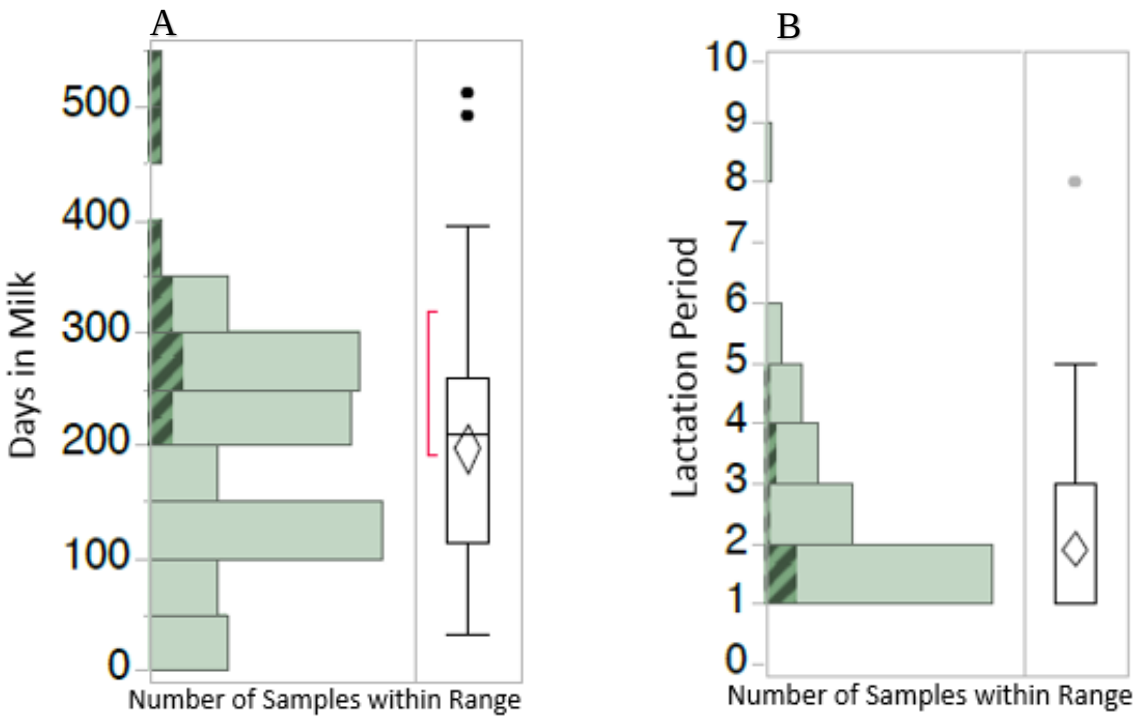

Figure 3.1 Distribution of days in milk and lactation period of cows in the same herd.

Cows were housed in the under the same conditions and fed the same diet once a day. Difference in color is not significant.

Rumination Collars

Cows were fitted with accelerometer based rumination collars, MooMonitor+, manufactured by Dairymaster Inc. Collars were fitted to Dairymaster specifications with the accelerometer on the left side of the neck and tightened firmly. Feeding behavior was monitored continuously and summarized as percentages of 15-minute intervals. Data was collected via a wireless modem at the barn and processed by Dairymaster.

Fatty Acid Analysis

Fat was extracted from fat cakes using a hexane:isopropanol solution according to Hara and Radin (1978), and the fatty acid methyl esters were prepared by base-catalyzed transmethylation according to Chouinard et al. (1999). Fatty acid methyl esters were quantified by gas chromatography with an Agilent 6890A gas chromatograph (Agilent Technologies, Palo A lot, CA) equipped with a fused-silica capillary column (SP-2560; 100m x 0.25mm i.d. with 0.2µm film thickness; Supelco Inc., Bellefonte, PA) and a flame-ionization detector with a hydrogen gas carrier. Oven temperature was set at 80˚C and increased to 190˚C at a rate of 2˚C/minute, and held for 15 minutes. Inlet and detector temperatures were 250˚C with a 100:1 split ration. Gas constant flows were held at hydrogen carrier 1 mL/min and detector hydrogen 25 mL/min, airflow 400 mL/min, and nitrogen plus carrier at 40 mL/min.

Fatty acid peaks were identified with gas chromatographic analysis using pure methyl esters standards (GLC 780, 461, and 566, NuChek Prep Inc. Elysian, MN and GLC110 Matreya, State College, PA). An equal weight reference standard (GLC 461; NuChek Prep Inc.) was used to determine correction factors for individual fatty acids. Milk fatty acid yield was calculated as

described by Glasser et. al (2007). The coefficient for correction for triglycerides was calculated for each sample instead of using the suggested fixed factor of 0.944. The mean proportion of fatty acids in total milk fat was calculated for each sample by multiplying 0.9885 as a correction for other milk fat fractions, based on the difference between the mean proportion of fatty acids in milk triglyceride and in total milk fat (Glasser et. al 2007).

Statistical Analysis

Data were analyzed using the JMP 12 (SAS Institute, Cary, NC). Distributions were calculated to find the mean, standard error, 10^{th} percentile, 25^{th} percentile, 75^{th} percentile, and 90^{th} percentile for the following milk compositions: percent total fat, percent true protein, percent *trans*-10 18:1, percent *trans*-9 18:1, percent fatty acids shorter than 16 carbons, percent fatty acids 16 carbons in length, and percent fatty acids longer than 16 carbons. Linear and quadratic regressions were run to analyze specific fatty acids against total fat percentage. The specific fatty acids of interest include *trans*-10 18:1, *trans*-11 18:1, *cis*-9, *trans*-11 CLA, fatty acids less than 16 carbons in length, fatty acids 16 carbons in length, and fatty acids greater than 16 carbons in length. Linear regressions were run for milk yield against percent fat, percent true protein, percent lactose, percent fatty acids longer shorter than 16 carbons, percent fatty acids 16 carbons in length, percent fatty acids longer than 16 carbons, percent *cis*-9, *trans*-11 CLA, *trans*-10 18:1, and *trans*-11 18:1 to assess the trends of these components with milk yield.

Chapter 4

RESULTS

Milk Composition

Milk composition was appropriately analyzed by gas chromatography and mid infrared spectroscopy for all 156 samples. Distributions of true protein and fat concentration are shown in Figure 4.1, distributions of *trans*-11 18:1 and *trans*-10 18:1 are provided in Figure 4.2, and distributions of fatty acids from de novo synthesis (less than 16 carbons), performed fatty acids (greater than 16 carbons), and fatty acids 16 carbons in length are shown in Figure 4.3. All overall appeared to be normal distributions and no major skews are present, however, total fat percentage (Figure 4.1B) and *trans*-10 18:1 concentration (Figure 4.2A) skewed slightly with more outliers than the rest of the components analyzed. Statistical parameters of the mean, standard error, and 10^{th} and 90^{th} percentile for each distribution are summarized in Table 4.1.

The fatty acids *cis*-9, *trans*-11 CLA, and *trans*-12 18:1 were analyzed against total percent fat by linear fit in Figure 4.4. The fatty acids iso C17:0, *trans*-10 18:1, fatty acids less than 16 carbons (fatty acids from de novo synthesis), fatty acids 16 carbons long, and fatty acids more than 16 carbons (preformed fatty acids) were analyzed by linear fit and quadratic fit against total fat percentage, shown in Figure 4.5. The percent of *trans*-11 18:1 was compared against total fat by quadratic fit in Figure 4.6.

