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Differences in Fatty Acid Content of Homogenized and Non-Homogenized Milk from Holstein and Jersey Cows

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DIFFERENCES IN FATTY ACID CONTENT OF HOMOGENIZED AND NON-HOMOGENIZED MILK FROM HOLSTEIN AND JERSEY COWS

By

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The objective of this study was to investigate fatty acid concentrations of homogenized and non-homogenized milk from Jersey and Holstein cows. Twenty-two, lactating Holstein (n=11) and Jersey (n=11) cows were fed the same ration with Calan® gates twice daily. Jerseys were fed 25kg DM and Holsteins were fed 30 kg DM. Feed offered was adjusted daily according to previous day orts amount. Body weight and measurements, blood, and ruminal samples were collected weekly as were feed and orts samples. Ruminal fluid was collected from six Holstein and six Jersey cows weekly (n=42). Milk weights were collected daily and samples were taken at 0300 and 1500 hours and pooled by week. There was no difference in fatty acid concentrations from milk of Holsteins compared to Jersey. There was a tendency ($P < 0.08$) for greater concentration of linolenate between breeds and of stearate between processes.

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CHAPTER I

INTRODUCTION

As the cost of milk production has increased, many producers have found a niche market of processing milk on farm. Their product is typically pasteurized, but not homogenized. This has gained popularity with consumers, perhaps because of nostalgia, or perception of this type of production to be healthier.

Currently there is little published data to indicate a difference in quality of pasteurized milk that has been homogenized to that of milk that has not been homogenized. It is possible that concentration of fatty acids, proteins, minerals and other components may be different, due to homogenization.

Homogenization is a mechanical process that uses pressure to force milk through a small opening in the pump to reduce particle size of the fat globules from 10 to 20um down to <1μm. After homogenization, fat and proteins enter a colloidal state of dispersion. Homogenization is achieved once milk has reached a temperature of 65.5°C and pressure is between 17,237 and 20,684kPa.

Figure 1 Homogenizer, photograph taken by Dr. William Gillis

Originally, homogenization was developed to reduce the amount of fat consumed through dairy products. Homogenization of milk also increased resistance to oxidized flavor from pasteurization and decreased curd tension, producing a creamier taste.

Pasteurization is a mechanical process that uses high heat to remove harmful bacteria that can be found in raw milk. Three types of pasteurization processes are conducted in the United State. First is low temperature long batch where milk is heated to 63°C for 30 minutes or 68°C for 35 minutes. This type of pasteurization is used mostly for ice cream mixes. In high temperature short time pasteurization, milk is heated to 72°C for 15 seconds or 79°C for 25 seconds. This process is used for fluid milk and some ice cream mixes. Ultra high temperature milk is heated to between 93°C and 149°C for 1-2 seconds. This process is used for fluid milk to increase the shelf life.

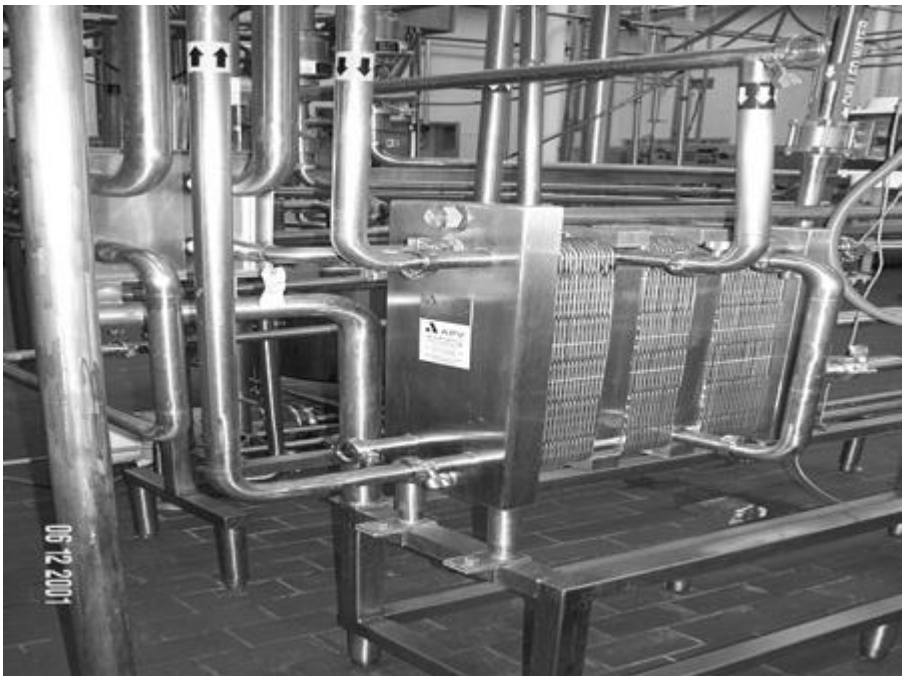

Figure 2 Plate pasteurizer, picture taken by Dr. William Gillis

Milk Consumption

In 1965, the United States Department of Agriculture (USDA) conducted a household food consumption survey and reported 30% of households did not meet their required recommended daily allowance of calcium. The average diet of girls aged 15 to 17, and women over the age of 35, were 34to 37% below the recommended allowances for calcium (USDA, 1969). Phillips et al., (1975) reported a decreased consumption of milk products, and an increase in the amount of soft drinks and alcohol consumed in the U.S. The authors attributed these trends to myths that surround consumption of milk and gave the following as examples: adults do not need milk, non-white people should not drink milk, raw milk is better than pasteurized milk, saturated fat and cholesterol 'cause' heart disease, cows are ecologically unsound, and nutrition does not sell milk.

Gerrior et al., (1998) reported a decrease of the amount of fluid milk being consumed, but an increase of the amount of cheese consumed. In 1970, annual per capita

milk and cheese consumption in America was 109 kg and 5.2 kg of cheese. In 1994, that decreased to 86 kg of milk and increased to 12.2 kg of cheese. In 1997, there was another decrease in the amount of milk to 83 kg and increase in cheese consumption to 13 kg.

The United States Department of Agriculture compiled a book of facts describing all of the USDA programs. Americans consumed 38% less milk, and ate nearly four times the amount of cheese in 2000 compared to the 1950s. Figure 1 shows amount of milk, cheese and milk products consumed by Americans over 50 years. This data indicates there was a decrease in the amount of fluid milk consumed from 1950 through present, but a steady increase in the amount of cheese consumed from 1950 through present. However, even though there was a decrease in the amount of total dairy products consumed from 1950 through 1979, with an increase of total milk production from 1980 to present.

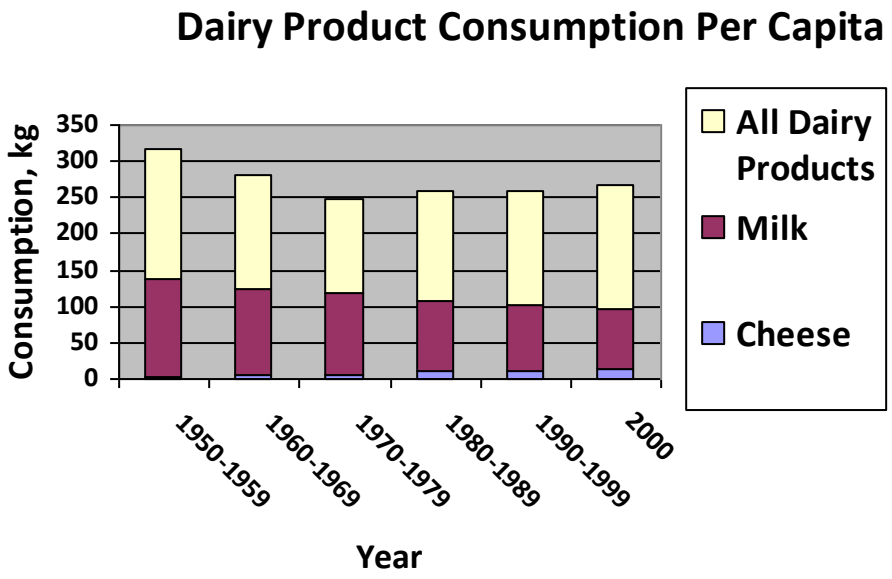

Figure 3 Americans are consuming less milk, and consuming more cheese. Adapted from USDA fact sheet 2001

Dairy Product Consumption and Health

Milk consumption and cardiovascular disease

Ness et al. (2001), conducted a retrospective study of 5,765 men aged 35 through 64, from workplaces in West Scotland between the years of 1970 through 1973. Participants were given a questionnaire and answered questions about health, lifestyle and milk consumption. Participants were given a medical examination, also. For data analysis milk consumption was separated into three categories: none, 473 mL per day, and more than >473 mL per day. Data of these men was evaluated during a 25 year period. During the duration of the study, 2,350 men died; 892 of coronary heart disease (CHD), 196 of stroke, 266 of lung cancer, 448 of other cancers and 548 of other causes. Milk consumption was inversely associated with all causes, cardiovascular, CHD, non-lung cancer and other mortality. Ten percent of subjects that consumed more than 473

mL per day died, while 15 and 17% of men who consumed 473 mL or less (respectively) died during the study (Figure 4).

