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DETERMINING THE RELATIONSHIP BETWEEN *trans*-10 FATTY ACID INTERMEDIATES AND MILK FAT PERCENT IN JERSEY COWS

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ABSTRACT

In the current dairy market, the value of milk is largely determined by its fat and protein content. Milk fat percent can vary among breeds, individual cows, and diets. The Jersey breed has become increasingly popular in the United States due to its high milk fat content. One of the factors that can influence milk fat content and, hence, the profit seen by producers, is diet-induced milk fat depression. Milk fat depression (**MFD**) is a condition that causes up to a 50% decrease in milk fat concentration, while milk production by weight remains the same. The biological pathway leading to MFD is caused by dietary or environmental changes that stress the rumen microbes. Under these stress conditions, an alternative, and slower bio-hydrogenation pathway is used by the bacteria, which increases production of *trans*-10, *cis*-12 conjugated linoleic acid (**CLA**) intermediates. Previous work has developed a model that can predict milk fat percent from *trans*-10 C18:1 in Holsteins. Currently, little information about MFD is available for Jerseys. The ability to use milk *trans*-10 C18:1 content to predict milk fat percent in Jerseys would allow us to compare the data to Holsteins and diagnose bio-hydrogenation induced MFD in Jersey herds. For this study, over 450 samples of Jersey milk were obtained from four dairy farms in Pennsylvania and analyzed for milk fatty acid content. The objective of this project was to measure the presence of *trans*-10 intermediates in milk from Jersey herds as an indicator of MFD. We hypothesized that an exponential relationship between milk fat and milk *trans*-10 C18:1 concentration would be observed in Jerseys, similar to that of Holsteins. We found little indication of MFD while fat concentration still varied widely. There are likely other factors that influence milk fat concentration in Jerseys more than MFD. In this study, we were

not able to establish a relationship between low fat content in milk and *trans*-10 C18:1 as an indicator of *trans*-10, *cis*-12 CLA.

Keywords: milk fat depression, Jersey cows, milk fat, fatty acid

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LIST OF ABBREVIATIONS

CLA- conjugated linoleic acid

DIM- days in milk

DHIA- dairy herd improvement association

FA- fatty acid

GC- gas chromatograph

MFD- milk fat depression

OBCFA- odd and branched chain fatty acids

TMR- total mixed ration

VFA- volatile fatty acid

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Chapter 1

Introduction

Milk fat concentration is an economically important component of milk. It is a major factor that determines the price a producer will be paid for their product. It also represents a significant energy expenditure by the cow and feed cost for the producer. This means that low milk fat production, such as occurs during diet-induced milk fat depression (**MFD**), threatens the profit of a dairy operation. Milk fat depression is a disease of dairy cattle that causes a drastic decline in the milk fat produced by a cow, while her total volume of production remains approximately the same. The condition is commonly caused by improper ration formulation, specifically fat content, that causes a change in the biohydrogenation pathway that metabolizes unsaturated fatty acids (**FA**) in the rumen of the animal. The normal pathway produces intermediates such as *cis*-9, *trans*-11 CLA and *trans*-11 $C_{18:1}$, while the alternative pathway produces *trans*-10, *cis*-12 CLA and *trans*-10 $C_{18:1}$. These alternative FA are less efficient in producing $C_{18:0}$ and therefore lead to a lower yield of milk fat.

There have been many studies that investigated the effect of MFD in Holstein cows. It has been found that when Holsteins have MFD, they have about a 50% drop in milk fat concentration (Bauman & Griinari, 2001). In addition, MFD can be artificially induced by infusing specific fatty acids into the rumen or abomasum of a cannulated cow (Baumgard, Sangster, & Bauman, 2001; Rico & Harvatine, 2013).

The current study was developed to investigate MFD in Jersey cattle using trans-10 $C_{18:1}$ as a biomarker. Jerseys are returning to popularity in United States dairy herds because of their ability to produce high fat milk. Holsteins, the most commonly used dairy breed, produce high

volumes of milk that is less rich in milk fat. The current market pays farmers for their milk components (fat and protein) while often charging for the hauling of the liquid portion of milk, thus giving Jerseys an advantage due to their high fat and protein milk. As MFD would decrease this fat content, it is crucial to understand how this disease affects Jerseys and methods of detection.

Milk samples were obtained after dairy herd improvement association (**DHIA**) testing from a number of Jersey farms in Pennsylvania. These samples were then extracted and methylated and run on a gas chromatograph (**GC**) to determine the FA content and JMP Pro was utilized for data analysis.

Chapter 2

Literature Review

Milk fat synthesis

The major components of cows' milk include water, fat, protein, lactose, vitamins, and minerals. Over the last century, the understanding of synthesis and biological activity of the different components of milk has advanced through a number of scientific studies (Lucey, Otter, & Horne, 2017). Dairy cows have been genetically selected to produce a high quantity of milk fat and protein compared to historic cows. This has been in large part achieved by increasing fluid milk production, which is part of what drives component yield.

Milk fat originates both from FA absorbed from the blood (preformed) and is produced in the udder of the cow (de-novo synthesis). All FA that are less than 16 carbons in length are produced in the mammary gland, while FA that are greater than 16 carbons are preformed. Fatty acids that are 16C are a mix of de-novo and preformed FA.

Preformed FA originate from either the diet of the cow or from adipose tissue that has been broken down. The diet of a dairy cow contains a large number of unsaturated fatty acids, which are not transferred into the milk produced by the cow (Luick, 1960). These long chain unsaturated fatty acids are hydrogenated by microbes in the rumen of the cow. The now-saturated FA are absorbed through the rumen wall into the blood and transported to the mammary gland of the cow. When a cow is in negative energy balance, meaning that not enough energy and nutrients are being obtained from the diet, she will start to mobilize lipids from fat

stores within her body. These fatty acids travel through the blood to the mammary gland and contribute to milk fat production.

The second pathway for milk fat production is de-novo synthesis in the udder of the dairy cow. This synthesis of short chain fatty acids is dependent on the concentrations of volatile fatty acids (**VFAs**) produced by fermentation of carbohydrates in the rumen and absorbed into the blood stream of the animal. As well as the capacity of the mammary gland to synthesize milk fat. The main VFAs of importance are acetate, propionate, and butyrate. Acetate and butyrate (in the form of *beta-hydroxybutyrate*) contribute the most to the synthesis of short chain fatty acids in milk. These two VFAs provide the majority of the carbon for de-novo milk fat synthesis (Bauman & Griinari, 2001). As the VFAs are broken down, they are then rebuilt into short chain fatty acids (C6:0 to C14:0) that appear in milk.