Regression values and *P*-values for the linear regressions and quadratic regressions are shown in Table 4.2 and Table 4.3, respectively. Linear regressions showed significant relationships between the increase of *trans*-10 18:1 ($R^2 = 0.09$, $P < 0.001$) and *trans*-12 CLA (R^2

= 0.06, P < 0.001) and decreases in milk fat. A noteworthy increase in iso C17:0 (R^2 = 0.05, P < 0.01) and *cis*-9, *trans*-11 CLA (R^2 = 0.06, P < 0.01) was correlated to a decrease in milk fat. The quadratic regressions show a significant relationship in the decrease of fatty acids longer than 16 carbons (R^2 = 0.08, P < 0.01) and increases in milk fat. There was no major linear relationship between fatty acids from de novo synthesis (R^2 = 0.01, P = 0.34). However, a quadratic correlation of de novo fatty acids increases with milk yield (R^2 = 0.04, P < 0.05).

Milk Yield

The distribution of the milk yield for all samples is shown in Figure 4.7. Statistical parameters of the mean, standard error, and 10^{th} and 90^{th} percentile for the distribution of milk yield is also summarized in Table 4.1. The linear regressions for milk yield against percent fat, percent lactose, and percent true protein are shown in Figure 4.8. Linear regressions of milk yield in relation to percent fatty acids from de novo synthesis, 16 carbons in length, and preformed are provided in Figure 4.9. The linear regressions of *cis*-9, *trans*-11 18:12, *trans*-10 18:1, *trans*-12 CLA are shown in Figure 4.10.

Summarization of the linear regression statistics for milk yield are provided in Table 4.4. Significant negative correlations were measured in true protein concentration (R^2 = 0.33, P < 0.001) and preformed fatty acids (R^2 = 0.05, P < 0.01). Fatty acids from de novo synthesis had a significant positive correlation to milk yield (P < 0.01). No significant change in milk yield was measured for lactose (R^2 = 0.01, P = 0.18), *trans*-10 18:1 (R^2 = 0.003, P = 0.50), *trans*-11 18:1 (R^2 = 0.0005, P = 0.77), and *cis*-9, *trans*-11 CLA (R^2 = 0.002, P = 0.26).

Gas Chromatography vs. Mid Infrared Spectroscopy

Gas chromatography is a highly accurate and precise method for fatty acid analysis, but is time consuming and expensive. The Pearson correlation coefficients (Table 4.5) provides an analysis of the strength of the relationship between fatty acid profiles obtained through mid infrared spectroscopy (MIR) and gas chromatography. MIR and GC were positively related for specific fatty acids: C16:0 (r = 0.284), C18:0 (r = 0.31), and 18:1 (MIR) to cis-9 18:1 (GC) (r = 0.31). However, some unexpected results occurred. A positive correlation exists between the *trans* fatty acids (MIR) and C18:0 (GC) (r = 0.385). Also *trans*-10 18:1 and *trans*-11 18:1 had opposing correlations to the MIR *trans* fatty acid profile: (r = -0.128) and (r = 0.164), respectively.

Rumination Data

An issue had occurred with the algorithm processing with Dairymaster. The issue was fixed at the Pennsylvania State Dairy Farm, but there were delays with data processing. Therefore, no results on rumination time will be in this current study.

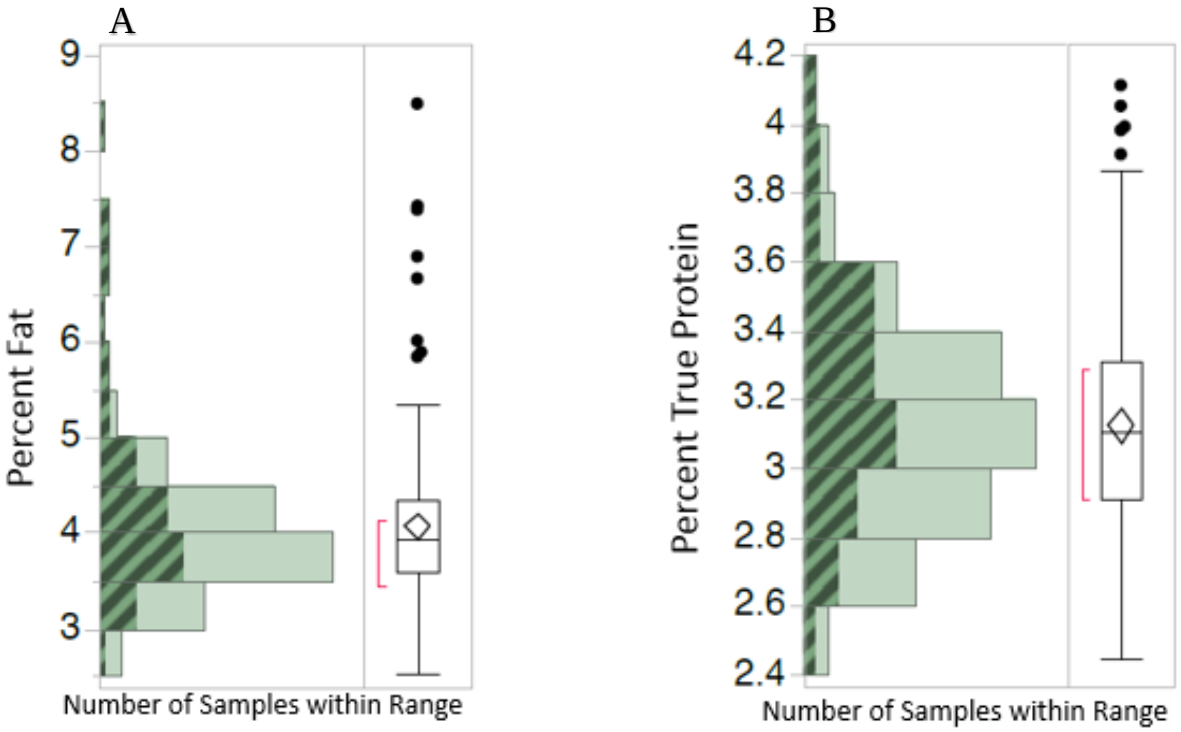

Figure 4.1: Distributions of fat and true protein concentration in milk across cows within the same herd.

Milk was collected twice from cows milked twice per day and housed in the same conditions and fed the same diet once per day. Milk was analyzed for fat and true protein by mid-infrared spectroscopy analysis. The light green area of the distribution contains the milk samples (n=91) taken on April 19, 2016. The dashed green distribution contains milk samples (n=65) taken on October 17, 2016.