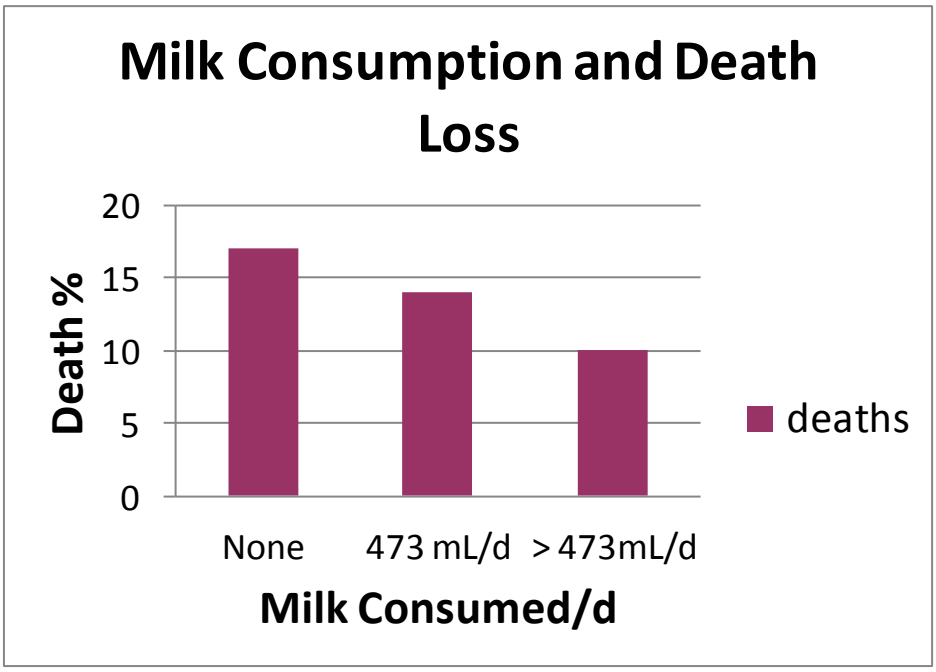

Figure 4 Milk consumption and death loss

Individuals reported to have above 140mg Hg systolic BP, and/or above 90mg Hg diastolic were three to four more times likely to develop CHD, and seven times more likely to develop a stroke (Pfeuffer et al., 2000, Griffiths, 2010) In conclusion, there was no evidence that regular consumers of milk are at increased risk of death from heart disease or death from all causes (Ness, 2001). When aged adjusted, risk of mortality from cardiovascular disease was lower in those drinking between 473 mL and one and 631 mL a day. The inclusion of milk in the diet reduced the number of deaths caused by heart disease and there could be several reasons why.

Casein and whey proteins found in milk decreased blood pressure in mice (McBean, 2006). Blood pressure was increased when angiotensin II increased, which constricted vascular smooth muscle. Casein and whey proteins are rich sources of angiotensin converting enzyme (**ACE**) inhibitory peptides. ACE converted inactive angiotensin I hormone into angiotensin II. Inhibiting ACE decreased blood pressure, due to reduced conversion of angiotensin I to angiotensin II (Pfeuffer et al., 2000). Studies of spontaneously hypersensitive rats demonstrated that potent ACE inhibitory peptides hydrolyzed from individual casein and whey proteins reduced systolic blood pressure by 2 to 34 mm Hg (McBean, 2006).

With the increase of high blood pressure and the potential of developing CHD on the rise in consumers (Pfeuffer et al., 2000), there is much debate over the consumption of lower fat dairy products in higher quantities.

Dairy product consumption and heart disease

Nestel (2008) found individuals that consumed dairy foods derived from unaltered milk had greater concentrations of total cholesterol and low density lipoprotein cholesterol (LDL-C) in their plasma. Low density lipoprotein cholesterol collects in the walls of blood vessels, which cause blockages of atherosclerosis; the greater levels of LDL-C present in the human body the greater risk for a heart attack from a sudden blood clot in an artery narrowed by atherosclerosis (American Heart Association, 2011). Nestel (2008) also stated that cheese consumption was an exception to the link of cardiovascular disease observed in epidemiological studies due to the possibility of decreased LDL-C raising effect compared with butter. Tholstrup (2004) evaluated three diets containing different energy sources and found that LDL-C was least in the cheese energy diet.

Individuals that consumed conventional dairy foods had plasma LDL-C concentrations of 4.49mmol/L, while unsaturated dairy foods had plasma LDL-C of 4.25mmol/L. Participants in this trial had a base line of 3.95 mmol/L of LDL-C.

Tholstrup et al. (2004), evaluated three different diets containing 20% of energy from whole milk, butter or hard cheese. Fourteen healthy human males were used. Each consumed the diet for a three week period with a washout period in between each treatment. At the end of each three week period, a fasting blood sample was obtained from each individual. Individuals consuming diets which contained butter and/or hard cheeses had a greater fasting plasma LDL-C concentration (0.21 vs.0.06 vs.-0.14, respectively; $P<0.04$) and a borderline difference in total cholesterol (0.20vs. 0.07 vs. -0.13, respectively; $P<0.05$).

Dairy product consumption and other health conditions

The July/August 2006 edition of the National Dairy Council digest (McBean, 2006), reported that consumption of dairy protein by humans stimulated whole body and/(or) muscle protein synthesis. The digest also reported on the better quality of dairy protein and the similarity of its amino acids composition to that found in the human body. Dairy proteins contain relatively large amount of leucine, which has a unique role for increasing fat loss, promoting lean muscle tissue and regulating blood glucose. Leucine also promotes normal maintenance and growth of muscle by humans. Dairy proteins have the ability, at least in the short term, to suppress food intake as a result of the ability to improve recovery time from exercise.

Milk Composition

Table 1 shows the composition of milk from different species.

Table 2 Composition of Milk of the Cow and other Mammals

	Cow	Goat	Human	Sheep	Mare	Pig	Ass
Water	87.7	87.0	88.2	81.3	89.9	81.9	90.1
Fat	3.6	4.6	3.3	6.9	1.2	6.8	1.3
Lactose	4.7	4.2	6.8	5.2	6.9	5.5	6.5
Protein (N x 6.38)	3.3	4.4	1.5	5.6	1.8	5.1	1.6
Ash	0.8	0.8	0.2	1.0	0.3	0.7	0.5

Adapted from Ensminger, 1993

Fatty Acids in Milk

Several factors affect the fatty acid composition of milk, including: animal genetics, stage of lactation, grain, amount and composition of dietary fat (Palmquist et al., 1993). Genetic correlations among traits for milk production are high; as milk production increased (volume), milk fat decreased. Milk fat production can be genetically selected for, but not at the same time. Cows in negative energy balance cause mobilization of adipose fatty acids and incorporate long chain fatty acids into milk. Short chain fatty acids with the exception of C_4 are low during early lactation, reaching >90% of maximal proportions by week eight of lactation. Increase of starch intake resulted in a depression of milk fat. In fat depression short chains decreased, while C_{18} increased. When dietary fats were added to the ration, fatty acids produced by the de novo process decreased when dietary fats were increased from 1 to 5%. C_{16} and C_{18} changes were dependent on the C_{16} and C_{18} ratios of the supplemented fats (Palmquist, 1993).

Table 3 Effect of dietary fat source and level on milk fatty acid composition in a feeding trial

Fatty Acid	Basal	Animal Vegetable blend	Calcium Soap	Hydrogenated animal fat	Saturated Fatty Acids	Tallow	P<
Octanoate (C8:0)	1.75	1.34	1.35	1.39	1.41	1.34	0.03
Caprate (C10:0)	3.97	2.51	2.57	2.63	2.72	2.60	0.001
Laurate (C12:0)	4.64	2.75	2.84	2.88	3.03	2.89	0.001
Myristate (C14:0)	13.01	9.33	9.54	10.28	10.10	10.30	0.001
Palmitate (C16:0)	29.87	26.45	34.15	28.42	32.67	28.41	NS
Palmitoleate (C16:1)	1.68	1.64	1.64	1.72	1.99	1.80	NS
Stearate (C18:0)	9.05	11.50	7.71	11.68	9.86	10.43	NS
Oleic (C18:1)	17.22	25.74	22.80	22.89	20.30	23.26	NS
Linoleate (C18:2)	2.24	2.00	2.58	1.67	1.74	1.59	0.05
Linolenate (C18:3)	0.55	1.16	0.63	0.72	0.62	0.91	0.01

Triacylglycerols control several properties important to milk quality. The melting point of milk is affected by amount of triacylglycerol, crystallization behavior, and rheological properties of milk fat as globules (Jensen, 1995). Unlike minerals, amount of triacylglycerol is not greatly affected by ordinary changes in diet (Jensen, 1995).