Importance of milk fat

Fat concentration of milk varies more than milk protein and lactose concentration, which fluctuate very little (Forsbäck et al., 2010). Lactose is the main osmotic regulator of milk and, as plasma osmolarity is tightly regulated, milk lactose concentration has little variation. Since lactose concentration does not vary, lactose yield is directly proportional to the total liquid volume of milk. Milk yield, protein and fat percentage vary by breed, with Holstein cows producing the most fluid milk while Jersey cows produce milk with the highest fat and protein concentration. There can be daily and seasonal variation in milk fat that results from diet, environment, and health status of the animal.

Milk fat is important to consumers because of the palatability that it provides in dairy products and to producers as it is one of the largest energy requirements in the production of

milk on a dairy farm. There have been historic concerns about the correlation between saturated fat consumption and the incidence of cardiovascular disease, but more recent studies have shown that there is no correlation between these two factors (De Souza et al., 2015). Further, it has been found that consuming full fat milk products may decrease the risk of obesity in children and obesity and diabetes mellitus in adult men and women (Beck, Heyman, Chao, & Wojcicki, 2017; Holmberg & Thelin, 2013; Rautiainen et al., 2016). Milk fat is the main component of butter and leads to the desirable smooth texture as well as contributing to the organoleptic properties of other dairy products such as cheese and fluid milk.

The variability in milk fat is a concern for dairy farmers, as one of the largest influencers on the price they are paid for milk is the fat content (Bailey, Jones, & Heinrichs, 2005). There are a large number of factors that can affect milk fat synthesis such as diet, environmental conditions, metabolic status, and breed of the animal. Salfer et al. found that there was a seasonal pattern of milk fat and protein yield (Salfer, Dechow, & Harvatine, 2019). Knowledge of these trends is important for producers as they are formulating diets that will adequately meet the nutritional needs of their cows throughout the year.

Biohydrogenation

Biohydrogenation is the conversion of unsaturated FA to saturated FA by the microbes in the rumen of an animal. Biohydrogenation of FA and the formation of bioactive intermediates in the diet of a cow is one of the determinants of milk fat concentration. There is some amount of fat in almost all feedstuffs but the type and amount varies greatly based on the source of the feed (Glasser, Doreau, Maxin, & Baumont, 2013). The most important factor in the biohydrogenation of dietary fats is the population of microbes within the rumen of the cow. These microbes

include bacteria, protozoa, and fungi that all contribute to the digestion of the diet consumed by a cow. Unsaturated fatty acids are the main substrate for rumen biohydrogenation. There is a fine balance between too much and too little unsaturated fat in the diet because too much can be toxic to the microbes and overwhelm the volume that is able to be biohydrogenated, while too little dietary fat means that the animal is not getting enough energy and her production can suffer (K. J. Harvatine, 2016).

The normal biohydrogenation process includes multiple steps that transform dietary unsaturated fatty acids into saturated fatty acids, with formation of *trans* FA as intermediates to the reaction. The main fatty acids in ruminant diets are linoleic (*cis*-9, *cis*-12-18:2) and linolenic (*cis*-9, *cis*-12, *cis*-15-18:3) acid, which occur at high concentrations in dairy feedstuffs such as grass and hay. These fatty acids, though, do not end up in the meat and milk of ruminant animals, which sparked the investigations into biohydrogenation. The normal pathway through which these unsaturated fatty acids are saturated produces *trans*-11 intermediates that terminate with stearic acid (C18:0) if the pathway runs to completion as seen in Figure 1 (K. J. Harvatine, Boisclair, & Bauman, 2009).

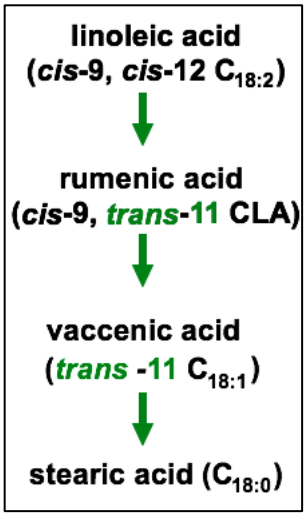

Figure 1. Pathway for the biohydrogenation of linoleic acid to stearic acid in the ruminant animal (Adapted from Harvatine, Boisclair & Bauman, 2009)

Milk Fat Depression

One of the most prevalent diseases of dairy cattle that affect the synthesis of milk fat is diet-induced MFD, or low-fat syndrome, which was first observed over a century ago, but the mechanism was not well understood until twenty years ago. MFD causes up to a 50% decrease in the milk fat content of milk that is not accompanied by a decrease in the total pounds of milk produced. More specifically, this condition is called biohydrogenation induced milk fat depression as it is caused by specific *trans* intermediates of the biohydrogenation pathway. At its root, MFD is generally caused by a shift in the microbial populations of the rumen so that the normal microbes that perform biohydrogenation are replaced with different microbes which produce alternative intermediates, including a large increase in *trans*-10 intermediates. This shift in rumen microbial environment can be caused by many factors, the most important of which is the diet of the animal. Since rumen microbes are replaced very quickly, as they divide, die, and are washed out of the rumen, it is easy for a shift in the microbial population to happen very rapidly. MFD causes a greater decrease in de novo fatty acids compared to preformed fatty acids due to the importance of microbes in their production.

With the shift in rumen microbes, there is a subsequent change in the process of fat biohydrogenation, which causes *trans*-10 bioactive fatty acids to be produced instead of the normal *trans*-11 intermediates. Studies have found that *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA are two molecules that can be measured in milk samples as indicators of MFD (Bauman & Griinari, 2001; K. J. Harvatine, 2009). The altered pathway of FA biohydrogenation can be seen in Figure 2, compared to the normal pathway. Although the altered pathway results in the same final product, the last step is inefficient and leads to other biological effects that also cause a decrease in the amount of milk fat produced.