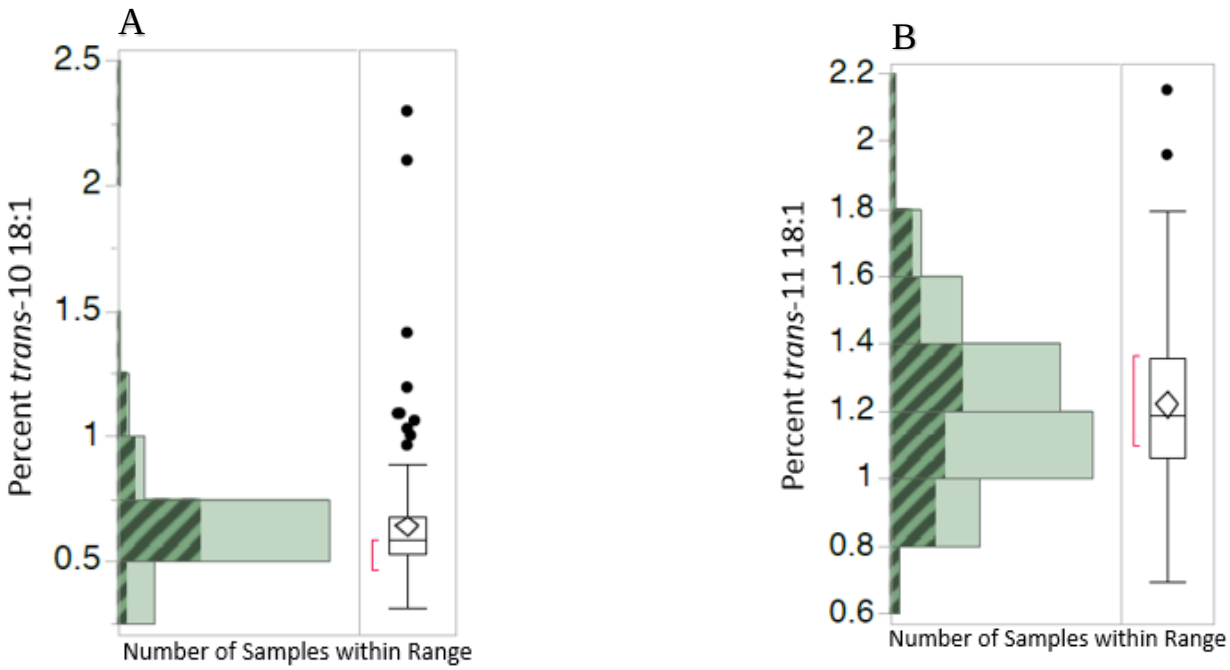

Figure 4.2: Distributions of *trans*-11 18:1 and *trans*-10 18:1 concentration in milk fat of cows within the same herd.

Milk was collected twice from cows milked twice per day and housed in the same conditions and fed the same diet once per day. Milk was analyzed for fatty acid profile by gas chromatography. The light green area of the distribution contains the milk samples (n=91) taken on April 19, 2016. The dashed green distribution contains milk samples (n=65) taken on October 17, 2016.

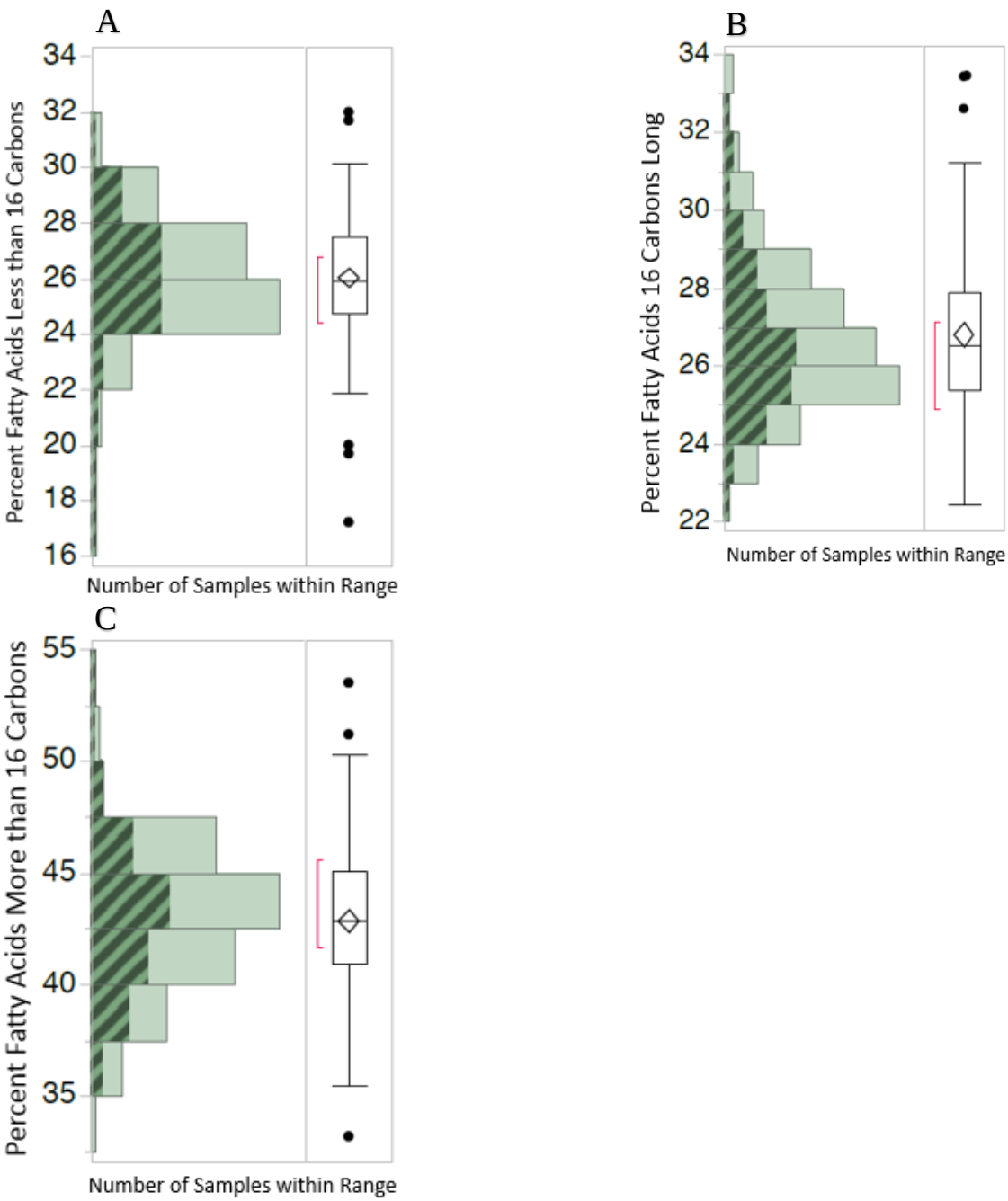

Figure 4.3: Distributions of fatty acids from de novo synthesis (less than 16 carbons in length), both de novo and preformed (fatty acids 16 carbons in length), and performed fatty acids (greater than 16 carbons in length) in milk fat of cows within the same herd

Milk was analyzed for fatty acid profile by gas chromatography. The light green area of the distribution contains the milk samples (n=91) taken on April 19, 2016. The dashed green distribution contains milk samples (n=65) taken on October 17, 2016. In both samples, milk was collected twice from cows milked twice per day and housed in the same conditions and fed the same diet once per day.

Table 4.1: Summary of milk components cows within the same herd[1].

			Percentiles			
Component	Mean[2]	Std. Error	10th	25th	75th	90th
Fat	4.08	0.07	3.36	3.59	4.35	4.91
True Protein	3.13	0.03	2.74	2.91	3.31	3.48
trans-10 18:1	0.64	0.02	0.49	0.53	0.68	0.80
trans-11 18:1	1.22	0.02	0.93	1.06	1.36	1.53
FA <16	26.05	0.17	21.72	24.76	27.49	28.79
C16	26.81	0.16	24.66	25.38	27.91	29.37
FA >16	42.84	0.26	38.21	40.97	45.04	46.48
Milk Yield	79.22	1.86	52.96	66.14	90.82	109.34
Days in Milk	197.02	10.70	114	210	259	304.9
Lactation Period	1.92	1.31	1	1	3	4

[1]Milk was collected twice from cows milked twice per day and housed in the same conditions and fed the same diet once per day. Milk composition was analyzed by mid-infrared spectroscopy analysis and fatty acid profile by gas chromatography.

[2]Mean values of fat and true protein are reported as percent of milk composition. Fatty acids are reported as percent of fat composition. Milk yield is reported in pounds. Both sample collections were analyzed together. Cows were fed the same diet and housed under the same conditions. Mean values of fat and true protein are reported as percent of milk composition. Fatty acids are reported as percent of fat composition.