Results from Hermansen (1995) concluded that bovine milk contained more than 500 fatty acids, with 12 to 15 present in predominant amounts. Of fatty acids contained in milk 75g/100g were saturated fatty acids, 21g/100g were monounsaturated fats and 4g/100g were polyunsaturated fatty acids.

Most saturated fats were produced by the mammary gland by de novo synthesis, with 50% of palmitic acid being produced by de novo synthesis. Fatty acids can be derived directly from preformed fatty acids of blood lipoproteins. These fats are derived primarily from plasma via digestion and absorption of dietary fat (Mansbridge, 1997). Three major preformed fatty acids found in blood of ruminants are 16:0, 18:0 and 18:1 (Duncan & Garton, 1963). It had been shown that polyunsaturated fats are not evenly distributed in plasma lipoprotein (Mansbridge, 1997).

Proteins

In bovine milk six major proteins are found: α_{s1}-casein, α_{s2}-casein, β-Casein, γ-casein, χ-casein, α-Lactalbumin, and β-Lactoglobulin. All six of these proteins are found in the mammary gland of the cow and, therefore are transferred to the milk.

Caseins represent more than 50% of protein found in milk, but several proteins make up the membranes of fat globules including: caseins, α-lactalbumin and β-lactalbumin. The composition of proteins that form the milk fat globule membrane, are not affected by homogenization pressure or fat concentration, but significant difference in the composition of the milk fat globule membrane were caused by the heat treatment that was applied before homogenization (Cano-Ruiz et al., 1997). The temperature of homogenization also has an effect on the proteins that are absorbed. Once temperature is increased the amount of α-lactalbumin and β-lactalbumin increases, compared to the amount of caseins absorbed at the higher temperature (Cano-Ruiz et. al. 1997). Once the temperature decreased the amount of caseins increased and the amount of α-lactalbumin and β-lactalbumin decreased (Cano-Ruiz et. al. 1997).

Minerals

Gaucheron (2005) reviewed mineral concentrations in bovine milk from several different breeds. Minerals found in bovine milk were divided into 2 groups, cations and anions. Cations included calcium, magnesium, sodium and potassium And anions included inorganic phosphate, citrate and chloride. These minerals are largely responsible for the structure and stability of casein micelles.

Mineral content varied in bovine milk based on breed and stage of lactation. Normandy cows have a greater mineral content in milk compared to Friesian, Red Pied and Holstein cows (Gaucheron, 2005). Mineral concentrations change during lactation and are greatest in colostrum and decrease for the remainder of lactation. In cows with mastitis, mineral concentration was elevated, to even greater concentrations than late lactation (Gaucheron, 2005).

Minerals were partly bound to casein micelles, in the aqueous phase of milk, or were diffusible. Potassium, sodium and chloride were diffusible, but inorganic phosphate and magnesium were partly bound to micelles. One third of calcium, 50% inorganic phosphate, 66% magnesium and over 90% of citrate were found in aqueous phase of milk (Gaucheron, 2005). Cerbulis and Farrell (1976) showed that breed effected mineral profile of milk.

According to Gerrior and Bente (2002), milk contained large amounts of calcium and provided more than 70% of the calcium available in the food supply in the United States. Decreased Ca concentrations in the average American persons' diet can be a precursor to many diseases or disorders that are related to calcium deficiency.

Minerals including sodium, magnesium, calcium and other milk components such as vitamins, protein, and essential fatty acids had a beneficial effect on blood pressure

regulation (Huth et al., 2006). These authors also stated that the combination of minerals found in milk seem to be more effective than that of minerals taken alone, or being taken as supplements.

Breed Differences

Milk fat is different among breeds and within breeds. Ensminger (1993), reported that each of the five dairy breeds has a different amount of fat. The amount of fat can also vary due to physiological factors including: stage of lactation, breed, and age of the cow, calving interval and the relative size of the cow within breed. Environmental factors that affect composition include: feed, underfeeding, deficiency of nutrients, and length of dry period, frequency of milking, season of the year, day-to-day variation, diseases and drugs.

Table 4 Composition of Milk from Different Breeds

Breed	Fat	Protein	Lactose	Ash	Total Solids	Solids not fat (SNF)
	%	%	%	%	%	%
Ayrshire	4.1	3.6	4.7	0.7	13.1	8.52
Brown Swiss	4.0	3.6	5.0	0.7	13.3	8.99
Guernsey	5.0	3.8	4.9	0.7	14.4	9.01
Holstein	3.7	3.1	4.9	0.7	12.4	8.45
Jersey	5.1	3.9	4.9	0.7	14.6	9.21

Table adapted from Ensminger, 1993.

White et al., (2001) examined the effect of either confinement or pasture feeding on milk fatty acids of both Holstein and Jersey cows. Milk samples were collected from nineteen Holstein and eighteen Jersey cows to determine fatty acid concentrations. This trial was conducted for four consecutive weeks beginning June 16 and ending July 7, 1998. Milk samples were collected twice per day, once per week. One sample set was used to determine fatty acid composition, the second set was sent to dairy herd information association (DHIA) to determine fat, protein, lactose, somatic cell count, milk urea nitrogen, other solids and solids not fat (SNF). The fats were determined using the Milk-o-Scan 6500. The milk samples that were used for fatty acid composition were thawed and centrifuged until separation occurred. Fatty acids found in the fats were converted to methyl esters and separated with gas liquid chromatography (GLC). There was effect of breed for fatty acids $C_{6:0}$ through $C_{14:0}$ ($P < 0.05$, $C_{6:0}$= 1.71, 1.74, $C_{8:0}$=1.17, 1.23, $C_{10:0}$=2.68, 2.89, $C_{12:0}$= 2.65, 3.14 and $C_{14:0}$=10.44, 11.47) with Jerseys producing greater concentrations. The pasture group produced greater concentration of conjugated linoleic acid (CLA) than the confinement group (0.66 vs. 0.36% ± 0.05% of total fatty acids, $P < 0.05$). Holsteins produced greater concentration of CLA than Jerseys (0.56 vs. 0.46% of total fatty acids, $P < 0.05$) and concentrations of $C_{16:1}$ and $C_{18:1.}$

DePeters et al., (1995) evaluated milk fatty acid concentrations in three different breeds (Holstein, Jersey and Brown Swiss) from a single commercial herd. All animals were fed same ration and managed the same. The portions of short chain fatty acid (SCFA) and long chain fatty acid (LCFA) did not differ among breed, but there was an increased amount of C_6 to C_{14} in Jersey milk ($P<0.05$).

Effect of Dietary Changes

White et al., (2001) demonstrated the effect dietary changes can have on milk fatty acid composition. Results showed that Holsteins and Jerseys fed the pasture diet had a greater amount of $C_{10:0}$ through $C_{14:1}$ compared to those fed corn silage ($P < 0.05$). Most fatty acids are derived either directly or indirectly from the diet while very few are synthesized in the body. Forages, oil and oilseeds, fish oil and fat supplements added to the diet have been shown to alter the fatty acid content of animal products, including milk. Those effects are discussed below.

Forages

The fermentation of carbohydrates from forages in the rumen results in the production of three volatile fatty acids acetate, butyrate, and propionate. Acetate and butyrate are precursors for the de novo synthesis of fatty acids in the mammary gland (Mansbridge, 1997). Even though low in the amount of oil present in forages, 25% up to 35% of fatty acids are derived from forages during early lactation, due to being consumed in large amounts (Mansbridge, 1997). Fatty acids present in grass forages, when contrasted to grass silage, are highly unsaturated with 18:3, 60g/100g and 18:2 13g/100g (Mansbridge, 1997). Potentially ruminants that are fed grass instead of grass silage have increased levels of n-3 polyunsaturated fatty acids. Still to be determined, is how much polyunsaturated fats survive the ensiling or drying process (Mansbridge, 1997).

Oils and Oilseeds

Plants store energy as either starch or in the form of oil (Mansbridge, 1997), creating several differences in amounts and types of fatty acids found in a particular plant. Interest has been raised and focused on feeding diets containing either whole seed

or seed oils. Whole seeds and seed oils are rich in oleic, linoleic, and linolenic acids. (Mansbridge, 1997) The introduction of oils or oilseeds into the diet of lactating dairy cows results in reduced levels of C4-C16, also resulting in an increase in levels of one or more of the long chain fatty acids, including 18:0, 18:1, 18:2 or 18:3 (Mansbridge, 1997). Oils fed as whole oil seeds have an increased affinity for protection during hydrogenation in the rumen compared to the protection that extracted oil has for 18:0 and 18:2 (Mansbridge, 1997).

Fish Oil

Fish oil has the ability to desaturate fatty acids and create polyunsaturated fatty acids. Unlike animals; plants have the ability to synthesize de novo linolenic acids. Research has shown differences in how fish oil is presented in milk; some research has shown cod liver oil that has been intravenous infusion is poorly transferred to milk fat (Storry et. al, 1969). Menhaden oil that has been infused post-rumen has been shown to have a good amount of transfer to milk fat (Hagmeister et al, 1988). In a third study dairy cows were fed cod liver oil, with no transfer of 20:5 or 22:6 to milk fat. However there was an increase in amounts of 20:0, 20:1, and 22:0 transferred (Brumby et al. 1972).

