Genotype and Breed Trend Influences on Citric Acid and Coagulation Times of Raw Milk

by

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Abstract

The objective of the study was to determine if citric acid levels measured in milk was related to genetic variants identified in Holstein and Jersey cows. The data used were milk samples collected from both Holstein and Jersey cows at Cal Poly, San Luis Obispo. Citric acid levels and other constituents were measured using FTIR methods with the FOSS Milkoscan™ FT2 on each sample. Genotypes were obtained for the DGAT 1 locus using polymerase chain reaction and an enzymatic digestion using the MWO I restriction enzyme. Observations were obtained on 13 Holsteins and 12 Jersey cows. Results indicated that citric acid level, as a percentage was higher for the Jersey cows than for the Holstein cows -- 0.18 and 0.14, respectively. However, when protein and percent fat were included as independent variables in the statistical model, the difference between Holstein and Jersey for citric acid level was not significant. This indicated that the between breed difference was due to usual breed milk concentration differences.

Acknowledgements

I would like to thank Dr. Rafael Jimenez-Flores for his enthusiasm on this research project. It was his enthusiasm that encouraged me to learn more about a topic that was entirely new to me. I would also like to thank Andrea Laubscher, Erin Doherty, and Becca Mellema for their dedication on finding a solution to the research that continued to go wrong in one way or another.

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Introduction

Coagulation properties of milk are important in a biological sense in that the body has a longer time in order to digest the food and absorb the nutrients. In dairy foods, coagulation is important in cheese, sour cream, yogurt, etc. In order to achieve a high quality product, processors need to know the chemical, physical and thermodynamic properties of their raw material. There properties are dictated by the milk composition. This includes the fat, protein, lactose, as well as those smaller components such as citric acid. In order to determine the quantity of these components in our laboratory, we take a sample be taken and analyze via a quick determination using an FTIR method, or by individually analyzing the components using methods such as the Babcock fat determination and the Kjeldahl for protein determination. Once a processor is aware of what the general components of the milk are and how it behaves, it will be easier to know what needs to be done in order to obtain a higher quality end product.

Stage in lactation as well as feed intake likely play a role in the amount of citric acid present in milk. However, finer analysis of milk components such as proteins and enzymes demonstrate genetic variation, and with a variation in the properties of milk. Further understanding on how these variants influence the properties of milk, we need to analyze these variations. To analyze genetic variants in cows, there are many processes that have been proven to be effective. All techniques start by analyzing the DNA of the cow. On the market today, there are kits specifically designed to ensure a quick and easy extraction of the DNA from blood sample. This allows a somewhat quicker analysis in the laboratory, and creates a less stressful environment for the students. Though there are not many shortcuts that can be taken, the analysis of DNA is rather easy to follow. It involves the extraction of DNA followed by a

polymerase chain reaction focusing on one particular gene, and finally the addition of a restriction enzyme in order to observe whether or not the there are heterozygous or homozygous characteristics present. Of course, the primers for the PCR are specifically designed around the important genes that are selected for study.

Fat is one of the major components of milk and is primarily composed of triglycerides, a typical storage form of lipids Triglycerides account for over 95% of the total milk fat (Jensen, 2002).

In cattle, DGAT1 is considered to be an important gene related to the for fat percent and production of milk. It is located on the centromeric end of the bovine chromosome 14, within a region that contains quantitative trait locus (QTL). This influences milk yield and composition. The DGAT1 gene is important in milk yield, and is therefore used in many studies involving milk analysis. When doing a polymerase chain reaction, the DGAT1 gene is essential to observe in order to fully obtain information about genetic makeup of the cow (Grisart et al., 2003).

Comparing the components of citric acid and genotype has a possibility of providing future knowledge for breeding or selecting dairy cows. Our objective is to find whether or not there is a trend between citric acid levels measured in milk and genetic variants identified in the cows in order to improve coagulation properties in dairy processing.

Literature Review

Coagulation importance in the production of dairy products

Coagulation of milk is important for the manufacture of many dairy products manufactured today. In order to produce yogurt, cottage cheese and other various products, it is important to keep in mind the specific components necessary to allow specific coagulation time and adequate end results. If coagulation requirements are not met via temperature, time, acidity level, and the possible addition of a starter culture, the milk will not reach a curdling point at the precise time, and will fail to become a desirable product for the target market (Walstra, 1999).

The composition of milk components varies among breed of cow. However, the typical composition of milk is as follows:

Water 87.1%
Fat 4.0%
Lactose 4.6%
Protein 3.25%
Minerals 0.7%
Organic Acids 0.17%
Miscellaneous 0.15%

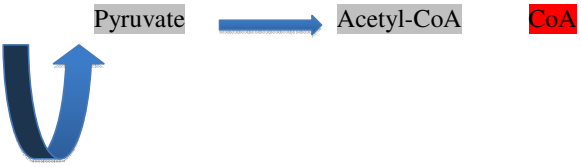
Using this information, it is appropriate to observe the variances in milk component composition varying among the breeds observed. Changes in composition occur from seasonal changes, feed intake of the cow, as well as the genetic makeup of the cow. These changes must be taken into consideration when analyzing the composition amongst Holstein and Jersey cows (Walstra, 1999).

