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Milko-Tester Analysis of Milkfat Content of Churned and Nonchurned Samples from Jersey and Holstein Dairy Cattle

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MILKO-TESTER ANALYSIS OF MILKFAT CONTENT OF CHURNED AND NONCHURNED SAMPLES FROM JERSEY AND HOLSTEIN DAIRY CATTLE

by

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Kazuko Monobe

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Milko-Tester Analysis of Milkfat Content of Churned and Nonchurned Samples from Jersey and Holstein Dairy Cattle

by

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Utah State University, 1977

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Department: Nutrition and Food Science

Milk samples from three Jersey and three Holstein herds were used to determine if the degree of churning correlated with the initial milkfat assay, and also to determine if the churned milkfat could be measured as accurately as the initial milkfat using the Milko-Tester.

Regression lines for the initial milkfat test versus churned milkfat test fit polynomial curves. The degree of churning was greater for Jerseys than Holsteins and correlated with the initial milkfat test.

The repeatability of initial milkfat tests was 0.98 for Jerseys and 0.99 for Holsteins. However, the repeatability of churned milkfat tests was lower for both breeds, especially for Jerseys. This indicated that the churned sample could not be measured for its milkfat content as accurately as the nonchurned sample. Churned milkfat samples all tested lower than initial milkfat samples, thus the Milko-Tester Mark III was unable to provide an initial fat test estimate following sample churning.

Cows were different from each other in the difference between initial and churned milkfat tests. This suggested that cows which produce milk

with a higher tendency for churning are apt to receive less reliable milkfat tests from central laboratories than other cows, when all milk samples are subjected to churning conditions.

(47 pages)

INTRODUCTION

Dairy farmers are paid on the basis of the milkfat content of the milk produced, thus breeding and culling of cows takes milkfat content into account. Bulls are proven on the basis of the performance of their daughters in total pounds of milk and milkfat produced each year.

In order to measure the milkfat content of milk from each cow in the herd, many dairy farmers in Utah and many other states send their milk samples to Dairy Herd Improvement Association (DHIA) central laboratories which are equipped with Foss Milko-Testers (automatic electronic milkfat testers, manufactured by Foss Electric, Hillerod, Denmark).

Before the introduction of the Milko-Tester (M-T) in 1968, milkfat was measured in the USA by DHIA supervisors using the Babcock method. The milk was tested either at the dairy farm or at the supervisors' residence. Some states still practice this method, but there is a national trend toward employing the M-T in central laboratories. Khalil and Layton (1972) reported that the M-T has replaced the Babcock method because of: (1) simplicity of operation, (2) small space requirement, (3) elimination of the handling of strong chemicals, (4) determination of milkfat in shorter time, and (5) excellent correlation between the M-T and the Babcock method under normal plant conditions.

With the replacement of the Babcock method by the M-T, the DHIA changed to centralized testing laboratories. This was primarily due to the fact that the M-T was too expensive (in 1976 the cost was $15,500 for Mark III models and $19,600 for Industrial models) for the DHIA to provide one for each supervisor for field use. Also, greater test reliability was expected if all samples were run through fewer instruments.

One of the problems with a single testing laboratory serving a large geographical area such as Utah relates to getting the samples to the laboratory in good condition. Leamy and Nilson (1974) stated that from the time of the introduction of centralized testing with the M-T, there have been complaints about low milkfat readings. According to Kroger et al. (1968), the churning of milk samples during shipment was one cause of the low fat readings. In 1968 Kroger et al. reported that churning was caused by the well-known disruption of the milkfat emulsion in the milk samples. This made it impossible to introduce a representative sample into the M-T, thus producing low readings.

There are six factors known to influence churning, and therefore the milkfat test. They are: (1) temperature, (2) agitation, (3) reconstitution of milk, (4) chemical additives, (5) breed of cow, (6) size of milkfat globules, and (7) freezing.

Moyes et al. (1975) observed that lowered milkfat readings were more evident in Jersey than in Holstein milk samples. Since there are differences in the tendency for churning in different breeds, it is probable that there may be similar differences among individual animals within a breed. If so, cows which produce milk with a higher

tendency for churning would consistently receive lower fat test reports if churning occurred during delivery to testing facilities.

The objectives of this study were: (1) to determine whether the amount of milkfat loss due to churning was proportional to the total milkfat content in Jersey and Holstein samples, (2) to determine whether the apparent milkfat content of churned samples can be measured with the same precision as nonchurned samples using the M-T, and (3) to evaluate whether some animals would be consistently biased against if their milk were subjected to adverse milk sample shipment conditions.