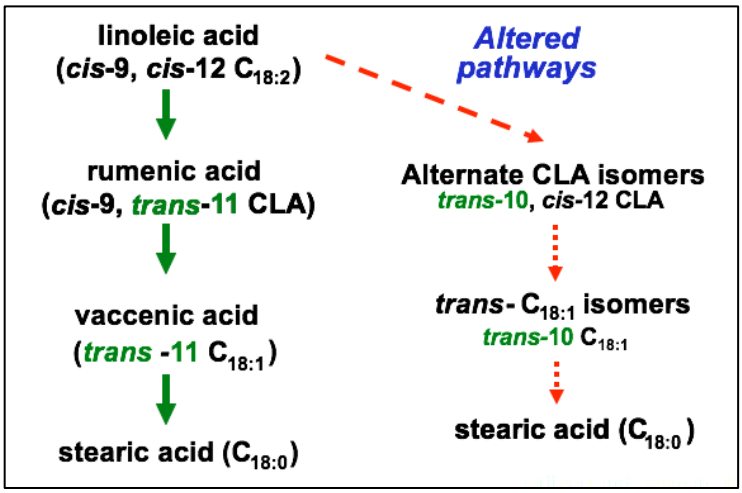

Figure 2: Comparison of normal rumen biohydrogenation to the altered pathway during milk fat depression (Harvatine et al., 2009)

The biological effects of these biohydrogenation intermediates are still being investigated, but studies have shown that certain proteins regulating fatty acid synthesis are downregulated in the presence of *trans*-10, *cis*-12 CLA (Bauman, Perfield, Harvatine, & Baumgard, 2008). The proteins that exhibited a decrease in expression and activity when CLA was added to the rumen of an animal include sterol response element binding protein 1 (SREBP1) and thyroid hormone responsive spot 14 (S14), (Bernard, Leroux, & Chilliard, 2008; Shingfield & Griinari, 2007). These are both proteins that regulate the expression of lipogenic genes in mammary tissue.

Rumination

Rumination is the process by which a ruminant animal regurgitates their partially digested feed (cud) and chews the material again (re-mastication) to aid in the breakdown of the tougher plant materials, such as cellulose. Rumination has become of interest recently because studies have found that increased rumination time can indicate cow comfort (Haley, Rushen, &

De Passillé, 2000), as well as increasing feed breakdown and subsequently milk yield. In addition, rumination is important for promoting saliva production, which acts to buffer the rumen. Bicarbonate and phosphate in the saliva keep the rumen within the optimal pH range of 6.0 to 7.0 (Allen, 1997), where rumen microbes function the best, as seen in Figure 3. If the pH gets too high or low, it can kill off these beneficial bacteria, therefore inhibiting digestion and possibly allowing detrimental bacteria to take over the rumen microbial populattion. Allen (1997) also observed a positive relationship between ruminal pH and milk fat percentage, expected to be the result of various dietary factors (Figure 3).

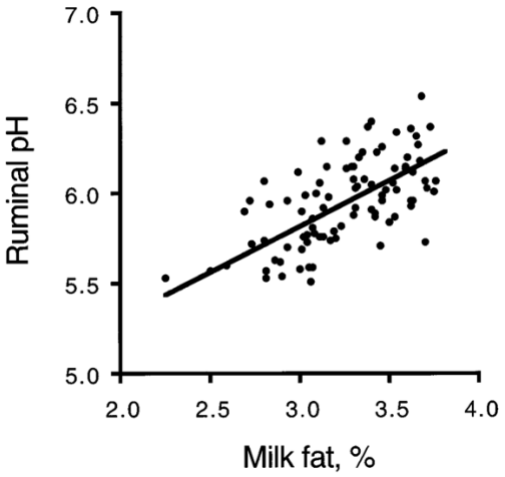

Figure 3: Relationship between ruminal pH and milk fat % from literature sources (Allen, 1997)

Rumination time can also be explained by factors outside the cow. One of these is particle size of the diet. Larger, more coarse feed particles take longer for the cow to break down and therefore require more rumination. Smaller particles are easily broken down by the rumen microbes and pass through the cow's digestive system rapidly, decreasing rumination time (Allen, 1997). This, in turn, is highly correlated with the incidence of MFD (Grant, Colenbrander, & Mertens, 1990).

Jerseys

Out of all of the traditional dairy breeds, Jersey cows are known for having the highest fat content of their milk. There have been a number of studies demonstrating that Jersey cows produce a higher percentage of milk fat (4.60%) than other breeds, such as Holsteins (3.65%) or Brown Swiss (4.04%) and, in addition, the fatty acid content of the milk also differed (DePeters, Medrano, & Reed, 1995; White et al., 2001; USDA, 2009). Due to this characteristic and the economic value of producing high fat milk, Jerseys have been increasing in popularity in U.S. dairy herds.

Although the Jersey breed was established hundreds of years ago and were the most numerous dairy breed through the 1930s, their numbers waned and Jerseys were a more minor breed compared to the high producing Holstein cow by 1980. Now, as Jerseys' popularity increases, more research is being and will have to be conducted on the unique characteristics of this breed. Milk fat depression is one of the disorders that has not been extensively researched in the past. It is yet to be determined how exactly MFD affects the fat content of Jersey milk and if the *trans*-10 biohydrogenation intermediates have different biological effects in this breed as compared to Holsteins.

Chapter 3

Materials and Methods

A total of 454 milk samples from Jersey cows across six Pennsylvania dairy farms were collected. High quality data was obtained on nearly all samples (n=450). These included 51 from Hillacres Jerseys (HA), 155 from Musser Run Farm (MR), 189 from Jersey Acres (JA), and 55 from previous Jersey cow studies (18EA6 and 2). The milk samples were obtained as part of routine, monthly Dairy Herd Improvement Association (**DHIA**) testing on the farms. Milk samples were preserved, stored frozen and shipped to the lab in State College, PA after DHIA testing was completed in Lancaster, PA or Ithaca, NY. Days in milk (**DIM**) and test day results (milk yield and milk fat and protein concentration) were obtained from PCDART, the herd management and records software used on each farm (PCDART operated by Dairy Records Management Service, Raleigh, NC).

Analysis was performed on the milk samples using the standard procedure for FA analysis as described in Rico and Harvatine (2013). Briefly, fatty acids were extracted from a measured quantity of milk using hexane isopropanol extraction and then methylated with sodium methoxide. Methylation products were analyzed by gas chromatography with a flame ionization detector and a 100mm column.