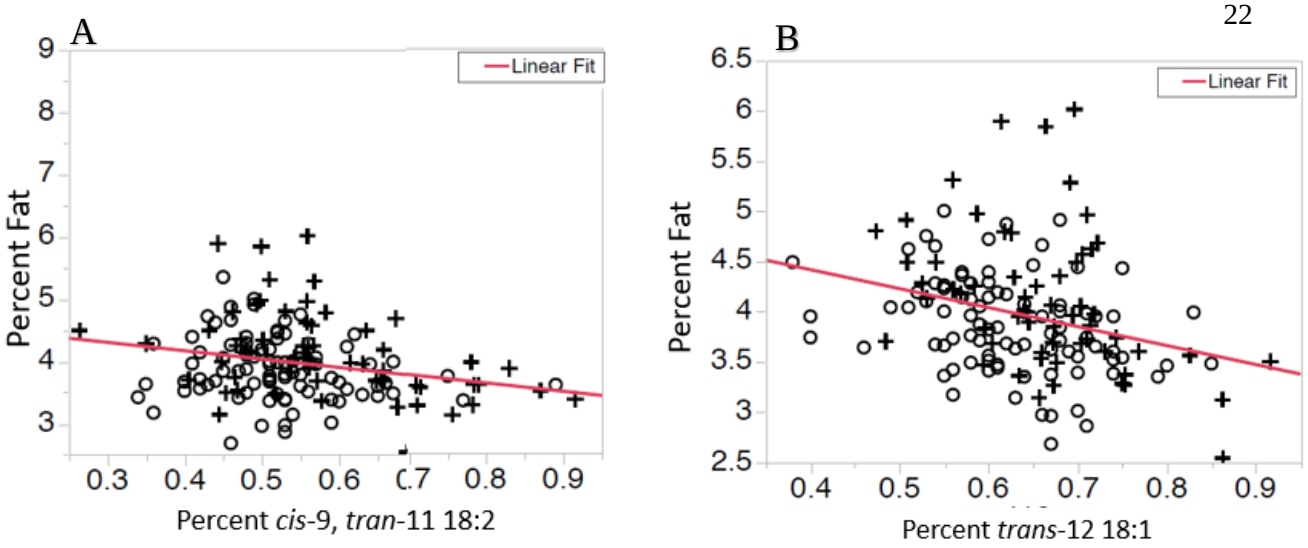

Figure 4.4: Relationship between milk fat percent and *cis*-9, *trans*-11 conjugated linoleic acid (CLA; 18:2) and *trans*-12 18:1 in cows within the same herd under the same management.

Open circles and plus symbols are used to signify data collected from the first sampling (n = 91) and second sampling (n = 65), respectively. Both samplings used cows fed the same diet, housed under the same conditions, and were milked twice per day. Milk was analyzed for fatty acid profile by gas chromatography.

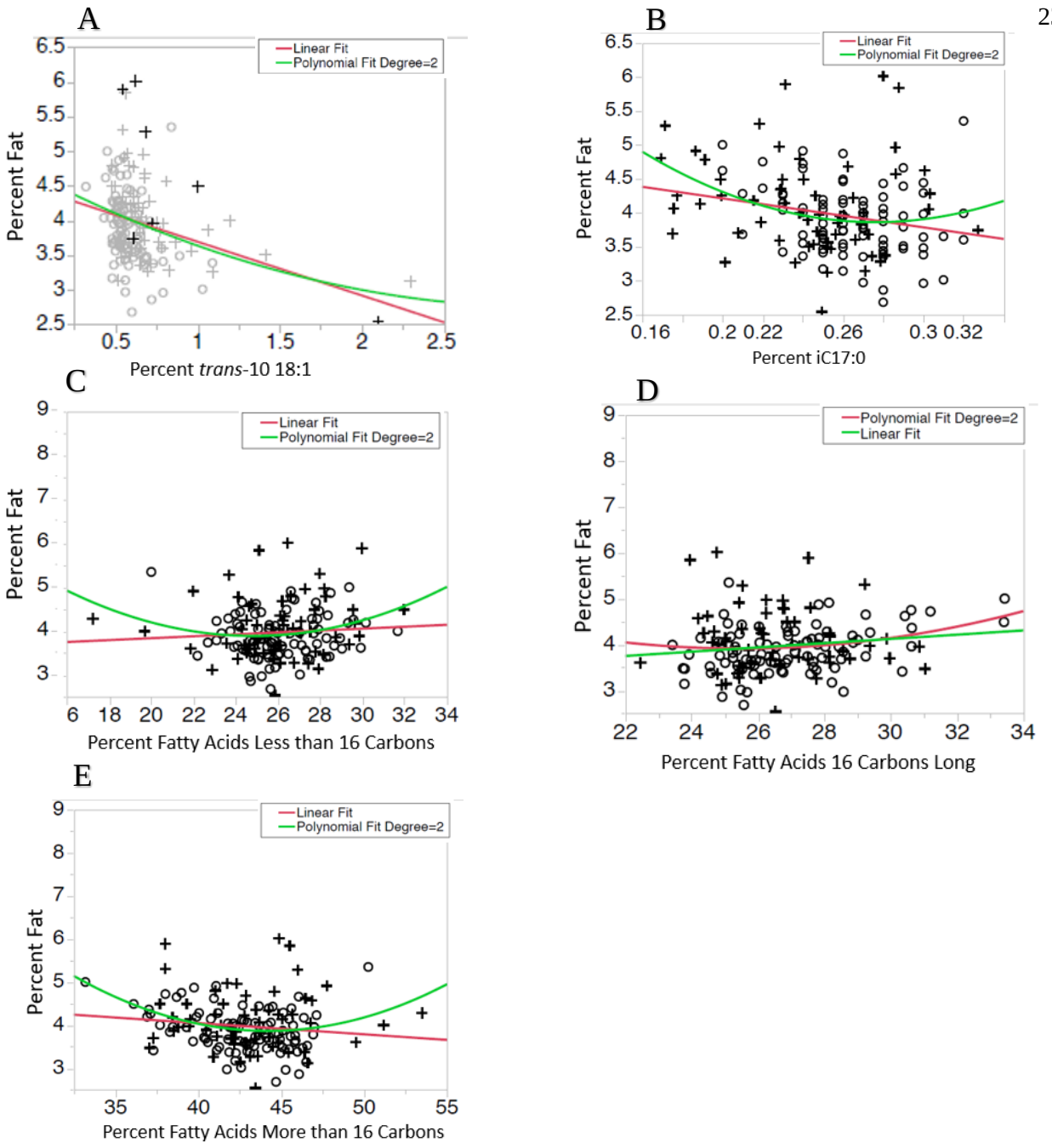

Figure 4.5: Relationship between milk fat percent and *trans*-10 18:1, iso C17:0, and sum of fatty acids less than 16 carbons, 16 carbon, and greater than 16 carbon in cows within the same herd under the same management.

Open circles and plus symbols are used to signify data collected from the first sampling (n = 91) and second sampling (n = 65), respectively. Both samplings used cows fed the same diet, housed under the same conditions, and were milked twice per day. Milk was analyzed for fatty acid profile by gas chromatography. Fatty acids less than 16 carbons originate from de novo synthesis, fatty acids greater than 16 carbon are taken up as preformed FA, and 16 carbon FA originate from both sources.

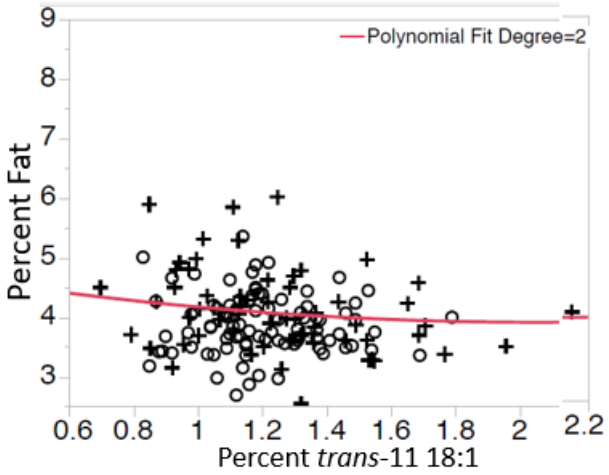

Figure 4.6: Relationship between milk fat percent and *trans*-11 18:1 in cows within the same herd under the same management.