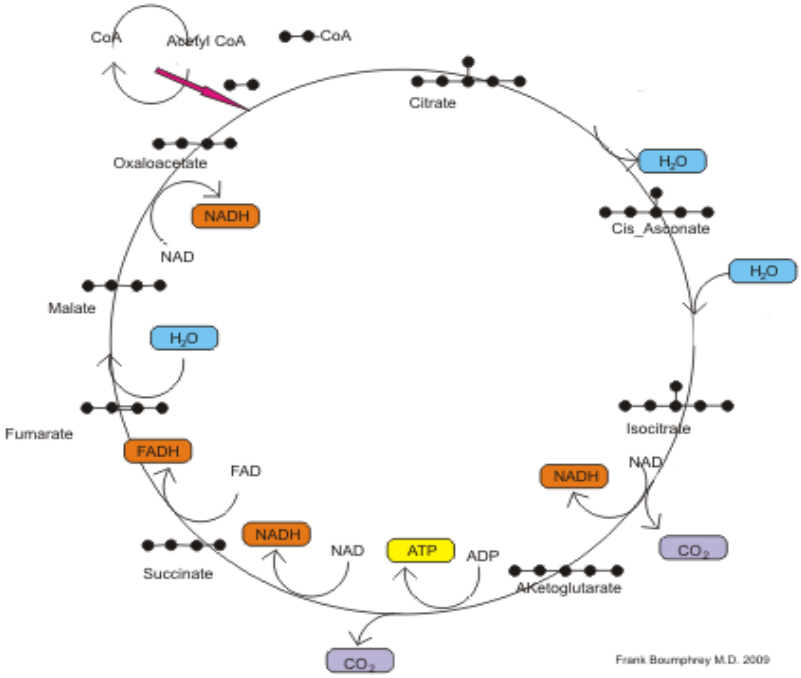
Citric Acid: Overview and Importance

Citrate is present in the milk of many animals. In bovine milk, the concentration of citrate present in the milk is approximately 150 mg per 100 mL. Citric acid is actively involved in the metabolism of plant and animal cells. Milk synthesis occurs in the alveolar cells of the udder, and there is approximately 90 fold the amount of citrate in milk as compared to that in blood (Davie, 1960). Therefore, the amount of citrate made readily available to the cow is an important measure in the determination of the coagulation characteristics of the milk produced. Mineral requirements of animals are influenced by stage of lactation. Lactation imposes large mineral demands on animals due to the mineral content of milk and minerals required in nutrient transport and metabolism (Larson, 1999).

Citrate is a constituent of milk that affects coagulation and flavor characteristics in milk processing. It is an intermediate in the Citric Acid cycle and plays an indirect role in fat synthesis by providing reducing equivalents in the form of NADPH. The citric acid cycle is an important process in the production of ATP, NADH and $FADH_2$, and the cycle directly follows Glycolysis (McMurry, 2009). This cycle contains a series of biochemical reactions that break down acetyl groups to product energy, which are carried by coenzymes and carbon dioxide. Milk contains varying amounts of citrate. In dairy foods, this is extremely important because fermentation products yield distinct aromatic flavors characteristic of fermented milk products (Rosenthal, 1991).

Figure 1. Citric Acid Cycle





Citric Acid Cycle

~~*Determination of citrate level in bovine milk* (Find ref. to HPLC methods and FT-IR methods)~~

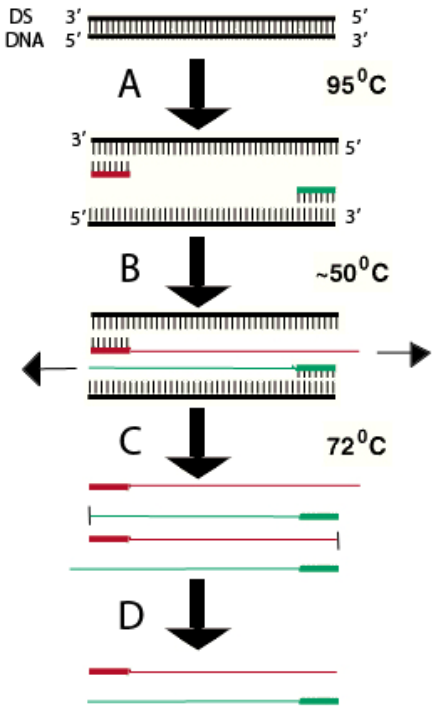
~~a. Today's methods in the genotyping of dairy cattle (Find references about genotyping)~~

Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a process of amplifying "a selected DNA sequence in a genome by a millionfold or more" (Snustad, 2003). This allows for scientists to obtain certainty on information about the DNA when there is not a large quantity of DNA readily available for analysis. Because significant amounts of a sample of DNA are necessary for molecular and genetic analyses, studies of isolated pieces of DNA are nearly impossible without PCR amplification. In a PCR reaction, specific primers are used, that are made in order to replicate specific base pairs of the loci of interest. Examples of Primers that can be used include Primer F (Forward) and Primer R (Reverse). These primers consist of a specific sequence of base pairs that are ample to the determination of the genetic makeup of the sample. Using this sequence, a restriction enzyme is eventually used to cut the base pairs at a specific site, yielding in an opportunity to observe the genotypic profile of the DNA. During the PCR process, a thermostable enzyme was used in order to simplify the process of amplifying the DNA. This enzyme is derived from *Thermus aquaticus*, and is useful so that the amplification temperatures can be done at higher temperatures, increasing yield of DNA. *Thermus aquaticus* (Taq) can survive incubation temperatures of 95°C (Yen et al, 2008). A schematic drawing of the PCR cycle can be found in Figure 3. (1) The first portion shows the denaturation occurring at 94-96 degrees C. (2) Annealing occurs at approximately 50 degrees C. (3) Elongation at 72 degrees C

(4) The first cycle is complete. The two resulting DNA strands make up the template DNA for the next cycle, thus doubling the amount of DNA duplicated for each new cycle (Meyer, 2005).

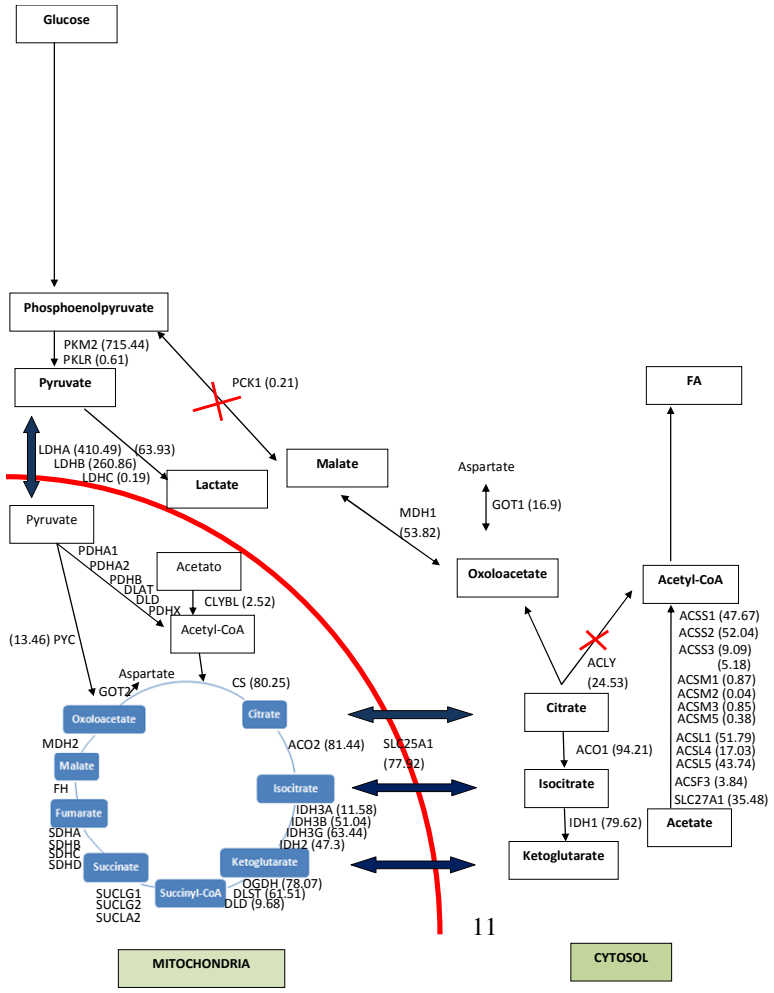
Figure 2. Polymerase Chain Reaction



Genotype Usefulness

An example of the usefulness of genotypes and milk processing quality was published by Medrano et al., where genetic variants of milk proteins such as kappa-casein were correlated with yield and properties of cheddar cheese (Medrano, 2010). Further studies concerning this topic are expected to be useful for the determination of the importance of fatty acids in cheese making.

Figure 3. Pathway of fatty acid synthesis in ruminant mammary tissue



Materials and Methods

Blood Collection:

Blood samples of Holstein cattle currently milked at California Polytechnic State University of San Luis Obispo were collected over the course of two days. The coccygeal blood samples were collected in the morning, directly after the 3:30 am milking shift had ended in the milking parlor. Specific safety measures were taken in order to ensure the safety of the cows following the blood drawing. This included the use of a hazardous sharps box to place the used needles in directly following blood drawing from each cow. Upon completion of milking, cows would return to the feed corral where they had an opportunity to freely consume a total mixed ration. Once cows arrived at the designated feeding corral, they were locked up using stanchion in order to allow for blood collection. The tail of the cow was raised and the surface of skin directly below the base of the tail was wiped with an alcohol wipe to allow a more sterile collection. The vein was found via touch, and a sterile needle with a vacuum tube was placed into the said area. A sterile blood collection vial containing a small amount of EDTA to help minimize blood clotting in the tube (BD Vacutainer Blood Collection Tubes, K2 EDTA (K2E) 10.8mg) was placed into the vacuum portion of the needle, and blood was then drawn into the tube. Following blood drawing, the tubes containing blood samples were inverted several times and were placed on ice before being taken to refrigeration for further analysis.