One study has suggested that polyunsaturated fatty acids found in fish oils may not be hydrogenated in the rumen (Ashes et al, 1992). In this study fish oil casein mixture was incubated with rumen contents from sheep for 24 hours, there was a decrease in the amount of 18:1 cis and trans; however there was no change in 20:5, 22:6 levels (Ashes et al. 1992)

Fat Supplements

Fat supplements are not widely used due to fats being given as an energy supplement, being used to minimize the adverse reaction on fiber digestion or microbial activity in the rumen (Mansbridge, 1997). The use of tallows in the United Kingdom is very limited due to the risks that are associated with bovine spongiform encephalopathy that can occur in cattle (Mansbridge, 1997).

Homogenization

Historically homogenization of milk was thought to be the reason that milk when tasted had the distinct flavor of being cooked. However, after testing, it was shown that pasteurization was the reason for the cooked flavor. Regarding human health, homogenized milk seems to be more digestible than untreated milk (Michalski, 2006).

Homogenized milk was first made available to the public in 1909 in Quebec, Canada. The process of homogenization was very different from that process that is practiced currently. The early process of homogenizing milk in 1919 was carried out by a Gaulin homogenizer and sterilized in bottles at 108°C for twenty minutes. In order for the milk to sell, it was labeled as the cure-all for some common sicknesses during that time period, not only a cure-all for children but also for older adults. Homogenized milk was not highly looked upon during this time, and was seen more to serve medicinal purposes, not many consumers purchased homogenized milk.

In 1927 milk started to become marketed as being homogenized, this time not for medicinal uses but now as milk that had good flavor. Again the process of homogenization had changed; the milk was properly homogenized this time, but was not properly sterilized, causing issues for consumers with sterilization. By this time consumers are realizing that homogenized milk is healthier, also realizing children in

school are not getting the adequate amount of milk fat in their school milk. The reason for this is because children were unable to shake the non-homogenized milk enough to break the fat globules apart. Also new mothers realized that infants were not getting adequate milk fat; the fat was clogging the bottle nipples that the mothers were using.

The accepted present day standard for milk homogenization is to pass milk through a high pressure pump to reduce the size of the fat globules. To achieve this size it is necessary to keep homogenization stable is between 17,237-20,684 kPa. The fat globule size for raw milk is between 10-20μm, while homogenized milk the fat globule size is <1μm. 66°C is optimal temperature for homogenization to increase efficiency of the process. Testing the efficiency of the homogenization process requires 24 hours of quiescent storage, and testing the fat volume of the top 25% and the bottom 25% of the milk. If fat difference is <.10% then the efficiency is very high. Visual examination also can be completed, if fat globules are smaller than 1μm. If visual and actual testing of the difference in fat globules proves to be the same, homogenization has occurred successfully and separation will not occur.

Pasteurization

Pasteurization was first commercially introduced in the United States in 1895, while the first compulsory pasteurization law for milk was passed in 1908 in Chicago. It was not until 1946 that vacuum pasteurization was perfected, and in 1948 ultra-high temperature pasteurization was first used. It was not until 1981 that ultra-high temperature (UHT) gained national recognition (Ensminger, 1993)

Three types of pasteurization are utilized today: low temperature long time, high temperature short time and ultra-high temperature

Low temperature long time can be completed two different ways: 63°C for 30 minutes, or 68°C for 35 minutes. This process is used for mostly ice cream mixes. High temperature short time can be completed two different ways: 72°C for 15 seconds, and 79°C for 25 seconds. This is used for a continuous system that also includes homogenization in the same machine. Ultra high temperature pasteurization requires a temperature of 121-149°F for one to two seconds and the product is rendered sterile, also allowing for the product to be kept at room temperature for up to six months.

Buchin et al., (1998), conducted a study investigating pasteurization on fat composition, and flavor found in semi-hard cheese. Milk samples were collected from two different sources, one farm located on the plain that fed pasture, and the second farm was located in the mountains and cows were fed hay. Milk samples were collected, and pasteurized or left as a raw product. Fatty acids were determined using gas chromatography, with slight modifications for the cheeses (Buchin et al.,1998) At the conclusion of the trail, it was determined that pasteurization of milk had no effect on the composition of milk fatty acids. They also discovered that cows from the plain location had higher concentrations of the short chain fatty acids or fatty acids found in both the cheese and milk samples. Cows from the mountain location that were fed hay had higher concentrations of long chain fatty acids and saturated fatty acids in both cheese and milk samples (Buchin et al., 1998).

Fidler et al. (1998), conducted a study investigating the effects of pasteurizing and sterilizing human breast milk from 12 mothers that gave birth to preterm (n=8) and term (n=4) babies. Each mother was at a different stage of lactation ranging from day five though 35 of lactation. Five mL was collected from each mother using an electric breast pump. Each sample was then divided into three different samples. One sample was not

treated (fresh milk), two were pasteurized at 62.5°C for 30 minutes or were sterilized at 120°C for 30 minutes.

Fatty acid analysis was determined by using gas-liquid chromatography (GLC). At the conclusion of the study, it was determined that fatty acid composition does not change with either the heat treatments of pasteurization or sterilization. However sterilization does result in the formation of surface skin, which tends to adhere to sides of containers. Total fat content includes surface skins, and the fat that is actually available to the recipient infant are different.

Gas Chromatography Mass Spectrometry

Gas chromatography mass spectrometry (GC/MS) has been used in several fields of study to investigate different amounts of oils and other substances found in samples. The field of forensic investigations utilized the GC/MS to determine amounts of drugs found in either a victim or a suspect. The GC/MS is used as a confirmatory test in this field when observing drugs, and drug residues found in samples.

The GC/MS utilizes samples by first taking sampled injected and ionizing the sample. Once sample has been ionized the gas chromatography separated samples based on ionization rates of compounds. After separated for ionization rates the mass spectrometry separated samples based on molecular weight.

This process was used by Jahreis et al (1999) to look for linoleic acid in bovine milk samples: sheep milk samples, goat milk samples, mare milk samples, sow milk samples and human milk samples. In this study they were looking at the anticarcinogenic conjugated linoleic acid.

Several other options exist for the flushing of the sample during the ASE step of F.A.M.E. analysis. Two other options for flushing include chloroform, and a chloroform methanol mixture. Each of these two options including the hexane flush will be examined to determine which flush produces the higher amount of oil separation during the extraction.

Dionex published two processes conducted on milk and cheese samples (Dionex, 2004). The first process uses one gram of fluid milk, instead of the dried powder milk used in the other processes stated above. Solvents used in the process are Petroleum ether, and isopropanol, in a (2:1) ratio. The advantage of using this process was the time spent on drying samples to powder. Drying samples times to have samples completely dried down to powder can range from 3 days to 4 days. Decreasing the time spent freezing and drying down samples, can cause a decrease in denaturing of the fats and proteins that are found. Decreasing fat breakdown is important since types and length of fatty acids is being studied in this project.

Instead of drying and freezing the milk samples, the milk will be able to be taken from the farm, transported back to the lab and be run immediately cutting down the prep time drastically using this process. Using the petroleum ether and the isopropanol as the solvent in the ASE decreases the time used for blowing down the solvent and oil that was removed from the sample.

The second process that Dionex published is using the same process as the hexane and chloroform methanol, except using a combination of hexane: dichloromethane: methanol. This process uses dried milk powder, like three other processes that have been conducted in the research. The process is the same, using 3.5 grams of hydromatrix, and using the ASE machine. The only difference is using liquid instead of powdered milk.

After trying several extracting solvents, it was determined that petroleum ether isopropanol alcohol was proven to be the solvent that extracted increased amounts of lipids for fatty acid analysis.

Summary

With heart disease being the leading killer of men and women in 2006, and more than half of all deaths among women due to heart disease, the thinking behind this study was to take into consideration milk and milk fats, and the possibility of relation to heart disease in consumers. It was predicted by the CDC in 2006 that the cost of heart disease would be around $316.4 billion (CDC, 2006). This total includes the cost of health care services, medications and lost productivity. In 2006, a list was produced documenting the percentage of people that have certain risk factors for heart disease. The list included inactivity (39.5%), obesity (33.9%), high blood pressure (30.5%), cigarette smoking (20.8%), high cholesterol (15.6), and diabetes (10.1%; CDC, 2006), and noted that 37% have more than one risk factor listed above.

With milk having higher amounts of poly-unsaturated fats and less short chain fatty acids, there is a possibility that if there is the ability to alter the fatty acid concentrations based on breed, or processing types, there is a possibility of lowering several of the risk factors in the future.

The objectives for this study were: To compare the fatty acid composition of milk from Holstein and Jersey cows that had either been homogenized or not.