Open circles and plus symbols are used to signify data collected from the first sampling (n = 91) and second sampling (n = 65), respectively. Both samplings used cows fed the same diet, housed under the same conditions, and were milked twice per day. Milk was analyzed for fatty acid profile by gas chromatography.

Table 4.2: Summary of R square and P values for linear regressions of fatty acids by fat percentage.

Fatty Acid	Linear fit (Fat =)	R Square	R Square Adjusted	*P*-Value
iso C17:0	5.0636-4.2681(i17:0)	0.053632	0.047238	0.0044
trans-10 18:1	4.4704-0.7755(t10)	0.093881	0.0878	0.0001
trans-11 18:1	4.4675-0.4074(t11)	0.02564	0.019101	0.0495
trans-12 18:1	5.1708-1.8896(t12)	0.084983	0.078759	0.0003
cis-9, *trans*-11 CLA	4.7893-0.0405(18:1c9)	0.062419	0.056127	0.0020
FA<16C	3.4033+0.0218(<16)	0.006136	-0.00053	0.3390
C16	2.7288+0.04639(C16)	0.0222	0.01563	0.0679
FA>16	5.0852-0.0260(>16)	0.018381	0.011793	0.0969

Milk samples (n=156) were collected on April 19, 2016 and October 17, 2016. All 156 samples were analyzed together. Cows were housed under the same conditions and fed the same diet.

Table 4.3: Summary of R square and P values for quadratic regressions of fatty acids by fat percentage.

Fatty Acid	Quadratic fit (Fat =)	R Square	R Square Adjusted	*P*-Value
iC17:0	4.7087-3.1937(i17:0)+ 76.9081(i17:0-0.2554)^2	0.089392	0.077003	0.0175
trans-10 18:1	4.5877 -0.9745(t10)+ 0.1964(t10-0.6441)^2	0.09642	0.08421	0.5200
trans-11 18:1	4.5394-0.3870(t11)+ 0.2326(t11-1.2205)^2	0.009735	-0.00321	0.7623
FA<16C	3.0649+0.0324(<16)+ 0.0133(<16-26.046)^2	0.04454	0.031628	0.0159
C16	3.1225+0.0302(C16) + 0.0111(C16-26.808)^2	0.032804	0.019734	0.2046
FA>16	4.9744-0.0255(>16)+ 0.0093(>16-42.8398)^2	0.078338	0.065883	0.0023

Milk samples (n=156) were collected on April 19, 2016 and October 17, 2016. All 156 samples were analyzed together. Cows were housed under the same conditions and fed the same diet.

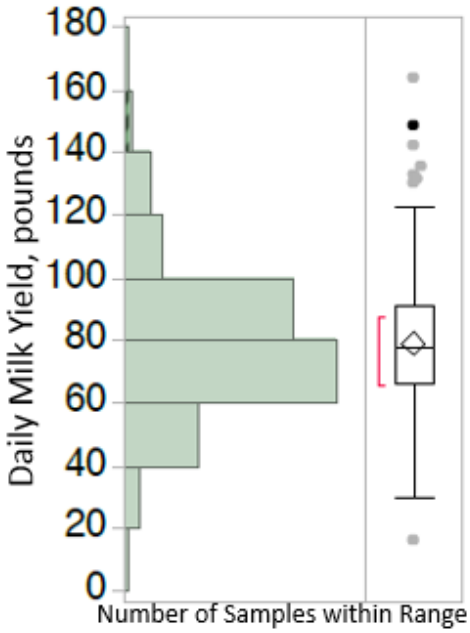

Figure 4.7 Distribution of milk yield, in pounds, of cows within in the same herd.

Milk weights were measured by an integrated milk meter at time of collection. In both samples, milk was collected twice from cows milked twice per day and housed in the same conditions and fed the same diet once per day. Color within the distribution is not significant.

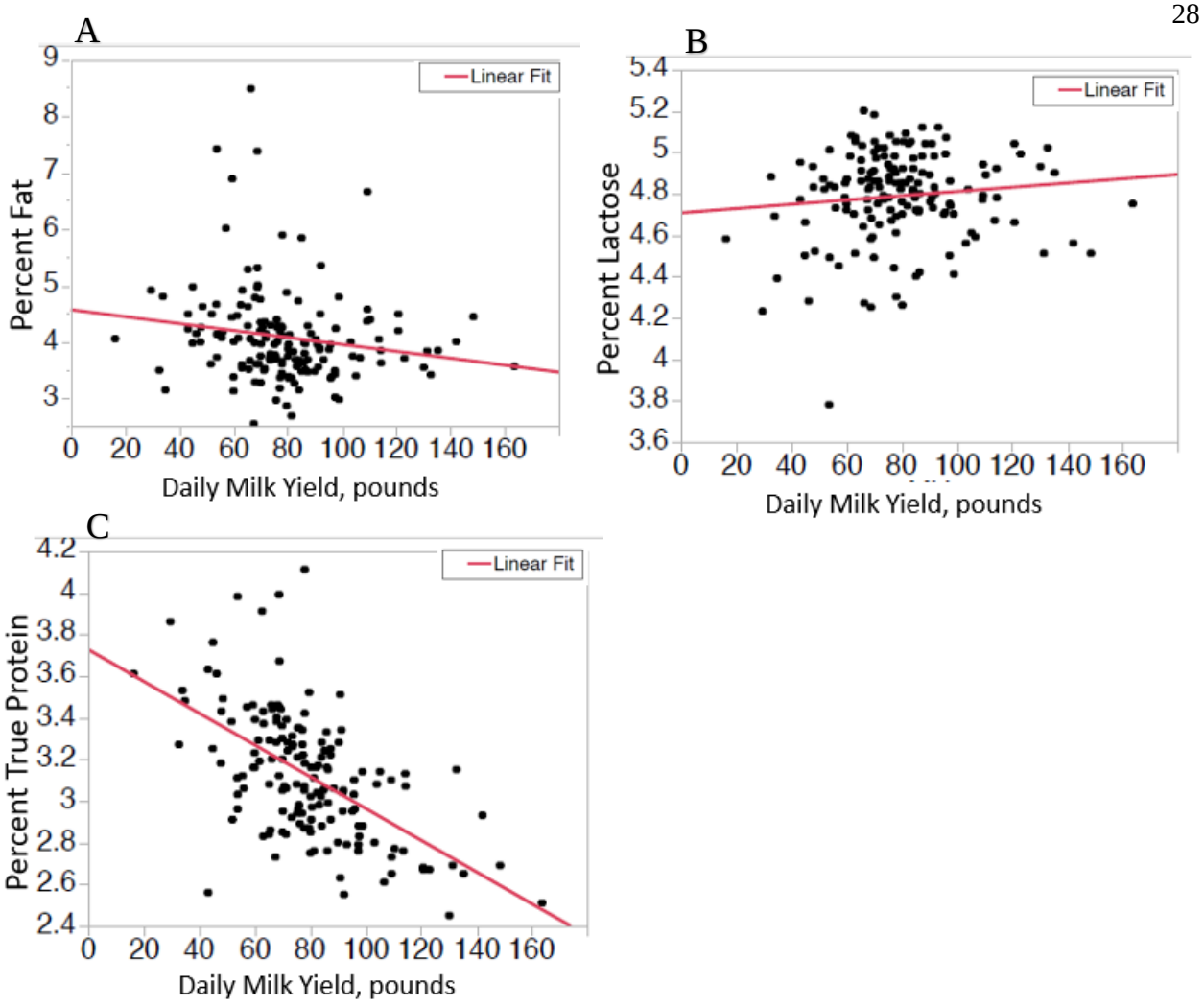

Figure 4.8: Relationship between daily milk production and concentration of fat, lactose, and true protein in the milk analyzed by linear regressions.