Different categories of FA were summed based on their origin (de novo, preformed, odd and branched-chain, and mixed). Straight chain even carbon FA with less than 16 carbons are made entirely in the mammary gland from acetate and butyrate and are categorized as de novo FA. Fatty acids with greater than 16 carbons all come from preformed FA that are absorbed from the blood. Mixed source fatty acids, C16:0 and C16:1, originate both from de novo synthesis and

from preformed FA in the blood. Odd and branched chain FA (**OBCFA**) are either preformed FA originating from microbial synthesis or are de novo synthesized starting with an odd or branched chain volatile fatty acid (**VFA**). Finally, the values for *trans*-10 C18:1 were log base 10 transformed (Logt10) to normalize the distribution of the data.

Statistical analysis was performed in JMP Pro v14.0. This program was used to calculate summary statistics for all of the FA and summary data as well as the data obtained from PCDART. Distributions were plotted using the Fit Distribution function. Simple regressions were performed between milk fat concentration and key variables of interest with linear and quadratic relationships tested.

Chapter 4

Results

Production by farm

The six farms were located across Pennsylvania and had different management and feeding practices. Aggregate data for all 450 cows, as well as data by farm is listed in Table 1. Milk production averaged 25.0 ± 6.7 kg/d (mean ± standard deviation) and ranged from 5.9 to 44.4 kg/d. Protein percent averaged 3.7 ± 0.4% and ranged from 2.1 to 4.9%, while milk fat percentage averaged 4.8 ± 0.9% and ranged from 1.3 to 7.7% (Figure 4). As seen in Figure 4, the mean (indicated by the diamond) for milk fat percent is greater than the median (indicated by the middle line in the plot), which implies a relatively greater number of samples with high milk fat than lower milk fat. The JA herd had the lowest milk fat percentage, averaging 4.55 ± 0.85% with a range of 1.3 to 6.9%, while HA herd had the highest milk fat percentage, averaging 5.56 ± 0.86% with a range of 3.2 to 7.7%.

FA Analysis

Gas chromatography results for FA concentration of the milk fat is presented in Table 1, with sums of preformed (sum FA > C16), mixed (sum FA = C16) and de novo (sum FA < C16) FA at the bottom of the table. De novo FA averaged 29.6 ± 2.64% of all FA, mixed averaged 34.9 ± 4.86% of all FA and preformed averaged 31.1 ± 6.30% of all FA. Figure 5 demonstrates the distribution of the sum of de novo FA which ranged from 13.8 to 35.8% of total FA content. Concentration of *trans*-10 C18:1 averaged 0.39 ± 0.34% with the highest herd concentration

averaging 0.48 ± 0.50% for JA. The *trans*-10 C18:1 concentration was log base 10 transformed (Figure 6) so that the data could be analyzed with a normal distribution.

Regressions

Various regression models were performed to determine the relationship between milk fat percentage and FA components of the milk fat. The best fit for *trans*-10 C18:1 compared to milk fat percent was a second-degree polynomial with the vast majority of the data falling below 1% *trans*-10 C18:1 (Figure 7). The best fit equation is: Milk fat % = 5.363- 1.509 * [trans-10 C18:1] + 0.200 * ([*trans*-10 C18:1] - 0.388)$_2$. When the concentration of *trans*-10 C18:1 was transformed by a log function (Logt10), there was a linear relationship with milk fat percent, with an equation of: milk fat % = 3.964 - 1.838 * Logt10. This linear relationship differed among the six farms (Figure 8). There was a weak linear relationship between milk fat and the sums of de novo, mixed and preformed FA as a percent of total FA concentration in the milk. The equation of best fit for milk fat by sum FA < C16 is: Milk fat % = 3.937 + 0.029 * Sum FA < C16; by sum FA = C16 is: Milk fat % = 3.519 + 0.037 * Sum FA = C16; and by sum FA > C16 is: Milk fat % = 5.683 – 0.028 * Sum FA > C16 (Figure 9).

Principal Components

Principal component analysis was conducted to explore relationships between the FA and milk measurements (Figure 10). This analysis is meant to show how a large number of variables are related on a 2D axis. The direction and length of each line signifies the strength and positive or negative relationship of each variable to the others on the graph. This was run to specifically look at the relationships between the different FA measured. Milk fat percent was closely related

to the concentration of *cis*-9 C16:1 while milk fat percent was opposite to *cis*-9, *cis*-21 C18:2, *cis*-12 C18:1, and *trans*-9 C18:1. The log transformed *trans*-10 C18:1 concentration was not directly inverse to milk fat percent, but it was a very large component and was directed mostly in the opposite direction.

Table 1. Summary of milk production and milk fatty acid profile for the combined dataset (All) and each farm individually

	Farm	HA	MR	JA	18EA6	2	All
n		51	155	189	38	17	450
DIM, d	Mean	158	148	191	169	174	170
	SD	104	90	119	37	89	103
Milk yield, kg/d							
Milk	Mean	21.4	26.1	24.5	20.9	27.7	25
	SD	7.67	5.38	7.28	3.85	6.33	6.7
Fat	Mean	1.17	1.24	1.09	1.02	1.35	1.2
	SD	0.43	0.24	0.31	0.22	0.33	0.3
Protein	Mean	0.83	0.95	0.87	0.75	0.99	0.9
	SD	0.26	0.17	0.22	0.11	0.18	0.2
Milk Composition, %							
Fat	Mean	5.56	4.84	4.55	4.94	4.80	4.8
	SD	0.86	0.81	0.85	1.00	1.03	0.9
Protein	Mean	3.97	3.69	3.63	3.62	3.61	3.7
	SD	0.40	0.40	0.42	0.40	0.35	0.4
FA							
C4:0	Mean	5.81	4.99	4.61	6.07	5.13	5.02
	SD	0.77	0.68	0.63	0.47	0.45	0.81
C6:0	Mean	2.96	2.73	2.63	3.03	2.98	2.75
	SD	0.29	0.21	0.27	0.18	0.24	0.28
C8:0	Mean	1.58	1.51	1.55	1.56	1.73	1.55
	SD	0.18	0.14	0.20	0.12	0.14	0.18
C10:0	Mean	3.46	3.59	3.73	3.14	4.26	3.62
	SD	0.55	0.44	0.66	0.30	0.38	0.58
C10:1c9	Mean	0.31	0.31	0.30	0.27	0.33	0.30
	SD	0.07	0.06	0.07	0.03	0.03	0.06
C11:0	Mean	0.06	0.12	0.11	0.05	0.13	0.10
	SD	0.04	0.05	0.06	0.02	0.05	0.06
C12:0	Mean	3.96	4.25	4.31	3.27	4.99	4.19
	SD	0.75	0.60	0.86	0.32	0.47	0.79
iC13:0	Mean	0.03	0.03	0.03	0.03	0.03	0.03
	SD	0.01	0.00	0.00	0.00	0.00	0.01
aC13:0	Mean	0.08	0.09	0.08	0.06	0.10	0.08
	SD	0.03	0.02	0.02	0.01	0.01	0.02
C13:0	Mean	0.10	0.15	0.15	0.08	0.17	0.14
	SD	0.04	0.05	0.06	0.02	0.05	0.06
iC14:0	Mean	0.10	0.06	0.07	0.07	0.09	0.07
	SD	0.04	0.01	0.02	0.02	0.03	0.03
C14:0	Mean	11.9	11.8	11.1	9.67	12.2	11.3