CHAPTER II

MATERIALS AND METHODS

Experimental Design

Animals and Diets

Twelve Holstein and twelve Jersey cows were used for this trial. Two cows were removed one Holstein was removed due to health issues and one Jersey was removed due to inability to use Calan® gate (American Calan®, Inc., Northwood, NH, USA). Animals were balanced by parity (1 to 4).

Cows were housed at Bearden Dairy Research Center and fed using Calan® gates for 42 days. Animals were fed total mixed ration used by the rest of the herd. Cows were fed twice daily, each animal fed for refusals between one and 2.3kg for the next morning. When an animal had a large amount of refusals (consisting of 4.5 kg. or more) for three consecutive days feed was decreases by 4.5 kg. The 4.5 kg were subtracted from the morning and evening feedings for that day. Refusals were weighed each morning before new feed was offered. Feed fed during the evening feeding were combined with feed that remained in containers from the morning. Decreasing feed offered was began the following morning feeding. Feed was decreased by 9.1 kg each time the feed was decreased. When an animal that had less than 2.3 kg of feed in the morning, amount of feed offered was increased that morning by 2.3 kg., and increased by 2.3 kg in the evening, resulting in a 4.5 kg increase for that day.

Sample Collection

Blood, feed and ruminal samples were collected on Thursday of each week for the duration of the trial. Rumen samples were collected from six randomly selected females of each breed for each week

Feed samples

Approximately 200 gram samples were obtained from the refusals from the night before from each animal. Each of these samples were taken and placed in a forced air oven at 60°C. Feed samples were kept in the forced air oven until the weight of the sample was within a half a gram of the previous weighing.

Blood/Serum samples

Blood samples were obtained by jugular venipuncture. Samples were placed on ice for collection until separation could occur. Samples were collected in serum separator tubes (15mL, vacutainer). Samples were centrifuged at 4330 G for 10 minutes; serum was separated, removed and placed in microcentrifuge tubes. A minimum of 3 mL was removed so samples could be run in triplicate. Samples were then stored at -18°C until analysis was completed.

Ruminal Fluid

Ruminal fluid was collected from twelve (n=12) randomly selected cows on collection day. Holsteins were sampled first, with the first six females through the chute being those animals that represented the Holsteins and the first six Jersey through the chute represented the Jerseys for that week. Rumenocentesis was performed by a veterinarian from the Mississippi State University College of Veterinary Medicine. A sixteen gauge, four inch long needle was injected into the rumen wall to obtain the

samples. Approximately 4 mL from each animal was obtained, pH was determined immediately after ruminal fluid was obtained. Ruminal samples were kept on ice during the sampling process until samples were centrifuged. Ruminal samples were centrifuged at 4330 rpm for 10 minutes. The portion of the sample that contained no particulate matter was removed and placed in microcentrifuge containers and placed in a freezer at -18°C until analysis was completed.

Milk Samples

Milk samples were obtained twice weekly for a total of four milkings. Samples were collected during Wednesday morning, and evening milking and Thursday morning and evening milking. During the third week of milk collecting, the sampling day was moved to Friday instead of Wednesday due to dairy herd information association sampling the whole herd milk. Each collection was combined into one 473 mL container. Once all collections were added to the 473 mL container, half was distributed into another container and labeled non-homogenized. Date, cow number and homogenized or non-homogenized was written on each container, samples were placed in a refrigerator until homogenization was completed. Homogenization was achieved by using a Kinematic homogenizer. Milk samples were removed from refrigerator, heated to 66°C, and homogenized. Samples that were homogenized and non-homogenized were pipetted into microcentrifuge tubes. Three microcentrifuge tubes were used for each homogenized or non-homogenized sample. Samples were placed into a -20°C freezer until analysis was run.

Analytical Analysis

Proximate Analysis

Proximate analysis was conducted on feed refusal samples and total mixed ration sample at Essig Nutrition Lab. Samples were analyzed for total dry matter, crude protein, neutral detergent fiber, acid detergent fiber and organic matter (ash). Organic matter is calculated when all liquid has been removed and sample is then weighed. Crude protein is calculated from nitrogen present in the sample. Kjeldahl converts nitrogen present in the sample to ammonia, which is determined by titration. The percentage of protein is obtained by the amount of protein multiplied by 6.25. Neutral detergent fiber is calculated by digesting (boiling) the sample in a so called neutral detergent solution that results in neutral detergent fiber. Acid detergent fiber is calculated by the residue after digested in a solution with sulfuric acid, ADF contains mostly cellulose and lignin.

Fatty Acids Extraction

Several tests can be used to determine the amount of fatty acids found in a milk sample. Milk-o-Scan and gas chromatography mass spectrometry have been used in previous studies to both quantify and qualify fatty acids. One gram of freeze dried sample is mixed with 3.5 gram of hydromatrix. Hydromatrix serves as a drying agent that removes excess moisture found in the sample. The sample was placed in an Accelerated Solvent Extractor (ASE; Dionex, 1228 Titan way, Sunnyvale, CA) tube it is then taken and placed in the ASE machine. The samples are heated up to 120°C and flushed at a rate of 60% hexane, at a kPa of 10,342. Each sample is subjected to this for 3 cycles of 15 minutes for each flush. The tubes containing oil and solvent were placed in a turbo-vap. Which removed hexane until <0.5mL oil is left.

Transesterification

Fatty acid methyl ester (FAME) esterification process can begin taking a subsample of 10µl of oil was mixed with 2mL of 1% H_2SO_4 (sulfuric acid) in MeOH, heated at 60°C for 2 hours. Then $NaHCO_3$ was added to increase pH and thereby halt the reaction. Once a pH of 8 was achieved, 2mL toluene (dichlorobenzene and BHT) was added as an antioxidant. Toluene caused a separation of the mixture into two distinct layers after several minutes. When layers were distinguished toluene layer (top layer) was removed and placed in a scintillation vial. The step of adding toluene is repeated to ensure a majority of the toluene and oil had been removed and placed in the scintillation vial. Once samples have been placed in vials, FAME analysis can begin. The samples are transferred from the scintillation vials to auto sampler vials, once labeled and placed in auto sampler vials samples were placed on the GCMS machine to have analysis completed. After the analysis had been run, each sample had a print out of the different fatty acids and concentrations in µg/mL (Table 4; figure 5, 6, 7).

Gas Chromatography Mass Spectrometry

In this study the GCMS machine was used to analyze for fatty acid, utilizing a Restek Stabilwax®-DA column (30 m X 0.25 mmID) with a 0.25µm film thickness. A standard and a blank were placed after every ten samples to flush column thereby minimizing contamination of samples. A blank was run at the end of the sample list to ensure that column was cleaned.

Standard solutions were prepared with 14 fatty acids; caprylic, capric, lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic, behenic, erucic, and lignoceric. The 14 fatty acid standard was diluted with BHT five times

creating a 5 point dilution. All chemicals used for this trial were purchased from Sigma Aldrich.

The GC/MS conditions for this trial were as follows: injector temperature: 260°C. Column oven temperature began at 50°C, held there for 2 minutes, increased by 10°C per minute until 250°C is reached, and held for eighteen minutes, until total time is 40 minutes per sample. External electrochemical ionization (EI) is used to ionize samples. From time zero to 7.5 minutes the MS is turned off, starting at time 7.5 minutes the MS turns on, until the end of the 40 minutes. Full scan was utilized and was scanned from 50 through 400 m/z.

Table 5 Retention time of Fatty Acids

Fatty Acid	Retention Time (minutes)
Octanoate	10.9
Dichlorobenzene	11.6
Caprate	13.8
Laurate	16.5
Myristate	18.8
Palmitate	21.0
Palmitoleate	21.3
Stearate	23.1
Oleic	23.4
Linoleate	23.9
Linolenate	24.8
Arachidate	25.5