Blood Analysis:

Blood samples were placed in groups of twelve and were spun down using a centrifuge spin filter in order to obtain the genetic material portion of the blood. This was done by using the UltraClean ™ BloodSpin Kit provided by Mo Bio Laboratories, Inc. The protocol, as found on the Mo Bio Laboratories, Inc. website, lists step-by-step instructions, along with materials for ordering new kits (MO-BIO, 2010). Following running of the BloodSpin kit, the centrifuge tubes containing the DNA samples were properly labeled and were placed in the freezer for further analysis.

DNA Agarose Gel

A 1.5% Agarose gel with EtBr was run for 40 minutes at 90 V to ensure that the intensity of concentration of the DNA obtained from the BloodSpin kit was enough to continue with the analysis. This included weighing out 1.5 grams of Agarose and dissolving it in 100 mL of TBE buffering solution. After the Agarose was completely dissolved in the buffer solution, between 3 and 5 μL of Ethidium bromide was added to the solution as a way to fluoresce the bands upon completion of running the gel. 10 μL (microliters) of spun DNA was mixed with a dyed loading buffer, and was loaded into predetermined wells of the Agarose gel. The gel was then run with the DNA samples running toward the positive end of the voltage, as DNA has a negative charge (because of the phosphate ions in its chemical backbone.) After the DNA had been run on the Agarose gel for 40 minutes, the DNA was looked at under UV Transillumination in order to observe the intensity of the bands formed, and a picture was taken for records. If the bands

appeared weak in intensity when observed under UV Transillumination, the bloodspin was completed a second time on the blood samples in order to ensure that the concentration of DNA obtained from the Blood Spin kit was enough to fulfill the following steps to determine the genetic makeup of the Holstein cattle. If the bands were clearly seen, those samples could be moved forward in order to complete a polymerase chain reaction.

Polymerase Chain Reaction:

Groups of twenty samples of Holstein DNA were set aside to perform a polymerase chain reaction (PCR) using specific primers for differentiating polymorphisms of the DGAT gene in order to amplify a single piece of DNA across several orders of magnitude, generating thousands of copies of the particular DNA sequence. The primers used were Primer F and R (Forward and Reverse). The base pair sequence are as follows:

DGAT F: 5' CCTGATGGTCTACACCATCC 3'

DGAT R: 5' CAGGATCCTCACCGCGGTAG 3'

Volumes of each reagent used and required are listed in the column farthest right of Table 2, "Volume (µL)," and a master mix was prepared to simplify the process. To determine the volume of each reagent to be mixed in the master mix, the quantities on the farthest right column were multiplied by 25. This slight excess allowed for slight error, in order to have the proper amount for each sample. This method can be used for each PCR set done. Prior to the preparation of the master mix, the ventilated hood to be used for a sterile environment was cleaned thoroughly with 70% Ethanol and Bleach prior to use. Once the master mix was

prepared with Taq added last, the samples were ready for preparation. 48 μL of the master mix, along with 2μL of template DNA were added to the designated well of autoclaved PCR strips. This process was done rapidly, as the samples were not placed on ice during preparation. The Taq reagent is very temperature dependent and should be kept to as close as 4 degrees Celsius as possible during use, in order to increase yields of DNA replication. Because the samples were not kept on ice during preparation, the samples were made as quickly as possible, and were run on the PCR machine directly after the DNA template was added to the reagents. This allowed the Taq reagent to better have the ability to aid in the replication of DNA during the PCR process. Among the DNA samples, the master mix as well as the hood in which it was prepared was tested for contamination by incorporating an "open" and "closed" well in the PCR strips. This entailed that two wells would contain the master mix sans the DNA template, and one would be left open during the strip preparation process, and the other would remain closed with the master mix included. This created an opportunity to determine if one of the reagents was not working properly, as well as to determine if the hood had any sort of contamination prior to preparation of the samples.

Following the addition of master mix and template DNA, the PCR strips were placed into the PCR machine and were run for the required temperatures and times involved in the PCR in order to successfully generate many copies of the DNA. These temperature and time changes required can be found in Figure 3.

Table 1. Blank template of PCR worksheet.

Sample

	1	2	3	4	5	6	7	8
A								
B								
C								

Table 2. Master Mix worksheet

Reagent	Total Volume (µL)	Volume (µL)
Template DNA	---	2
10 X Buffer		5
dNTP (10mM)		1
DMSO		2.5
MgCl2		3
Primer (F)		2
Primer (R)		2
NanoPure H20		32.25
Taq (5U/µL)		0.25

Volume of master mix to each: 48 µL

Following the Polymerase Chain Reaction, a 2.5% Agarose gel was run on the samples contained in the PCR strips in order to determine if the PCR process had successfully replicated

the DNA. This involved the same process as the original BloodSpin gel, but involved the addition of 2.5 grams of Agarose to 100 mL of TBE Buffer solution as well as 5 microliters of Ethidium Bromide. Ethidium bromide is used as a "dye" in order to see the DNA samples under UV light. The gel was run for 40 minutes at 90 V, and was then looked at under UV Transillumination to determine if the polymerase chain reaction successfully replicated the DNA. If this occurred, the replicated DNA contained in the PCR strips was used with a restriction enzyme in order to determine the genotype, using the DGAT gene.