	SD	1.29	0.93	1.27	0.49	0.50	1.26
iC15:0	Mean	0.19	0.18	0.18	0.21	0.16	0.18
	SD	0.03	0.02	0.02	0.02	0.02	0.03
aC15:0	Mean	0.38	0.32	0.36	0.40	0.35	0.35
	SD	0.07	0.04	0.04	0.03	0.05	0.05
C14:1c9	Mean	0.95	0.95	0.81	0.64	0.92	0.86
	SD	0.23	0.22	0.20	0.11	0.21	0.23
C15:0	Mean	0.86	1.23	1.06	0.75	1.18	1.07
	SD	0.27	0.33	0.29	0.10	0.27	0.33
iC16:0	Mean	0.29	0.19	0.21	0.20	0.24	0.21
	SD	0.08	0.04	0.08	0.04	0.06	0.07
C16:0	Mean	33.8	38.2	31.3	27.5	32.0	33.6
	SD	4.10	3.06	2.78	2.19	2.47	4.66
iC17:0	Mean	0.04	0.04	0.04	0.05	0.24	0.05
	SD	0.01	0.01	0.01	0.01	0.02	0.04
C16:1c9	Mean	1.28	1.48	1.09	0.86	1.06	1.23
	SD	0.29	0.27	0.23	0.20	0.24	0.32
aC17:0	Mean	0.37	0.33	0.38	0.38	0.34	0.36
	SD	0.04	0.04	0.06	0.03	0.04	0.06
C17:0	Mean	0.18	0.36	0.39	0.13	0.50	0.34
	SD	0.06	0.19	0.16	0.03	0.05	0.18
C17:1c9	Mean	0.22	0.22	0.16	0.13	0.14	0.19
	SD	0.08	0.05	0.07	0.02	0.03	0.07
C18:0	Mean	9.92	7.83	11.3	14.5	9.77	10.2
	SD	1.79	1.37	1.97	1.61	1.45	2.63
C18:1t4	Mean	0.01	0.01	0.02	0.04	0.02	0.02
	SD	0.00	0.00	0.00	0.01	0.00	0.01
C18:1t5	Mean	0.01	0.01	0.01	0.02	0.01	0.01
	SD	0.00	0.00	0.00	0.00	0.00	0.00
C18:1t6-8	Mean	0.24	0.19	0.27	0.31	0.25	0.24
	SD	0.03	0.02	0.07	0.03	0.03	0.07
C18:1t9	Mean	0.17	0.13	0.20	0.23	0.20	0.18
	SD	0.02	0.01	0.04	0.02	0.02	0.05
C18:1t10	Mean	0.33	0.28	0.48	0.42	0.36	0.39
	SD	0.07	0.05	0.50	0.04	0.08	0.34
C18:1t11	Mean	0.87	0.75	0.95	1.10	0.64	0.87
	SD	0.25	0.14	0.26	0.14	0.11	0.24
C18:1t12	Mean	0.33	0.31	0.39	0.49	0.42	0.37
	SD	0.07	0.05	0.09	0.05	0.04	0.09
C18:1c9	Mean	14.9	12.6	15.8	18.4	12.8	14.7
	SD	3.63	2.43	2.77	1.62	1.94	3.23
C18:1t15	Mean	0.00	0.00	0.17	0.00	0.25	0.08
	SD	0.00	0.00	0.12	0.00	0.03	0.12

C18:1c11	Mean	0.60	0.62	0.57	0.64	0.35	0.59
	SD	0.16	0.12	0.19	0.06	0.10	0.17
C18:1c12	Mean	0.25	0.18	0.28	0.32	0.31	0.25
	SD	0.05	0.03	0.05	0.04	0.05	0.07
C18:2c9c12	Mean	1.41	1.68	2.55	2.46	2.05	2.10
	SD	0.19	0.22	0.50	0.36	0.35	0.59
C18:3c6c9c12	Mean	0.14	0.13	0.24	0.14	0.13	0.18
	SD	0.02	0.01	0.09	0.02	0.02	0.08
C20:0	Mean	0.00	0.00	0.02	0.03	0.02	0.01
	SD	0.00	0.01	0.01	0.01	0.01	0.01
C18:3c9c12c15	Mean	0.20	0.27	0.30	0.24	0.34	0.27
	SD	0.05	0.03	0.10	0.03	0.07	0.08
C20:1c11	Mean	0.07	0.09	0.05	0.08	0.09	0.07
	SD	0.01	0.02	0.02	0.01	0.03	0.02
CLAc9t11	Mean	0.34	0.31	0.33	0.42	0.26	0.33
	SD	0.09	0.05	0.12	0.09	0.08	0.10
C20:2n6	Mean	0.01	0.02	0.02	0.02	0.03	0.02
	SD	0.00	0.00	0.01	0.01	0.01	0.01
C22:0	Mean	0.00	0.00	0.00	0.00	0.04	0.00
	SD	0.00	0.00	0.01	0.00	0.01	0.01
C20:3n6	Mean	0.09	0.09	0.11	0.08	0.10	0.10
	SD	0.02	0.02	0.06	0.05	0.02	0.04
C20:4n6	Mean	0.12	0.12	0.14	0.14	0.14	0.13
	SD	0.02	0.03	0.05	0.03	0.03	0.04
C20:5n3	Mean	0.03	0.03	0.03	0.02	0.00	0.03
	SD	0.01	0.01	0.01	0.00	0.00	0.01
Sums, %FA							
Sum FA<C16	Mean	20.9	30.1	29.0	27.6	32.5	29.6
	SD	2.62	1.93	2.95	1.32	1.38	2.64
Sum FA=C16	Mean	35.0	39.7	32.4	28.4	33.0	34.9
	SD	4.11	3.14	2.82	2.32	2.59	4.86
Sum FA>C16	Mean	30.1	25.6	34.2	40.2	28.5	31.1
	SD	5.38	3.89	4.64	2.34	2.50	6.30
Sum OBCFA	Mean	2.75	3.05	2.97	2.41	3.36	2.94
	SD	0.40	0.46	0.34	0.16	0.38	0.43
Logt10	Mean	(0.49)	(0.56)	(0.38)	(0.38)	(0.45)	(0.46)
	SD	0.09	0.07	0.19	0.04	0.07	0.16