Cows were fed the same diet, housed under the same conditions, and were milked twice per day. Milk was analyzed by mid-infrared spectroscopy.

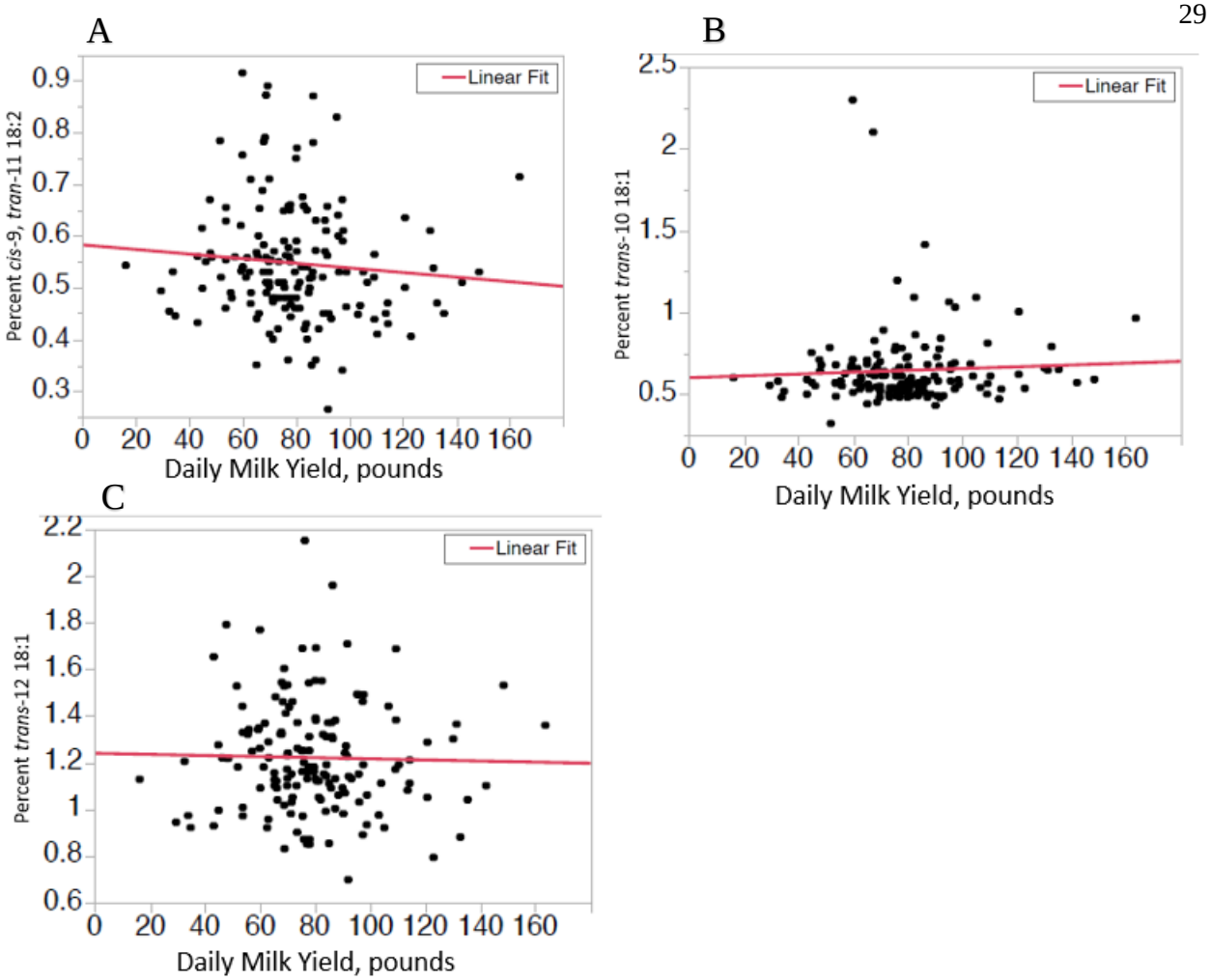

Figure 4.9: Relationship between daily milk production and milk fat *cis*-9, *trans*-10 18:2, *trans*-11 18:1, and *trans*-12 18:1 analyzed by linear regressions.

Both samplings used cows fed the same diet, housed under the same conditions, and were milked twice per day. Fatty acids were analyzed by gas chromatography.

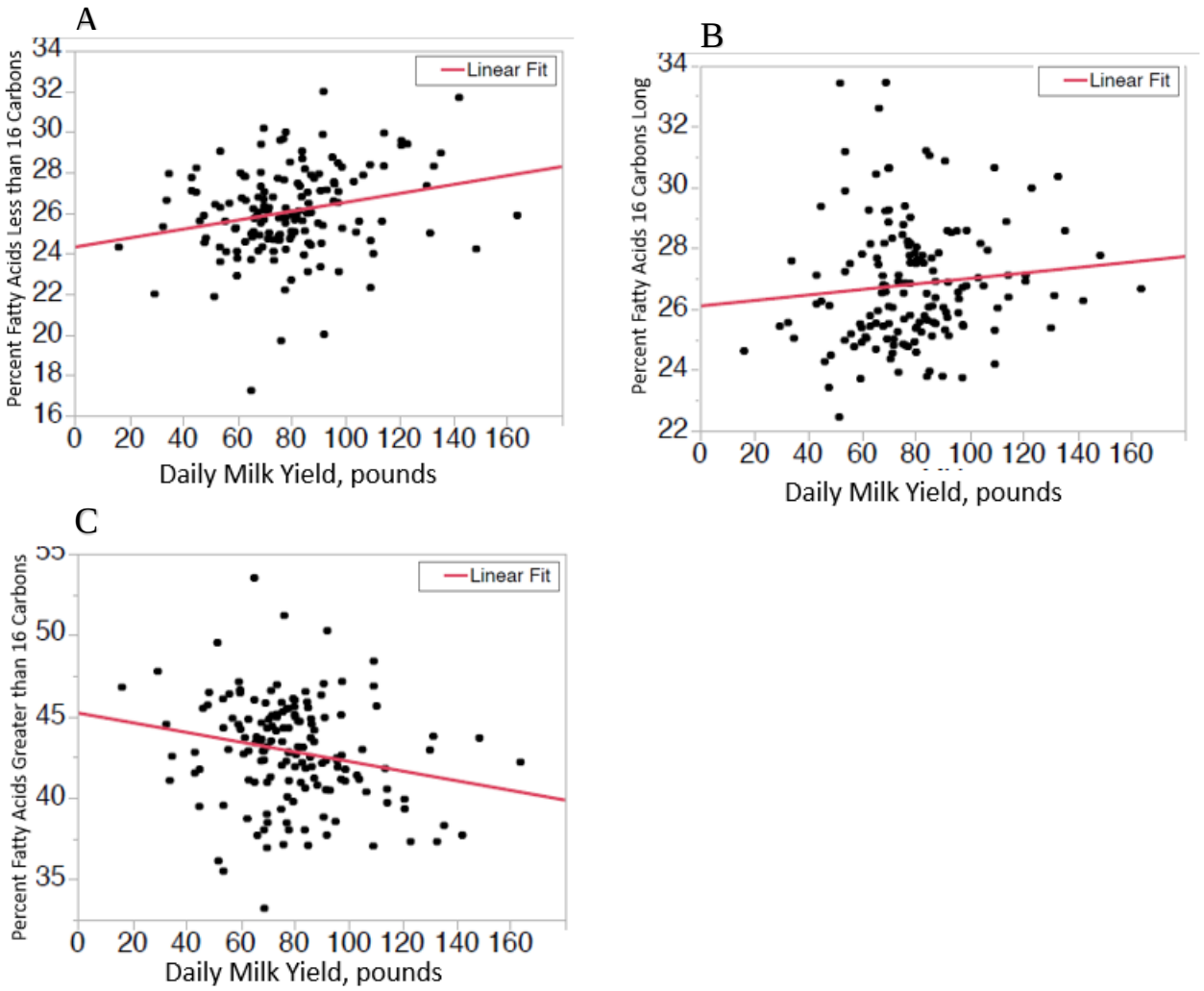

Figure 4.10: Effect of daily milk production on percent fatty acids from de novo synthesis (less than 16 carbons in length), fatty acids synthesized or preformed (fatty acids 16 carbons in length), and performed fatty acids (greater than 16 carbons in length), analyzed by linear regression.