Restriction Enzyme Digestion:

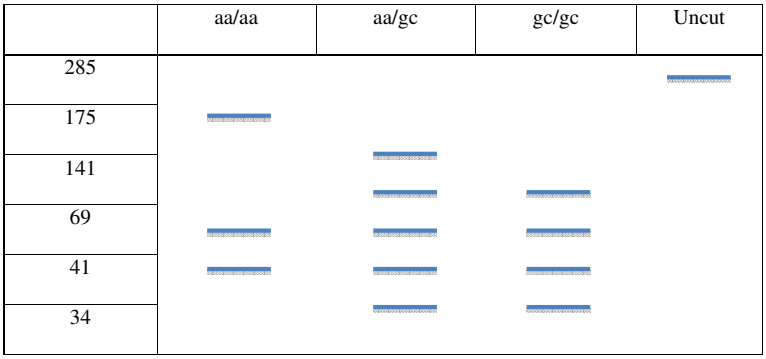
Upon completion of the PCR, the samples took part in a restriction enzyme digestion using the enzyme MwoI. This restriction enzyme cuts the sequence from the polymerase chain reaction at the following recognition site:

5' . . . G C N N N N N * N N G C . . . 3'

3' . . . C G N N N * N N N N C G . . . 5'

The source of MwoI is from an *Escherichia coli* strain that carries the cloned MwoI gene from *Methanobacterium wolfeii.* This restriction enzyme was used specifically in order to observe the genotypes AA, AG, and GG in the Holstein and Jersey DNA samples collected. After the sequence was cut, the following table (Table 3) can be used in order to observe the genetic variances. The bands on the 3.5% Agarose gel are clearly different for each genetic makeup.

Table 3. Genotypes gel band breakup using a 3.5% Agarose gel.



This was done in order to distinguish between the various genetic makeups of the Holstein dairy cattle at California Polytechnic State University in San Luis Obispo. It was determined with this step whether or not each individual cow was either heterozygous or homozygous, and if the genetic makeup yielded to be AA, AG, or GG.

Volumes required are as follows:

Table 4. Restriction enzyme master mix worksheet

Reagent	Total Volume (Master Mix)	Volume
Sterile nanopure Water	45 µL	1.5 µL
Buffer	75 µL	2.5 µL
MWO I	30 µL	1 (5U) µL
PCR product	---	20 µL

Master mix to each : 5 µL

A master mix was prepared, and 5 µL was added to each pre-autoclaved centrifuge tube to be used for the enzymatic digestion along with 20 µL of PCR product. The samples were then placed in a 60 degree Celsius water bath overnight, and a 3.5% Agarose gel was run for 40 minutes at 90 V the following morning. The gel was then looked at under UV Transillumination

and each sample was looked at individually to conclude the genetic variances of the Holstein dairy cattle.

Milk Collection and Analysis:

Samples containing at least 30 mL of milk were collected during an afternoon milking shift in the month of September for the Holstein cows when a California state milk inspector was present. Milk samples were collected in April, 2010 for the Jersey's samples. A vacuum collection system was put in place, and samples were collected and immediately placed into refrigeration to be tested the following morning.

The milk samples were tested using a FTIR FOSS Milkoscan™ FT2 system. Each sample was thoroughly inverted several times prior to testing. A spreadsheet of the components of the milk, including the citrate level was saved for future comparison with the restriction enzyme digestion results.

Statistical Analysis

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Citric acid level in milk was included as a dependent variable in the following statistical model with breed, genotype, and lactation number (same as age of cow) were included as class fixed effects and days fresh was included as a fixed linear regression. In a second analysis the independent variable of percent fat reported from DHIA test day was included as a fixed linear regression. In a third model, the independent variable of infrared percent protein was included in the first model as a fixed linear regression. The independent variables of test day fat and infrared

percent protein were analyzed in an attempt to determine if the effects observed in the first model were the result of factors such as differences in milk concentration (or % water). Data were analyzed using the PROC GLM procedure in SAS.

Results and Discussion

Table 5. Cow ID number and genotype by breed.

Jersey	Genotype	Holstein	Genotype
CP-105	AG	CP-1983	AG
CP-110	AG	CP-2033	AG
CP-128	AG	CP-2060	GG
CP-135	AA	CP-2066	GG
CP-158	AG	CP-2068	AG
CP-174	AA	CP-2083	GG
CP-807	AG	CP-2119	GG
CP-870	GG	CP-2123	AG
CP-880	AG	CP-2201	GG
CP-886	GG	CP-2214	AG
CP-891	GG	CP-2232	GG
CP-903	GG	CP-2237	GG
CP-939	AG		

Table 6. Count of breed by genotype.