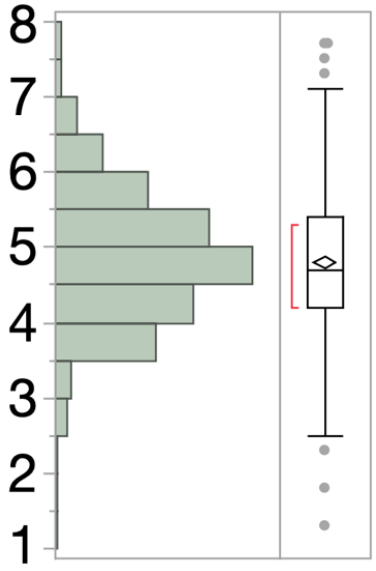

Figure 4: Distribution of milk fat concentration in 450 Jersey cows on one test day.

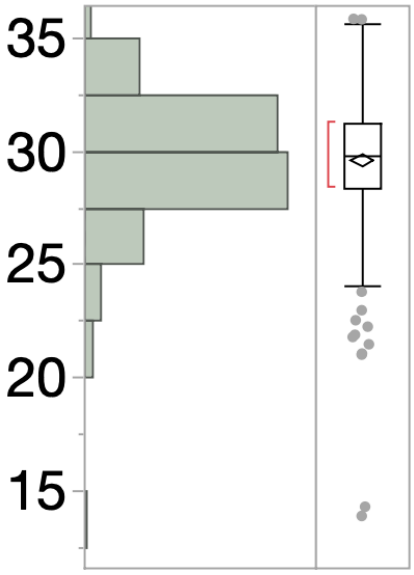

Figure 5: Distribution of FA < 16C (de novo FA) in milk fat of 450 Jersey cows on one test day.

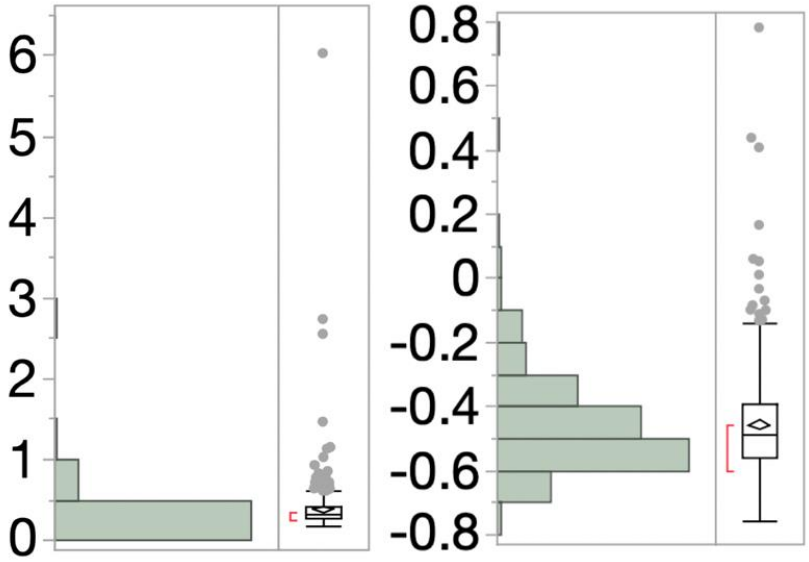

Figure 6: Distribution of concentration of *trans*-10 C18:1 (left) and log transformed *trans*-10 C18:1 concentration(right) in 450 Jersey cows on one test day.

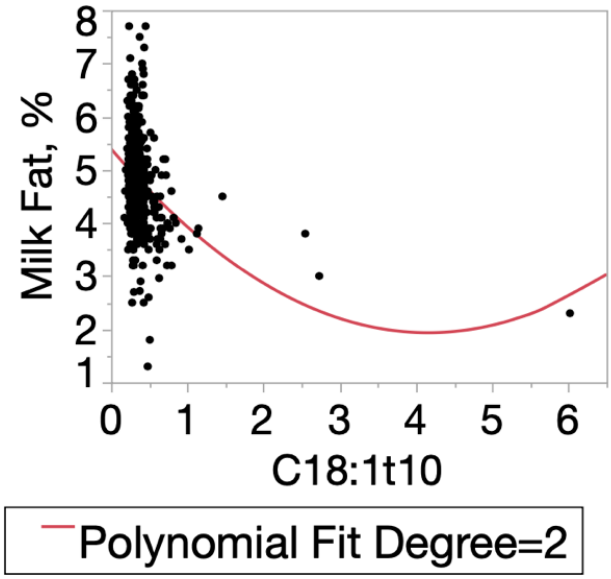

Figure 7: Relationship between milk fat concentration and *trans*-10 C18:1 in 450 Jerseys on one test day (R^2 = 0.097, P < 0.0001)