Both samplings used cows fed the same diet, housed under the same conditions, and were milked twice per day. Fatty acids were analyzed by gas chromatography.

Table 4.4: Summary of R square and P values for linear regressions of milk components by milk yield.

Milk Component (y)	Linear fit (y =)	R Square	R Square Adjusted	*P*-Value
Fat	4.5635-0.0062(MY)	0.028117	0.021765	0.0370
True Protein	3.7251-0.0076(MY)	0.330888	0.326515	0.0001
Lactose	4.7058+0.0010(MY)	0.011947	0.005489	0.1758
cis-9, *trans*-11 CLA	0.5826-0.0004(MY)	0.008363	0.001881	0.2578
trans-10 18:1	0.5997+0.0006(MY)	0.003007	-0.00351	0.4980
trans-11 18:1	1.2387-0.0002(MY)	0.000539	-0.00599	0.7743
FA<16C	24.3010+0.0220(MY)	0.055765	0.049594	0.0031
C16	26.0964+0.0090(MY)	0.010902	0.004437	0.1960
FA>16C	45.1940-0.0297(MY)	0.045927	0.039691	0.0074

Milk samples (n=156) were collected on April 19, 2016 and October 17, 2016. All 156 samples were analyzed together. Cows were housed under the same conditions and fed the same diet.

Table 4.5: Pearson correlation coefficient of fatty acid quantification by mid infrared spectroscopy (MIR) and gas chromatography (GC) analysis.

	C16_0	C18_0	C18_1	LCFA	MCFA	MonoUnsaturatedFA	PolyUnsaturatedFA	SaturatedFA	SCFA	Trans FA	TotalUnsaturatedFA
C16_0	1										
C18_0	0.677	1									
C18_1	0.59	0.77	1								
LCFA	0.68	0.896	0.9543	1							
MCFA	0.793	0.622	0.5847	0.691	1						
MonoUnsaturatedFA	0.638	0.785	0.982	0.959	0.636	1					
PolyUnsaturatedFA	0.296	0.507	0.5695	0.524	0.125	0.586	1				
SaturatedFA	0.968	0.735	0.6483	0.743	0.815	0.696	0.324	1			
SCFA	0.877	0.684	0.6186	0.697	0.75	0.68	0.437	0.949	1		
Trans FA	0.078	0.267	0.2224	0.2	-0.173	0.176	0.497	0.012	-0.014	1	
TotalUnsaturatedFA	0.646	0.807	0.9325	0.963	0.699	0.948	0.425	0.713	0.668	0.032	1
16 0	0.284	-0.003	-0.095	-0.101	0.108	-0.066	0.024	0.24	0.214	0.059	-0.169
18 0	0.117	0.31	0.0448	0.161	0.038	0.028	0.112	0.045	-0.037	0.385	0.057
t10	-0.351	-0.28	-0.059	-0.175	-0.254	-0.108	-0.184	-0.347	-0.35	-0.128	-0.095
t11	-0.271	0.101	0.047	0.057	-0.136	0.011	0.051	-0.241	-0.203	0.164	0.041
18 1c9	-0.333	0.025	0.28	0.212	-0.098	0.259	0.021	-0.312	-0.314	-0.041	0.308
LA	-0.147	-0.101	0.1706	0.107	-0.026	0.157	-0.067	-0.121	-0.11	-0.245	0.255
ALA	-0.269	-0.146	0.0007	-0.035	-0.193	-0.014	0.076	-0.249	-0.208	-0.049	0.049
9,11 cla	-0.401	-0.207	0.0515	-0.053	-0.177	0.033	-0.005	-0.342	-0.271	-0.11	0.037
<16	0.193	-0.202	-0.291	-0.273	0.055	-0.258	-0.046	0.243	0.329	-0.185	-0.3
16	0.29	0.014	-0.05	-0.062	0.133	-0.02	0.017	0.256	0.229	0.006	-0.114
>16	-0.282	0.13	0.2079	0.208	-0.116	0.171	0.026	-0.299	-0.34	0.143	0.245

MIR data on axis is labeled in red and GC data is labeled in black. MIR data was completed by Lancaster DHIA and the gas chromatography was completed in lab with an Agilent 6890A gas chromatograph. Data were taken using the same samples taken from Holstein cows fed and housed under the same conditions. Milk samples (n=156) were taken on April 17, 2016 and October 17, 2016.

Chapter 5

DISCUSSION

Milk Composition

The percent fat composition, 4.08% ± 0.068%, is consist with other publications. Maekawa et al. (2002) reported milk fat composition for their control group as 3.78% ± 0.10%. de Veth et al. (2004) reported total milk fat as 3.55% ± 0.12%. Byskov et al. (2015) obtained 3.9% ± 0.06%. The percent protein content (3.13% ± 0.025%) is also consistent with Maekawa et al. (2002), de Veth et al. (2004), and Byskov et al. (2015) who reported 3.31% ± 0.03%, 2.97% ± 0.18%, and 3.2% ± 0.03%, respectively. *Trans*-10 18:1 percent levels were significantly higher than reported by Maekawa et al. (2002). The 10^{th} percentile for *trans*-10 18:1 was 0.49% ± 0.02% and the literature showed 47% ± 0.03%. *Trans*-11 18:1 had the opposite trend to this study with the 90^{th} percentile as 1.53% ± 0.02% to an average of 1.56% ± 0.07%. The percent composition of de novo fatty acids (26.81% ± 0.16%) to performed fatty acids (42.84% ± 0.26%) is representative of published literature. Fats in milk, under normal conditions, arise equally from performed fatty acids and de novo synthesis (Harvatine et al., 2009). Fatty acids 16 carbons long originate from preformed fatty acids and synthesized de novo (Bauman and Griinari, 2001), which would show relatively equal production from both pathways.

The regressions of *trans* isomers and fatty acids from the data both support and refute the current literature. The linear regression of *trans*-10 18:1 and percent fat showed a significant relationship in milk fat depression ($P < 0.001$). However, results from de Veth et al. (2004)

showed that increases in *trans*-10, *cis*-12 CLA, the precursor for *trans*-10 18:1 through the alternative biohydrogenation pathway, causes an exponential reduction in percent milk fat. The quadratic regression for *trans*-10 was not significant ($P = 0.52$). Additional proof of the classical pathway of biohydrogenation can be exhibited through the regressions of *trans*-11 18:1. The quadratic regression for *trans*-11 is not significant ($P = 0.76$). However, the linear regression for *trans*-11 in milk fat depression has some significance ($P < 0.05$). Bauman and Griinari (2001) do not support these results. Abomasal infusions of *cis*-9, *trans*-11 CLA did not yield any remarkable changes in milk composition, specifically fat.