Count of Breed				
	AA	**AG**	**GG**	**Grand Total**
Holstein		5	7	12
Jersey	2	7	4	13
Grand Total	**2**	**12**	**11**	**25**

Table 7. FTIR results with the FOSS Milkoscan™ FT2

Cow ID	Breed	DSF	Lactation	Age (Months)	Last Fat %	305 me Milk	Pen No.	Fat %	Protein %	Casein %
CP-105	J	177	5	63.2	4.70	28610	1	4.8	3.64	2.73
CP-110	J	238	3	62.3	4.70	13970	1	1.521	3.71	2.91
CP-128	J	68	3	58	4.70	18170	1	0.861	3.41	2.67
CP-135	J	57	3	57.1	4.40	20580	1	4.34	3.3	2.64
CP-158	J	20	3	54.5	4.70	19290	1	3.811	3.96	3.1
CP-174	J	253	2	52.1	6.20	20680	1	4.507	4.3	3.3
CP-807	J	102	5	89.2	4.60	22540	1	4.467	3.21	2.43
CP-870	J	316	4	77	4.50	19080	1	1.965	3.72	2.83
CP-880	J	112	4	75.3	5.60	21950	1	1.292	3.7	2.97
CP-886	J	223	5	74.3	4.40	17050	1	1.328	3.61	2.83
CP-891	J	204	4	73.5	3.50	25320	1	2.551	3.33	2.48
CP-903	J	39	4	71.9	4.60	24940	1	1.437	3.11	2.46
CP-939	J	277	3	65.4	5.30	18540	1	1.185	4.06	3.12
CP-1983	H	218	5	101.9	5.5	20670	3	5.948	3.27	2.41
CP-2033	H	251	5	87.9	3.4	27410	3	2.901	3.52	2.61
CP-2060	H	65	5	81.4	5.2	17750	3	5.647	2.74	1.97
CP-2066	H	291	5	80.1	3.9	32410	3	4.119	3.38	2.37
CP-2068	H	254	5	79.5	4.8	28630	3	4.945	3.43	2.61
CP-2083	H	352	4	75.5	3.6	36760	3	3.358	3.44	2.37
CP-2119	H	230	4	67.7	3.5	14370	3	3.42	3.34	2.09
CP-2123	H	96	4	67.4	4.2	27270	3	3.7	2.89	2.2
CP-2201	H	166	3	63.3	4.4	26140	3	3.937	3.55	2.56
CP-2214	H	71	3	59	4.3	20560	3	4.596	3.44	2.58
CP-2232	H	128	3	54	3.9	30120	3	4.005	3.34	2.33
CP-2237	H	353	2	52.3	4.1	32220	3	4.295	3.78	2.61

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Table 8. FTIR results with the FOSS Milkoscan™ FT2 continued

Cow ID	Citric Acid %	Lactose %	Total Solids %	SNF %	Acidity	Urea	Density	FPD	FFA	Genotype
CP-105	0.2	4.72	14.3	9.32	18.63	312	1029.2	513	0.212	AG
CP-110	0.18	4.9	10.94	9.41	15.88	385	1030.8	504	0.318	AG
CP-128	0.22	4.93	9.97	9.22	19.18	482	1029.3	523	0.205	AG
CP-135	0.21	4.82	13.47	9.04	15.12	449	1027.6	521	0.114	AA
CP-158	0.18	5.1	13.78	9.91	18.19	392	1033.5	509	0.108	AG
CP-174	0.16	4.61	14.49	9.74	19.06	437	1031.2	511	0.49	AA
CP-807	0.19	4.62	13.31	8.72	14.98	461	1026.8	514	0.124	AG
CP-870	0.17	4.89	11.47	9.46	19.4	386	1030.9	520	0.565	GG
CP-880	0.22	4.84	10.68	9.41	17.27	378	1030.3	520	0.122	AG
CP-886	0.17	4.83	10.62	9.27	19.15	385	1030.1	511	0.394	GG
CP-891	0.16	4.78	11.54	8.94	18.14	458	1028.9	512	0.419	GG
CP-903	0.22	4.94	10.29	8.95	15.06	387	1029	515	0.271	GG
CP-939	0.12	4.77	10.79	9.55	20.2	525	1032.4	514	0.539	AG
CP-1983	0.1	4.74	14.97	8.76	14.41	310	1026.9	522	0.592	AG
CP-2033	0.15	4.68	12.08	9.06	15.29	341	1028.5	531	0.365	AG
CP-2060	0.25	4.34	14.06	8.22	15.94	474	1023.5	532	0.698	GG
CP-2066	0.16	4.64	13.27	8.93	14.14	326	1027.2	512	0.683	GG
CP-2068	0.15	4.79	14.27	9.13	17.82	413	1028.5	527	0.369	AG
CP-2083	0.14	4.49	12.32	8.79	15.2	349	1027.1	526	0.5	GG
CP-2119	0.12	3.64	11.55	7.88	15.72	288	1022.7	531	0.413	GG
CP-2123	0.14	4.75	12.25	8.51	15.48	487	1027.2	530	0.231	AG
CP-2201	0.14	4.79	13.33	9.22	19.28	347	1029.7	523	0.728	GG
CP-2214	0.16	4.98	14.02	9.26	14.89	357	1029.5	521	0.443	AG
CP-2232	0.15	4.65	13.02	8.88	16.73	301	1028.2	525	0.599	GG
CP-2237	0.15	4.61	13.77	9.32	17.52	310	1030.2	517	0.677	GG