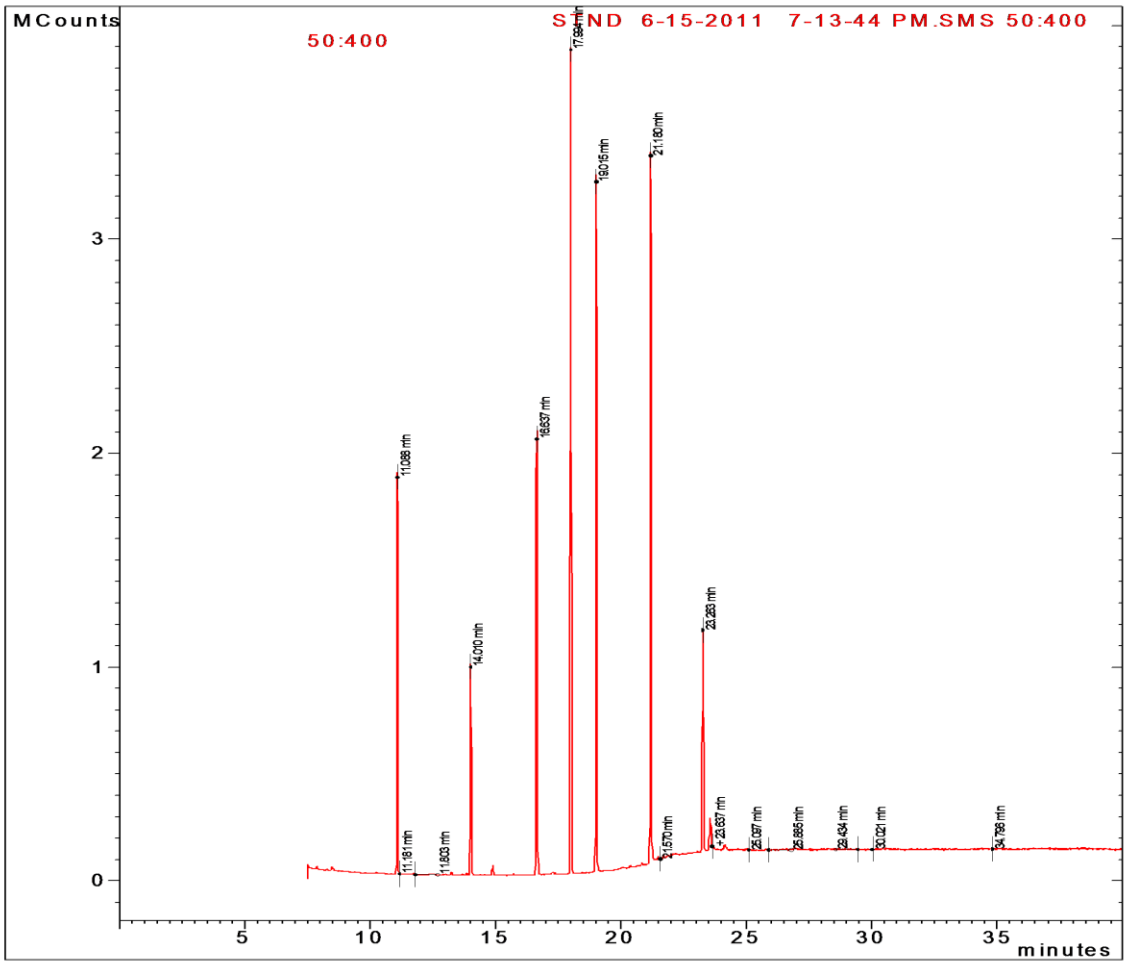

Figure 5 Chromatogram of Standard

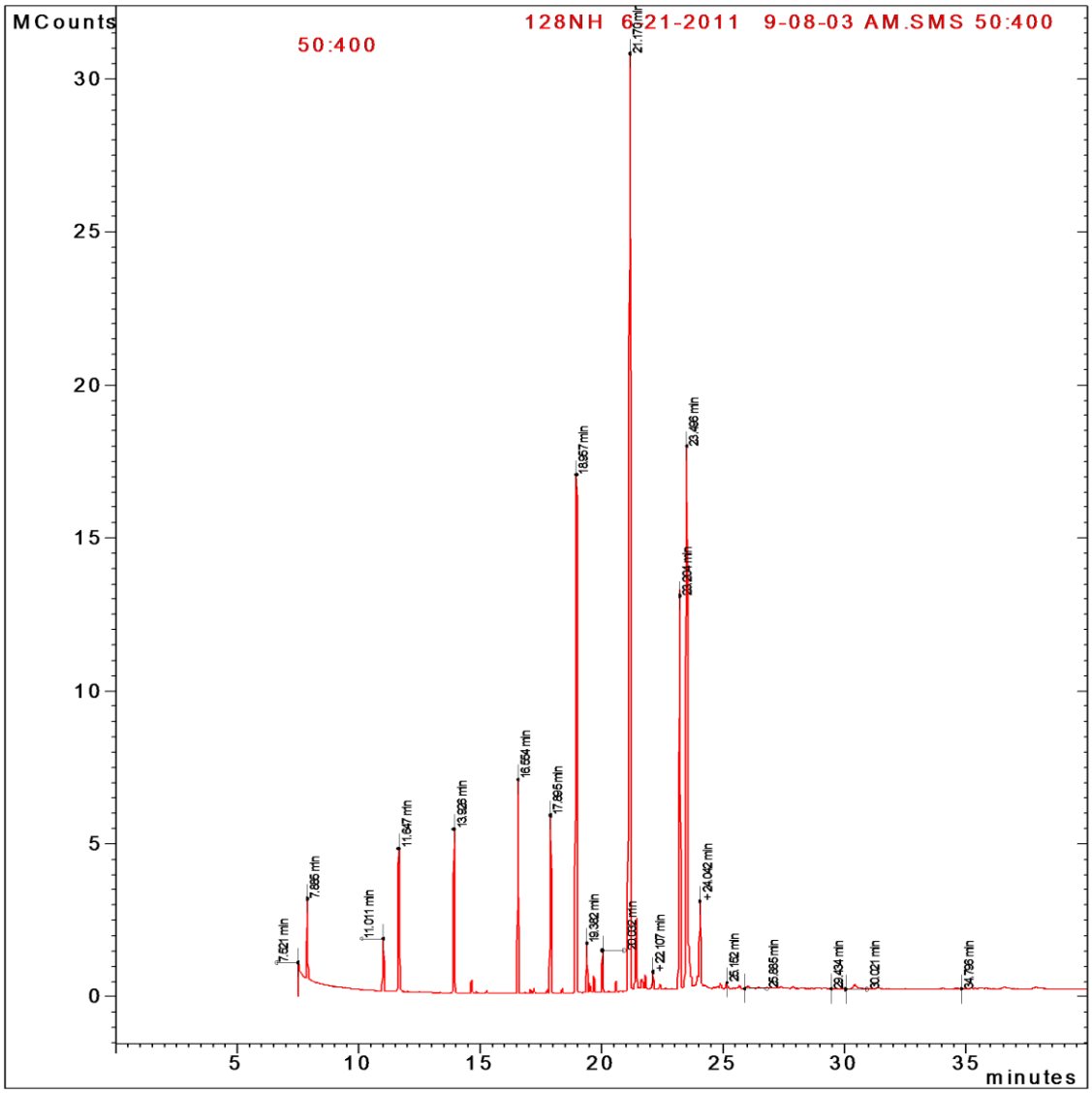

Figure 6 Chromatogram of Jersey, non-homogenized milk

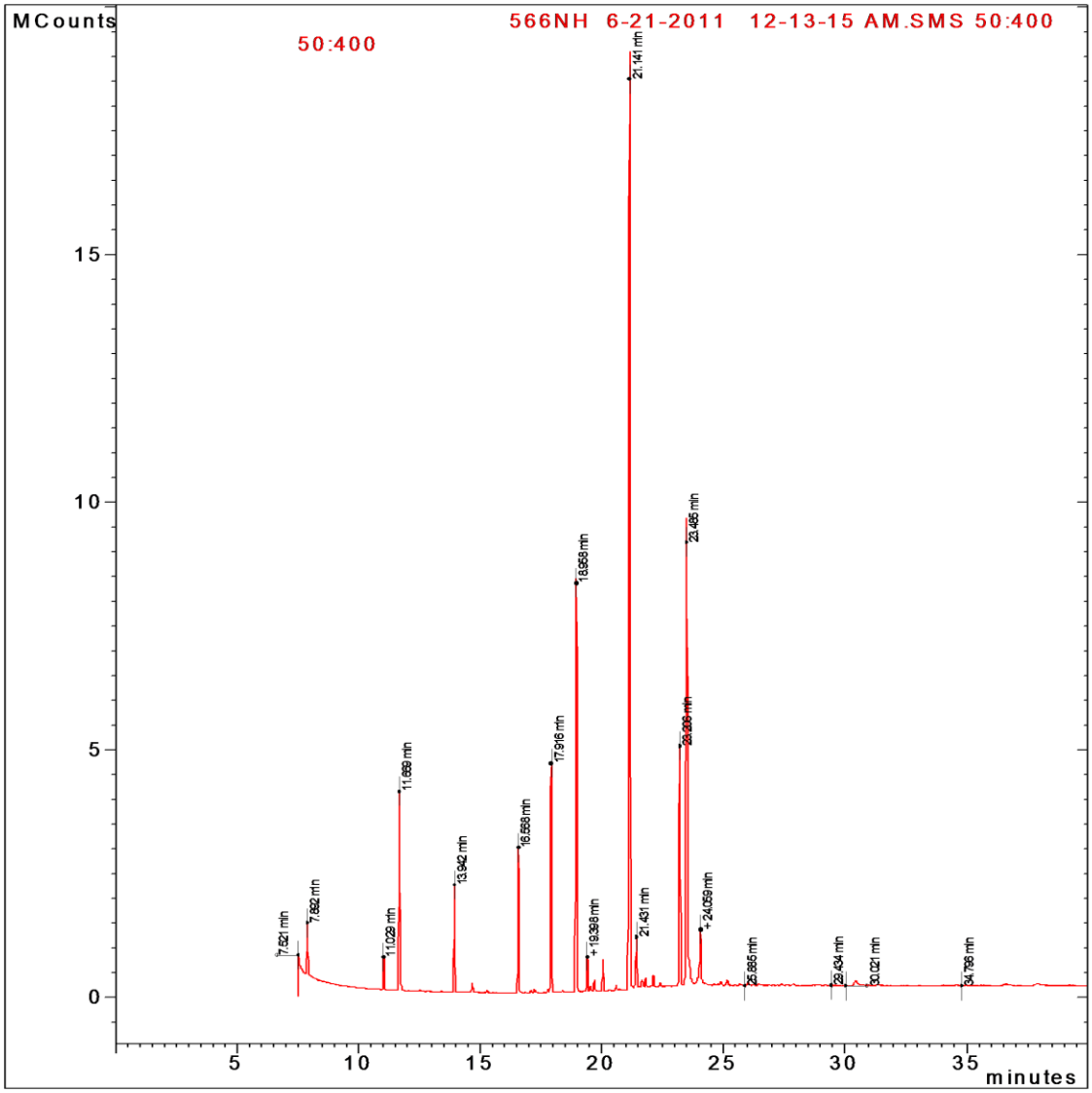

Figure 7 Chromatogram of Holstein, non-homogenized milk

Statistical Analysis

Data was subjected to ANOVA using Mixed Procedures of SAS with treatment, week, breed, and all interactions used as dependent variables. Ruminal, milk and serum data analysis did not use treatment because all animals were in both treatments.

Dry matter, body measurements, hip height (HH cm), wither height (WH cm), average daily gain (ADG) and milk yield were recorded and analyzed. Significance was

set as $P<0.05$. Model used: $Yikj= Ti+Bj+Wk+eijk$ With Ti representing treatment, Bj representing breed effects, and Wk representing week effects

CHAPTER III

RESULTS AND DISCUSSION

Breed Effects

Days in milk, Milk yield & Feed efficiency

When investigating the differences between breed as it is related to dry matter intake (DMI), milk yield (MY), and feed efficiency (FE), there was no significant difference between FE (H=2.39 DMI/MY, J=2.02 DMI/MY, P<0.41), or MY (H=30.13 kg/d, J=26.69 kg/d, P<0.21). However between the two breeds DMI was greater for Holsteins (H=62 kg) compared to Jersey (J=52 kg) P<0.001). From this data it is apparent that Jerseys have the ability to produce the same amount of milk, while consuming less feed, compared to Holsteins.

Feed Samples

There were no differences for in fatty acid concentrations found in the refusal of feed samples. Of fatty acids that were in the ration, most saturated fatty acids were present in smaller concentrations of feed samples and unsaturated fatty acids were found in greater concentrations in the feed samples (Table 5). In comparison of fatty acids found in the ration and fatty acids found in milk there was the incidence of having an increase in amounts of unsaturated fatty acids in the ration. In long saturated fatty acids, there was an increase in amounts of fatty acids present in milk compared to fatty acids found in the ration. Amounts of unsaturated fatty acids as a group were present higher in

feed than in milk (2,008.7 μg/ml, 728.7 μg/ml; respectively). Saturated fatty acids as a group were present higher in milk than in feed (871.5 μg/ml, 1302.19μg/ml, respectively).

Blood Samples

There was not a significant difference in fatty acid concentrations for breed in serum samples that were analyzed (Table 7). However fatty acid concentrations of blood samples, (except linoleate, linolenate and arachidate), was less than fatty acid in milk indicating that mammary gland had the ability to synthesize fatty acids. Saturated fatty acids of blood, (except palmitate and stearate), was less than ruminal fluid concentrations. On the other hand unsaturated fatty acids, (except palmitoleate) of blood was greater compared to ruminal fluid.

Rumen Samples

Palmitoleate was the only fatty acid which differed ($P<0.05$) between Holstein and Jersey ruminal fluid (Table 6). Of the samples that were found the rumen concentrations of fatty acids in ruminal fluid was greater than fatty acid concentrations of serum. Unsaturated fatty acid concentrations from ruminal fluid, (except palmitoleate), were less ($P<0.05$) than serum. Saturated fatty acid concentration of ruminal fluid (except palmitate and stearate), were greater ($P<0.05$) than serum ($P<0.05$). Because fatty acid concentration was less in ruminal fluid compared to milk, it can be concluded that the mammary gland can synthesize most fatty acids instead of obtaining fatty acids from the diet.

Milk Samples

Linolenate tended ($P<0.05$) to be the only fatty acid different in milk between Holsteins and Jerseys (Table 8). According to Ensminger, (1993) most if not all fatty acids are produced by de novo synthesis in the mammary gland. Data from the present trial supports the theory that cows synthesize most fatty acids in milk instead of using fatty acids from the diet.