The regressions of de novo synthesis fatty acids (<C16) and preformed fatty acids (>C16) are unexpected based on previous publications. *Trans*-10, *cis*-12 CLA has been shown to inhibit Δ^9-desaturase gene expression and activity (Baumguard et al., 2000b), which decreases de novo fatty acid synthesis. Fatty acids from de novo synthesis linearly increased with percent fat ($P < 0.01$) and preformed fatty acids quadratically decreased with percent fat ($P < 0.01$). de Veth et al. (2004) reported a greater decline for the incorporation of performed fatty acid than de novo synthesis. This unexpected change is due to low levels of *trans*-10, *cis*-12 CLA. At higher levels *trans*-10, *cis*-12 CLA has a greater reduction on de novo synthesis (Peterson et al., 2002).

The Pearson correlation coefficient for the mid infrared spectroscopy and gas chromatography data helped to assess the relationship between the two measurements. It is difficult to achieve high correlations provided that the MIR data was sampling more fatty acids in each sample. This elevated value, compared to the smaller GC data for a single fatty acid, decreases the strength of the relationship between the two values. There is a significant relationship between the short chain fatty acids of the MIR data and C16:0, fatty acids from de novo synthesis (<C16), and fatty acids 16 carbons in length. However, it is clear that some trends

are not a direct measure of fatty acids. For example, the *trans* fatty acids for MIR and C18:0 has a strong correlation (r = 0.385). However, this is not indicating that the methods of analysis are incorrect. Biohydrogenation of linoleic acid generates stearic acid (C18:0) (Harvatine et al., 2009). However, it is during this process that the *trans* isomers are escape the rumen. Therefore, the *trans* fatty acids are related to the production of C18:0.

Milk Yield

The effect of milk fat depression on milk components and milk yield has evidence both supporting and negating effects. Chouinard et al., (1999) and de Veth et al. (2004) infused *trans*-10, *cis*-12 CLA to induce milk fat depression and saw no changes in milk yield or percent protein. Harvatine and Allen (2006) and Rico et al. (2014) produced similar results when milk fat depression was induced by saturated and unsaturated fatty acids and palmitic acid supplementation, respectively. Conversely, some management programs have increased milk yield and percent protein during milk fat depression. These results are attributed to the repartitioning of energy, since fatty acid synthesis requires 50% of the energy of milk synthesis (Harvatine et al., 2009).

The linear regressions from the sample agree with the notion that milk fat depression does not affect milk yield. Percent fat composition of *cis*-9, *trans*-11 CLA ($P = 0.25$), *trans*-10 18:1 ($P = 0.50$), and *trans*-11 18:1 ($P = 0.77$) are significant in relation to milk yield. However, performed fatty acids ($P < 0.01$) has a significant negative correlation with milk yield. Also, fatty acids from de novo synthesis ($P < 0.01$) has a significant positive correlation with milk yield. It could be hypothesized that increased de novo synthesis means that the rumen is appropriately

buffered and fermenting. Byskov et al. (2015) found that time ruminating was positive related to milk fat percent with increasing ruminating time minutes per kilogram of dry matter intake ($P < 0.05$).

Rumination Collars

Without the data from Dairymaster, there can be no definitive discussion about the effects rumination has on milk fat depression during this study. However, previous publications can help develop an explanation for the obtained results. If individual cows could be identified by their rumination time then there may be insight to how some *trans* isomers were far from the mean. A relative decrease in detected rumination time may be able to explain regressions of the de novo and preformed fatty acids. If the findings from Peterson et al. (2002) explain the reduced performed fatty acid absorption due to lower percentages of *trans*-10, *cis*-12 CLA. Then it could be expected that those cows would have moderately reduced rumination time because the pH of the rumen has not dropped enough to for optimal biohydrogenation.

Chapter 6

CONCLUSION

`The objective of accessing milk fat depression based on time spent ruminating was not accomplished. The experiment is ready to continue and compare rumination time to prevalence of *trans*-10, *cis*-12 CLA and milk fat depression as soon as the data is processed. However, the composition of milk fat for the samples was analyzed to assess the trends of *trans* isomers and milk fat depression. Milk yield was compared through linear regression to analyze the changes in production that may accompany milk fat depression. Further research can be conducted to better assess the time ruminating has on the biohydrogenation of unsaturated fats in the rumen.

Chapter 7

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ACADEMIC VITA

JAMES FURNESS MCNEW

EDUCATION

THE PENNSYLVANIA STATE UNIVERSITY **University Park, PA**
Bachelor of Science in Veterinary & Biomedical Science **2013 – Present**

- Admitted to the Schreyer Honors College in 2015.
- Scholarships and Awards: Kenton D. Rexford Honors Scholarship in the College of Agriculture (2016-2017), N. C. Harris Scholarship Fund (2016-2017), Paul R. and Ethel S. Guldin Agricultural Scholarship (2015-2016), Veterinary Founders Academic Scholarship (2015-2016), The Provost's Award (2013-2017).

EXPERIENCE

THE NEW BOLTON CENTER **Kennett Square, PA**
"Shadow" of Dr. Raymond Sweeney VMD DACVIM **Summer 2015 and 2016**

- Assisted in diagnostic tests for large animals, focused on internal medicine, neurology, ophthalmology.
- Observed ultrasounds, muscle biopsies, endoscopic procedures, enucleation, exploratory and dental surgery.
- Assisted doctors and students in treatments for horses, cows, alpacas, goats, swine, sheep.
- Communicated with doctors and students to better understand the workings of large animal hospital and the veterinarian profession.

KENT VETERINARY CENTER **Millington, MD**
"Shadow" of Dr. Judy Tubman VMD **Summer 2016**

- Handled horses for examination and medical procedures.
- Introduced to reproductive and sports medicine: artificial insemination, ultrasound, lameness exams, joint injections, shockwave therapy, freeze firing.
- Scored horses' body condition and topline to provided nutritional consultation to owners.
- Experienced horse dentistry and the principles of power floating teeth.
- Observed vaccinations and physical exams for horses, cats, dogs.

TRI-STATE BIRD RESCUE & RESEARCH, INC. **Newark, DE**
Wildlife and Rehabilitation Intern **Summer 2015**

- Handled and humanely restrained 15 different species of birds to check body condition, administer medication, change bandages, band for release.

- Administered oral and intravenous medication to osprey and Canada geese.
- Trained to wash oiled birds and implemented skills on silted laughing gull.
- Provided husbandry daily for species of passerines, water fowl, raptors, vultures.
- Worked with a team of staff, interns, and volunteers to care for 300 birds at the center.

PI LAMBDA PHI **State College, PA**

Vice President **Spring 2016**

- Organized and assisted in running monthly chapter meetings to explain current status and upcoming activities to our 90 member brotherhood.
- Enforced risk management policy and chapter constitution to maintain safety and productivity.
- Scheduled and oversaw weekly brotherhood house clean ups to maintain cleanliness.

Director of Risk Management **Spring 2015**

- Worked with my President and Vice President to create a risk management plan following the Interfraternity Council by-Laws for all social functions.
- Oversaw all social functions to enforce risk management policy.

Sustainability Chair **Fall 2014**

- Enacted a corrugated cardboard recycling program at chapter house.

ADDITIONAL INFORMATION

Honors Societies: Gamma Sigma Delta (Agricultural), Order of Omega (Greek).
Community Service: Eagle Scout, Challenger Baseball, PSU Pre-Vet Club.
Hobbies: Camping, Canoeing, Fishing, Traveling, Sport Shooting, Scuba Diving.
Pets: Great Dane, Labrador Retriever, Chickens, Peafowl