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Table 9~~6~~. Citric acid values – Breed vs. Genotype

Genotype	AA		AG		GG			
Breed	Avg of Citric acid	StdDev of Citric acid	Avg of Citric acid	StdDev of Citric acid	Avg of Citric acid	StdDev of Citric acid	Total Avg of Citric acid	Total StdDev of Citric acid
H			0.14	0.0235	0.15857	0.042200	0.15083	0.0355370
J	0.185	0.0354	0.187142	0.034	0.18	0.027080	0.18461	0.02961288
Grand Total	0.185	0.0353	0.1675	0.0377	0.1663	0.03748	0.1684	0.0362491

Table 10~~7~~. Fat percentage values – Breed vs. Genotype

Genotype	AA		AG		GG			
Breed	Average of Fat	StdDev of Fat	Average of Fat	StdDev of Fat	Average of Fat	StdDev of Fat	Total Average of Fat	Total StdDev of Fat
H			4.418	1.1690	4.11157	0.7614	4.23925	0.9155332
J	4.4235	0.1180	2.5624	1.7167	1.82025	0.5609	2.6203846	1.5201065
Grand Total	4.4235	0.1180	3.3355	1.7371	3.27836	1.3336	3.39744	1.4902772

Table 11~~8~~. Protein percentage values – Breed vs. Genotype

Genotype	AA		AG		GG			
Breed	Average of Protein	StdDev of Protein	Average of Protein	StdDev of Protein	Average of Protein	StdDev of Protein	Total Average of Protein	Total StdDev of Protein
H			3.31	0.251694	3.3671428	0.31731	3.3433333	0.280756
J	3.8	0.70710	3.67	0.294052	3.4425	0.27584	3.62	0.3482575
Grand Total	3.8	0.70710	3.52	0.323363	3.3945454	0.29101	3.4872	0.341571

Table 12~~9~~. Casein percentage values – Breed vs. Genotype

Genotype	AA		AG		GG			
Breed	Average of Casein	StdDev of Casein	Average of Casein	StdDev of Casein	Average of Casein	StdDev of Casein	Total Average of Casein	Total StdDev of Casein
H			2.482	0.178241	2.328571	0.23154	2.3925	0.2168839
J	2.97	0.4667	2.8471	0.2505137	2.65	0.20800	2.805384	0.2718950
Grand Total	2.97	0.4667	2.695	0.2848444	2.445454	0.26729	2.6072	0.3206961

Table 13~~0~~. Lactose percentage values – Breed vs. Genotype

Geno-type	AA		AG		GG			
Breed	Average of Lactose	StdDev of Lactose	Average of Lactose	StdDev of Lactose	Average of Lactose	StdDev of Lactose	Total Average of Lactose	Total StdDev of Lactose
H			4.788	0.114	4.451	0.38451	4.5917	0.339754814
J	4.715	0.15	4.84	0.157	4.86	0.06976	4.8269	0.13362788
Grand Total	4.715	0.15	4.8183	0.137	4.6	0.36423	4.714	0.276088754

Table 14~~1~~. Total Solids percentage values – Breed vs. Genotype

Genotype	AA		AG		GG			
Breed	Avg of TS	StdDev of TS	Avg of TS	StdDev of TS	Avg of TS	StdDev of TS	Total Avg of TS	Total StdDev of TS
H			13.518	1.2847	13.0457	0.861101	13.2425	1.0313727
J	13.98	0.7212	11.9671	1.7615	10.98	0.621664	11.97307692	1.6405811
Grand Total	13.98	0.7212	12.6133	1.7118	12.2945	1.283366	12.5824	1.5007561

Table 15~~2~~. Solids Non Fat percentage values – Breed vs. Genotype

Genotype	AA		AG		GG			
Breed	Average of SNF	StdDev of SNF	Average of SNF	StdDev of SNF	Average of SNF	StdDev of SNF	Total Average of SNF	Total StdDev of SNF
H			8.944	0.3042	8.74857	0.521933	8.83	0.438592791
J	9.39	0.495	9.36286	0.3592	9.155	0.254624	9.3030769	0.334374656
Grand Total	9.39	0.495	9.18833	0.388	8.89636	0.474284	9.076	0.449731401

Table 16~~3~~. Density values – Breed vs. Genotype

Genotype	AA		AG		GG			
Breed	Average of Density	StdDev of Density	Average of Density	StdDev of Density	Average of Density	StdDev of Density	Total Average of Density	Total StdDev of Density
H			1028.12	1.06395	1026.94	2.87915	1027.4333	2.30230451
J	1029.4	2.5456	1030.33	2.21187	1029.73	0.9535	1030	1.83348484
Grand Total	1029.4	2.5456	1029.41	2.09131	1027.95	2.6864	1028.768	2.41311003