Processing Effects

Milk Samples

When investigating homogenization effect on fatty acid concentration, only a tendency ($P<0.08$) was observed for the amount of stearate. In observing the difference in processing effects, the same trend was reported that was reported in milk and breed effects. Starting with fatty acid concentrations in ruminal fluid the same is true. Concentrations of saturated fatty acids (besides Palmitate) of ruminal fluid were greater compared to serum. Saturated fatty acid concentration of serum samples were all less numerically in concentrations, compared to milk. Two unsaturated fatty acids (linoleate and linolenate) had decreased numerically in concentration in milk. Breed did not affect fatty acid concentrations of milk. This also supports that theory that the cow will synthesize most fatty acids in the mammary gland. Of the 11 fatty acids that were analyzed, only one (palmitoleate) had a greater numerical concentration in ruminal fluid compared to milk. This leads to the idea that fatty acids are produced by de novo synthesis in the mammary gland of the cow.

Table 6 Diet ingredients and nutrient composition found in total mixed ration

Ingredient	**% Diet DM**
Hay	2.89
Baleage	8.15
Seed	3.6
Silage	36.1
Corn Grain	46.9
Water	0.1
Nutrient Composition	
Dry Matter% As-Fed	100
ASH % DM	7.8
Protein % DM	17.2
NDF % DM	48.4
ADF % DM	16.4

Table 7 Dry matter and nutrient intake in Holstein and Jersey cows

	Holstein	Jersey	SE	P <
Dry Matter, kg/d	62.0	52.0	2.18	0.001
Organic Matter Intake, kg/d	57.9	48.0	1.92	0.001
CP Intake, kg/d	10.8	8.9	0.3	0.001
NDF Intake, kg/d	30.4	25.2	1.01	0.001
ADF Intake, kg/d	10.2	8.5	0.3	0.001

Table 8 Fatty acid concentration in diet fed to Holstein and Jersey cows

Fatty Acid	μg/ml
Octanoate (C8:0)	0
Caprate (C10:0)	0
Laurate (C12:0)	77.8
Myristate (C14:0)	87.6
Palmitate (C16:0)	517.9
Palmitoleate (C16:1)	134.4
Stearate (C18:0)	189.1
Oleic (C18:1)	660.0
Linoleate (C18:2)	998.6
Linolenate (C18:3)	215.7
Arachidate (C20:4)	0

Table 9 Fatty acid concentrations in rumen fluid from Holstein and Jersey cows

	Holstein	Jersey	SE	P<
Octanoate (C8:0) μg/ml	0.55	1.77	0.71	0.23
Caprate (C10:0) μg/ml	80.2	94.03	11.44	0.40
Laurate (C12:0) μg/ml	59.4	75.7	7.59	0.14
Myristate (C14:0) μg/ml	48.96	75.7	14.2	0.19
Palmitate (C16:0) μg/ml	133.6	170.2	23.9	0.28
Palmitoleate (C16:1) μg/ml	2.22	3.2	1.12	0.05
Stearate (C18:0) μg/ml	38.26	59.31	14.4	0.31
Oleic (C18:1) μg/ml	20.22	65.8	19.65	0.11
Linoleate (C18:2) μg/ml	3.55	5.7	1.96	0.45
Linolenate (C18:3) μg/ml	0.0	0.0	0.0	N/A
Arachidate (C20:4) μg/ml	0.0	0.0	0.0	N/A

Table 10 Fatty acid concentration in serum from Holstein and Jersey cows

	Holstein	Jersey	SE	P<
Octanoate (C8:0) μg/ml	0.01	0.007	0.06	0.97
Caprate (C10:0) μg/ml	9.31	31.8	23.02	0.50
Laurate (C12:0) μg/ml	23.64	26.85	14.59	0.88
Myristate (C14:0) μg/ml	11.57	20.20	7.23	0.41
Palmitate (C16:0) μg/ml	159.07	147.53	16.82	0.63
Palmitoleate (C16:1) μg/ml	0	0	N/A	N/A
Stearate (C18:0) μg/ml	139.94	113.09	13.34	0.17
Oleic (C18:1) μg/ml	31.47	28.54	4.3	0.63
Linoleate (C18:2) μg/ml	216.15	160.23	39.47	0.33
Linolenate (C18:3) μg/ml	7.9	6.15	0.70	0.10
Arachidate (C20:4) μg/ml	0	0	N/A	N/A

Table 11 Concentrations of ruminal fluid, serum and milk samples

	Feed, Rumen, and Serum Fatty Acid Composition, μg/mL		
	Feed	Rumen[1]	Blood[1]
Octanoate (C8:0) μg/ml	0	1.16	0.0135
Caprate (C10:0) μg/ml	0	87.12	20.56
Laurate (C12:0) μg/ml	77.8	67.55	25.25
Myristate (C14:0) μg/ml	87.6	62.33	15.89
Palmitate (C16:0) μg/ml	517.9	151.9	153.3
Palmitoleate (C16:1) μg/ml	134.4	2.71	0
Stearate (C18:0) μg/ml	189.1	48.8	126.52
Oleic (C18:1) μg/ml	660.0	43.01	30.0
Linoleate (C18:2) μg/ml	998.6	4.63	188.19
Linolenate (C18:3) μg/ml	215.7	0	7.025
Arachidate (C20:4) μg/ml	0	0	0

[1] the blood and rumen were averaged for breed for this table.