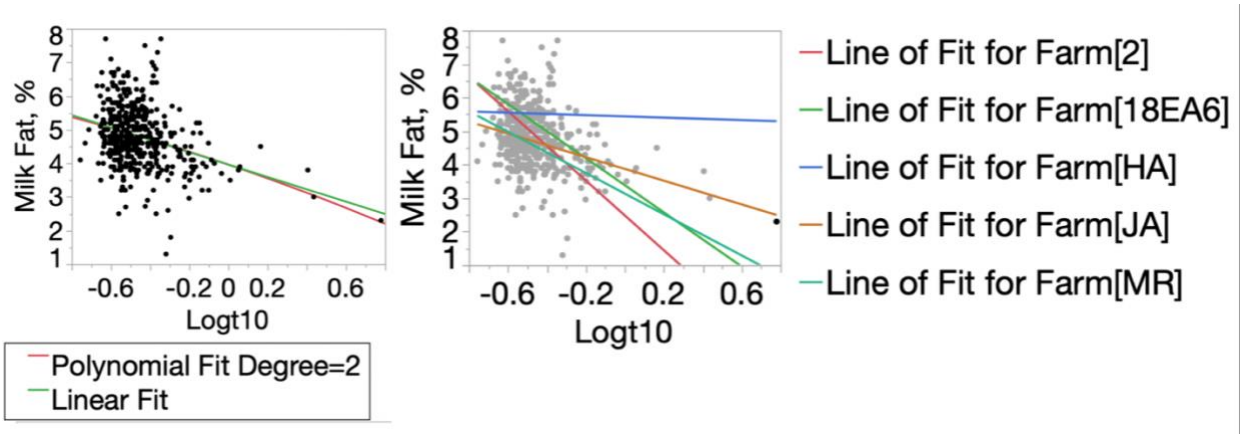

Figure 8: Relationship between milk fat concentration and log transformed *trans*-10 C18:1 in all farms (R^2=0.104, p < 0.0001) (left) and by farm (right)

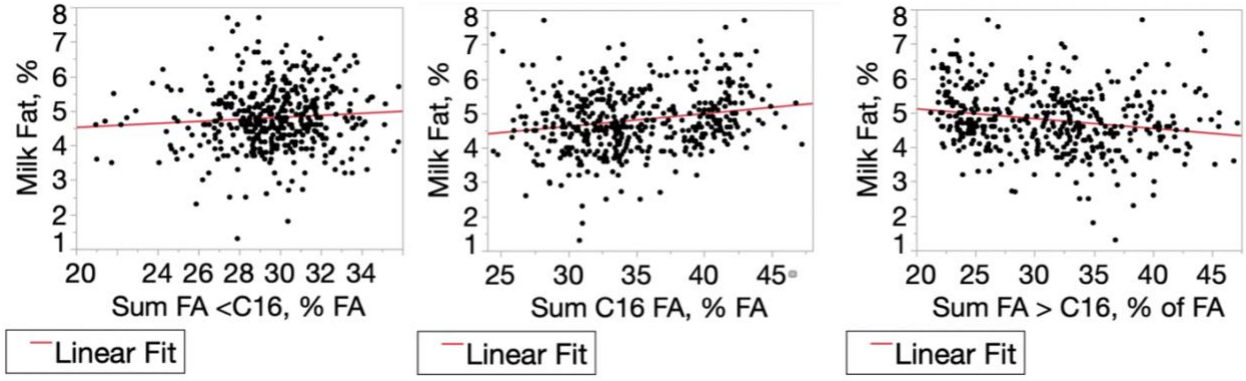

Figure 9: Relationship between milk fat concentration and fatty acid profile by source as a percent of total FA (by sum FA < C16, R^2=0.006, p < 0.1026 (left); sum FA = C16, R^2=0.038, p < 0.0001 (center); and sum FA > C16, R^2=0.0368, p < 0.0001 (right).

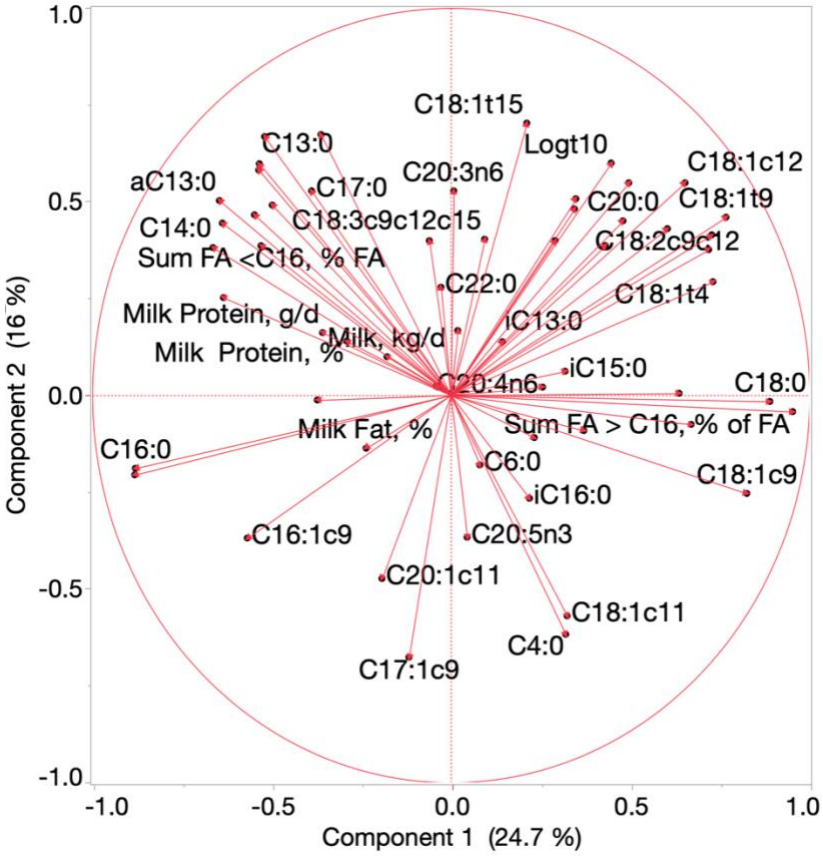

Figure 10: Principal components analysis of milk components and fatty acid profile of 450 Jersey cows sampled on one test day.

Chapter 5

Discussion

The purpose of this study was to evaluate the prevalence of diet-induced MFD in Jersey cows. Milk fat depression was indicated by milk FA content and specifically *trans*-10 C18:1 concentration. Previous studies have demonstrated that Holsteins experiencing MFD show up to a 50% decrease in fat concentration with a distinct increase in milk *trans*-10 C18:1 (Rico & Harvatine, 2013). The findings in Jersey cows from the current project differ from the previous results in Holsteins. Out of the 450 cows sampled for this study, less than 10 cows presented with MFD based on their milk FA content (elevation of *trans*-10 C18:1 > 0.5% of FA) accompanied by a significant drop in milk fat concentration. Despite the absence of MFD indicators, there was still a wide variation in the milk fat concentration within these Jersey herds (1.3 to 7.7%).