Table 17~~4~~. Least Squares Means of Citric Acid: Breed

Breed	Citric Acid LS Mean
Holstein	0.15254662
Jersey	0.18469337

Table 18~~5~~. Least Squares Means of Citric Acid: Genotype

Genotype	Citric Acid LS Mean
AA	0.16334761
AG	0.16158182
GG	0.18093057

Table 19~~6~~. Least Squares Means of Citric Acid: Lactation Number

Lactation No.	Citric Acid LS Mean
2	0.18030105
3	0.15497780
4	0.16052039
5	0.17868075

Table 20~~7~~. Least Squares Means of Citric Acid with fat: Breed*

Breed	Citric Acid LS Mean
Holstein	0.16144863
Jersey	0.18290371

* % fat added into the equation

Table 21~~18~~. Least Squares Means of Citric Acid with fat: Genotype*

Genotype	Citric Acid LS Mean
AA	0.17397562
AG	0.16200532
GG	0.18054756

* % fat added into the equation

Table 22~~19~~. Least Squares Means of Citric Acid with fat: Lactation Number*

Lactation No.	Citric Acid LS Mean
2	0.18728747
3	0.15501629
4	0.16027416
5	0.18612675

* % fat added into the equation

Table 23~~0~~. Least Squares Means of Citric Acid with protein: Breed*

Breed	Citric Acid LS Mean
Holstein	0.14906809
Jersey	0.19110078

* % protein added into the equation

Table 24~~18~~. Least Squares Means of Citric Acid with protein: Genotype*

Genotype	Citric Acid LS Mean
AA	0.16359430
AG	0.16611523
GG	0.18054378

* % protein added into the equation

Table 25~~19~~. Least Squares Means of Citric Acid with protein: Lactation Number*

Lactation No.	Citric Acid LS Mean
2	0.19210117
3	0.15937489
4	0.15555327
5	0.17330841

* % protein added into the equation

In the first statistical model where the dependent variable of citric acid level in milk was evaluated with the independent variables of breed, lactation number, days since fresh (DSF), and genotype, it was found that both breed and the length of days since fresh had a significant effect on citric acid level. The coefficient of determination for this model (R^2) was 0.59, which means that approximately 59% of the variation in the observed citric acid data can be described by the linear regression model used. The p-values for breed and DSF were 0.0186 and 0.0025, respectively. The regression coefficient for citric acid level on DSF indicated an inverse relationship such that for every 1 day increase in days in milk, the citric acid level found in the milk decreased by 0.000239%. When contrasting the three various genotypes of interest (AA versus AG, GG), it was found that there was no difference between genotypes for citric acid level (p-value= 0.7590. Tables 17-19 show the least squares means of citric acid by breed, genotype and lactation number.

When test day fat percentage was included in the model (second model) it was found that only the length of days since fresh was significant (p-value = 0.0024) and the model had an R^2 value of 0.60. The regression of citric acid level on DSF in this model was -0.000262%. Again, genotype was not statistically significant (p-value= 0.9251). Tables 20-22 show the least squares means of citric acid when percent fat was added to the general linear model.

When infrared protein percentage was included in the model breed was significant (p-value = 0.0184). It was also found that days since fresh is ~~directionally significant~~has potential significance~~,~~ -with a p-value of 0.0777. The R^2 was 0.61. The regression coefficient for citric acid level on DSF was -0.0001761717%. When contrasting the three various genotypes of interest (AA versus AG, GG), it was found that the AA genotype was not statistically significant

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(p-value= 0.7073). Tables 23-25 show the least squares means of citric acid when percent protein was added to the general linear model.

Discussion

In the sample size used in this study (n=25), it was found that both breed and days since fresh play a significant role (P=0.0186, P=0.0025, respectively) in the citric acid level found in milk. As the cow continues to be milked throughout the 305-day milk period, the citric acid level will likely decrease each day. – We expect citrate to depreciate over time, both during milking as well as during processing techniques used during manufacture of dairy products. Therefore, there is a chance that the trend between genetic variances and level of citrate varies from cow to cow based on the length of days in milk after freshening.

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Each Holstein cow was fed the same diet at the time in which the milk was collected on September 11, 2010. However, the milk collected from the Jersey's was collected 5 months prior on April 10, 2010. This created an opportunity for the feed composition to change, and this may have allowed the Holstein cows to produce more or less citrate based on the diet fed. In order to determine if there is a significant trend, further studies need to be conducted.

Upon obtaining results from the FTIR methods with the FOSS Milkoscan™ FT2, the results for the fat percentage found in Jersey milk appeared to be much different than a typical fat percentage in jersey cattle. Some cows yielded over 10% fat while others yielded less than 1%. This caused concern for the reliability of the FTIR methods, and it was decided that the fat percentages obtained would not be sufficient for analysis. Therefore, the fat percentage used in the statistical analysis was that obtained from the DHI records recorded in September 2010 for Jersey cows. It is recommended that further analysis on the reliability of the FOSS Milkoscan™ FT2 be done in the future.

Conclusion

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Based on the data obtained, both days in milk and breed significantly influenced the citric acid percentage found in raw milk obtained from both Holstein and Jersey dairy cows. For each day in milk, the citrate level is likely to have decreased.

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