Table 12 Fatty Acid Concentration in milk from Holstein and Jersey Cows

	Holstein	Jersey	SE	P<
Octanoate (C8:0) µg/ml	14.78	16.48	2.30	0.60
Caprate (C10:0) µg/ml	139.36	135.43	6.89	0.68
Laurate (C12:0) µg/ml	144.53	140.82	8.30	0.75
Myristate (C14:0) µg/ml	298.07	283.44	17.88	0.56
Palmitate (C16:0) µg/ml	509.94	475.15	25.70	0.34
Palmitoleate (C16:1) µg/ml	45.32	26.36	7.75	0.86
Stearate (C18:0) µg/ml	263.11	269.30	20.10	0.83
Oleic (C18:1) µg/ml	432.64	342.49	46.77	0.17
Linoleate (C18:2) µg/ml	86.77	58.90	13.27	0.14
Linolenate (C18:3) µg/ml	5.51	2.27	1.30	0.08
Arachidate (C20:4) µg/ml	0.25	0.04	0.1	0.13

Table 13 Fatty Acid Composition in Homogenized and Non-homogenized milk from Holstein and Jersey Cows

	Homogenized	Non-Homogenized	SE	P<
Octanoate (C8:0) µg/ml	14.25	17.0	2.03	0.23
Caprate (C10:0) µg/ml	134.82	140.0	5.77	0.40
Laurate (C12:0) µg/ml	138.73	146.62	6.92	0.29
Myristate (C14:0) µg/ml	279.37	302.14	14.96	0.16
Palmitate (C16:0) µg/ml	483.47	501.62	21.55	0.43
Palmitoleate (C16:1) µg/ml	37.75	33.92	6.93	0.65
Stearate (C18:0) µg/ml	251.55	280.86	16.50	0.08
Oleic (C18:1) µg/ml	357.05	418.08	39.51	0.16
Linoleate (C18:2) µg/ml	77.66	67.0	12.65	0.57
Linolenate (C18:3) µg/ml	4.42	3.36	1.22	0.50
Arachidate (C20:4) µg/ml	0.25	0.05	0.1	0.14

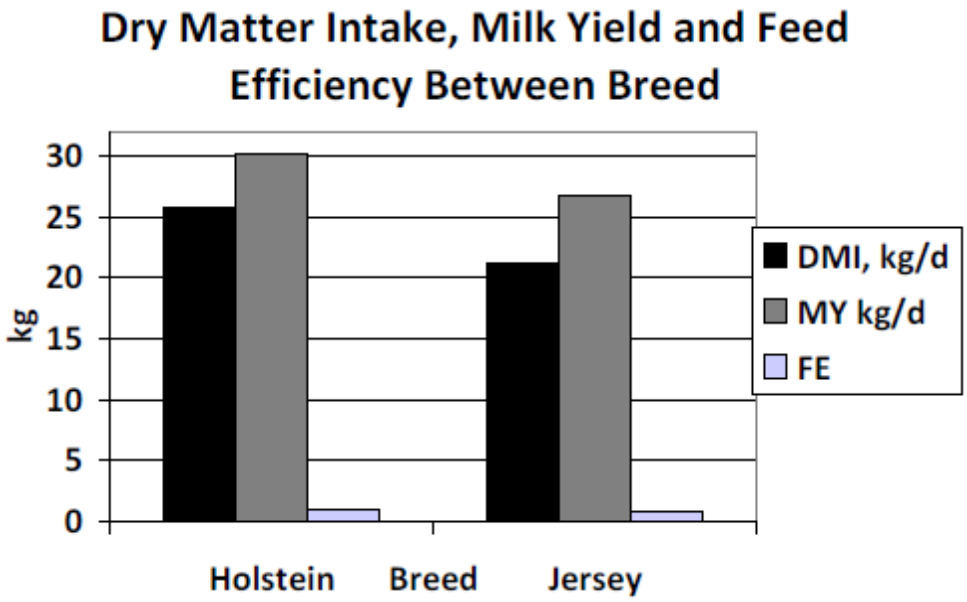

Figure 8 Dry matter intake, milk yield and feed efficiency in Holstein and Jersey cow

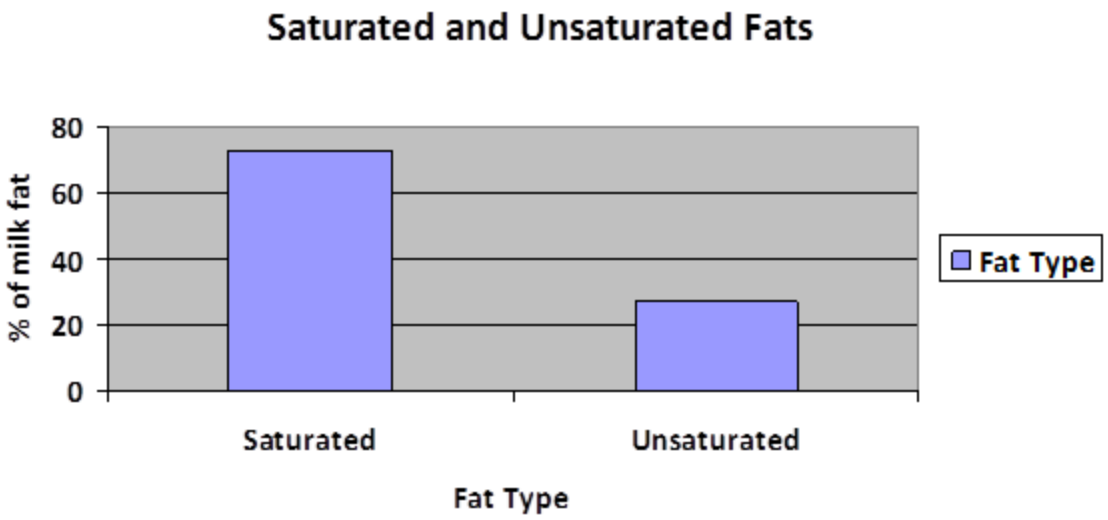

Figure 9 Saturated and unsaturated fatty acid % found in milk

CHAPTER III

CONCLUSIONS AND IMPLICATIONS

In conclusion, there is no significant difference in breed or processing that affects fatty acid composition. Data that was compiled and analyzed in this study showed that there is not a significant difference in processing of the milk that will alter the composition of milk that is consumed by the human population.

People should not believe everything that they hear or about the milk industry. After completing this study, when people make the claim that non-homogenized milk has better health effects on persons regarding high cholesterol, blood pressure or coronary heart disease, that indeed that is not the case. In regards to amounts and types of fatty acids found there is also no significant difference.

Research has shown that there is a positive correlation between the consumption of poly unsaturated fats and the decreased risk of these heart related issues.

With the information that was gathered during the White et al., study, the next step would be to conduct a study that investigates not only grazing versus confinement, but also altering the diet of those animals on the confinement diet. With the addition of oil seeds or the grasses from the grazing portion of the study considering grasses have increased amount of poly unsaturated fats compared to grass forages. A second portion would involve of feeding grasses versus forages. Currently there is no data available to indicate amounts of the polyunsaturated fats that survive the ensiling process. Analytical analysis of samples using GC/ MS would be adequate to complete. Running the GC/MS,

would give the breakdown of the samples, showing the differences in fatty acids and their concentrations that differ between grass and the dried forages. Milk samples could be run in the similar fashion as the study presented in this paper, looking at both the processing treatments, and also looking at the feeding treatments.

At the conclusion of this study, and based on data from other studies that have been conducted in the past, there is the ability to manipulate the fatty acid composition of milk based on the diet. The second study suggested above would need to be conducted to determine amounts of the poly unsaturated fats lost during the ensiling process. Based on the increase of concentrations conducted in the White et al., project, the potential for a significant difference in amounts found in the forages and then subsequently found in milk samples could be determined

This trial indicates that non-homogenized milk has the same health benefits as homogenized milk, putting to rest the idea that non-homogenized milk is “healthier” for consumers.

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