The average milk fat concentration measured in this study (4.8%) was comparable to the average milk fat content for Jerseys seen in literature sources (4.64%), (USDA, 2009). These findings suggest that there are alternative explanations for the variability in Jersey milk fat. Some of these alternative explanations could include genetics, acetate supply from the rumen, stage of lactation, and lactose yield. Milk fat concentration is a fairly heritable trait in dairy cattle compared to other commonly measured production traits. The genetic selection programs from each farm were not recorded, so it is possible they were not selecting for fat concentration or were selecting for traits that are inversely related to fat concentration, such as fertility or health traits. Acetate is one of the primary VFAs that contributes to milk fat synthesis. If a diet is not

balanced correctly, rumen synthesis of VFAs may be limited or changed in profile, specifically the ratio of acetate to propionate. This may lead to a decrease in milk fat synthesis (Linn, 1988).

Stage of lactation and lactose yield are inter-related. A cow naturally peaks in milk production at approximately 80 DIM and then gradually trails off until she is dried off and no longer produces milk. Lactose is one of the main determinants of milk volume as it stays at the same concentration in milk throughout lactation. Simply, as lactose yield increases, milk yield follows a similar trend. Fat yield does not rise and fall like milk and lactose; instead fat yield remains about the same until about 150 DIM when it begins to decline (Moallem, Kaim, Folman, & Sklan, 1997). The change in volume of milk produced, while fat quantity (grams) remains essentially the same, causes fat concentration to be higher during the beginning and end of lactation and lower during peak milk production.

We hypothesized that Jersey cows would experience a 50% drop in milk fat when suffering from MFD and this condition would be identifiable by a measurable increase in concentration of *trans*-10 C18:1 in the milk used as an indicator of *trans*-10, *cis*-12 CLA. There was a large variation in milk fat concentration of sampled cows, but this was not accompanied by an increase in *trans*-10 C18:1 concentration. Based on the principal component analysis, log transformed *trans*-10 C18:1 may not be directly predictive of milk fat in Jerseys in contrast to Holsteins. There were other components that appeared more strongly related to milk fat in Jerseys than this traditionally measured FA. Other FA that may be better indicators of MFD include to *cis*-9, *cis*-21 C18:2, *cis*-12 C18:1, and *trans*-9 C18:1. This could be further evaluated by bivariate analysis of milk fat concentration by the concentration of these FA.

This study had an adequate sample size for the parameters that were measured and tested. A challenge encountered while selecting farms to obtain samples from was that additional on

farm rumination tracking systems were originally considered part of the criteria for inclusion in the study. This limited the number of farms that were chosen, however, the rumination portion of the experiment was not pursued. If more farms were added, they could have been specifically chosen because of problems with low milk fat in the herd. This would have produced a larger distribution in the milk fat concentration seen in the data set and ideally more animal that fell in the category of MFD based on their *trans*-10 C18:1 levels. Another limitation of this study is that there was no control or analysis of the diet for each of the farms. MFD can be caused by unsaturated fat content or particle length of the diet so it is unclear if any of the few cases of this condition were caused by improper formulation of the diet. MFD is a condition that develops over time. With only one time point represented in the data from each farm, we were unable to study trends in milk fat per cow over time. Future studies could investigate the incidence of milk fat depression specifically in Jersey herds that are having problems with low fat concentration and incorporate an analysis of the diet. In addition, samples could be collected at sequential time points to quantify the variations in milk fat concentration seen in Jerseys. In a more controlled research setting, milk fat can also be induced by infusing *trans*-10, *cis*-12 CLA into the abomasum of the animal (Baumgard et al., 2001; Kevin J Harvatine, Robblee, Thorn, Boisclair, & Bauman, 2014; Urrutia et al., 2018). These studies have been performed in a Holstein model in the past but more information needs to be gathered in Jersey cows. This will allow for a quantification of the milk fat concentration drop caused by MFD from the onset of the condition through the recovery to normal milk fat levels.

Chapter 6

Conclusions

In Holstein cows, *trans*-10 C18:1 is measured as an indicator of milk fat depression, a condition that can decrease milk fat concentration by 50%. This relationship has not been extensively studied in Jersey cows. In this study milk fat yield and percentage varied between and among six Jersey farms. Those cows on the low end of fat production did not necessarily demonstrate high concentrations of *trans*-10 C18:1 upon FA analysis. This large range of milk fat which was not accompanied by increased *trans*-10 C18:1 suggests that there were other factors that had a greater impact on milk fat concentration than this fatty acid, such as genetics, diet, parity and stage of lactation. Further studies that induce MFD with high unsaturated fat or low particle size diets in Jersey cows can be used to identify FA indicators that are more accurate in diagnosing this condition in Jerseys as compared to Holsteins.

Appendix A

Other Information

Table 2: Farm Information

Farm	Number of cows	Location	Date Sampled
JA	~210	Pine Grove, PA	5/28/19
HA	~60	Peach Bottom, PA	3/19/19
MR	~180	McVeytown, PA	10/16/19

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ACADEMIC VITA

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Education

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Experience

- **Dairy Nutrition Undergraduate Research Assistant** *State College, PA* Fall 2016- May 2020
  - Lab of Dr. Kevin Harvatine
  - Completing my research project on milk fat depression in Jerseys
- **Calving Corner at the Pennsylvania Farm Show** *Harrisburg, PA* January 2020
  - Educated visitors at the Farm Show about the dairy industry
  - Assisted with the care of 16 cows that calved at the exhibit
- **Hameau Farm** *Belleville, PA* Summers 2013-2016
  - Managed and milked a herd of 40 cows and cared for calves and heifers
  - Oversaw care of other farm animals including pigs, goats and chickens

Internships

- **STgenetics Ohio Heifer Center** *South Charleston, OH* Summer 2019
  - Rotated through maternity, lactating cows, ET, calf care and veterinary team
  - Learned about the dairy genetics industry
- **Maple Dell Farm** *Woodbine, MD* Summer 2018
  - Milked 120 cows in a double-ten herringbone parlor
  - Learned about cow and crop management
- **New Bolton Center** *Kennett Square, PA* March 2018
  - One-week at large animal hospital for Penn Vet
  - Shadowed fourth year veterinary students
- **Hillacres Pride Jerseys Farm** *Peach Bottom, PA* Summer 2017
  - Internship on a 60-cow Jersey cow farm and cheese business
- **Palmyra Farms** *Hagerstown, MD* March 2017

Activities

- **Penn State Dairy Science Club**, *President* August 2016- May 2020
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  - Assisted with The Nittany Lion Fall Classic sale
- **Coaly Honors Society**, *President* Fall 2017- May 2020
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