The definition of terms used in this thesis follows:

Initial: The percent milkfat assay on the M-T before churning.

Churned: The percent milkfat assay on the M-T after churning.

Difference: The mean Churned subtracted from the mean Initial of individual cows.

REVIEW OF LITERATURE

Milkfat

Physical properties of milkfat. The average density of milkfat has been found to be 0.8892 gr/ml at 60 C, and the average coefficient of expansion is 78.34 X 10-5/ml/ml/C for the temperature range of 30 to 60 C. The density of purified milkfat was found relatively constant and not affected to any marked extent by breed, season or feed (Jennes et al., 1942).

Milkfat globules have a surface membrane consisting of proteins, phospholipids and other substances. The phospholipids act as the primary emulsion stabilizers. They have both nonpolar and strongly polar groups, and therefore orient themselves on the surface of milkfat globules with their polar groups in the aqueous phase. According to Pyenson and Dahle (1938), the membrane is hydrated with 15 percent of the total bound water in the milk, thereby stabilizing the fat emulsion by keeping the fat globules separated. This separation of the milkfat globules is assured further by their electrical charges. The charge of milkfat globules is negative at pH above the iso-electric point (pH 4.0 to 4.5). This electrical charge inhibits the aggregation of globules by causing an electrical repulsion between them.

Milkfat globules vary from 0.1 to 22 micron in diameter, with the majority being from 1 to 5 micron. The size of the milkfat globules

varies among breeds, with the higher test breeds having larger milkfat globules (Webb, Johnson and Alford, 1974).

Table 1. Distribution of the fat phase in the milk of four breeds of cows according to the size of fat globules.[a] (Webb, Johnson and Alford, 1974)

Breed	Average size of fat globules in distribution classes					
	0-2.4μm	2.4-4.8μm	4.8-7.2μm	7.2-9.6μm	9.6-12.0μm	12.0-14.4μm
	Percentage of fat globules in each of the groups[b]					
Jersey	8.1	38.3	32.1	18.1	5.3	1.1
Guernsey	6.5	38.9	35.0	14.4	4.4	0.7
Aryshire	14.6	54.0	23.4	6.2	1.6	0.2
Holstein	14.5	54.6	24.5	5.1	1.1	0.2
	Percentage of total fat in each of the groups					
Jersey	0.1	11.3	26.1	30.7	23.9	7.9
Guernsey	0.1	11.3	33.2	29.7	25.7	-
Aryshire	0.3	34.0	41.6	17.8	6.3	-
Holstein	0.3	38.3	50.1	11.3	-	-

[a]Data from Van Slyke

[b]Approximately 1,000 globules counted

Smith (1959) also summarized that there is a tendency for the higher testing cows within each breed to produce milk with larger milkfat globules than do the lower testing cows. Fat globules become smaller with advancing lactation, particularly if cows are on dry feed. Hilditch and Hasperson (1943) observed that cows on dry feed and advanced in lactation have smaller milkfat globules that are packed more closely together, thus causing a shallow cream line.

Sommer (1951) explained the difference in globule size as follows: Triglyceride molecules secreted by the mammary gland combine with other triglyceride molecules. When phospholipids present in the fluid (or

simultaneously secreted) are deposited randomly on the growing globules, the surface of the milkfat globules are completely covered with phospholipids. The phospholipids on the surface of milkfat globules prevent further growth of milkfat globules. When triglycerides are secreted rapidly, the globules grow to a larger size before becoming sealed off with phospholipids.

Components of milkfat. The main milkfat components are triglycerides of fatty acids (Webb, Johnson and Alford, 1974). The triglycerides constitute 95 to 96 percent of all components. The remainder of the components include phospholipids (0.2 to 1.0 percent), sterols (0.25 to 0.40 percent), and a trace amount of free fatty acids, mono- and diglycerides, wax, squalene, fat soluble vitamins, and fat soluble pigments. The relative proportions of these compounds in milkfat are affected by the breed of cow, feed and stage of lactation.

There are 64 different fatty acids in milkfat, ranging from 4 to 26 carbon atoms with a relatively high proportion of short chain and saturated fatty acids, many of which are not found in other fats (Anon., 1965).

Morrison, Jack and Smith (1967) reported that the majority of fatty acids of phospholipids phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine are 16:0, 18:0 and 18:1 fatty acids.

The average milkfat content of milk from individuals within dairy breeds. Although there is more than 2 percent milkfat difference between a high testing breed (Jersey) and low testing breed (Holstein), Hastings (1944) stated that the difference in milkfat test between animals of the same breed is sometimes even greater as shown in Table 2 and Figure 1.

Table 2. Maximum, minimum values for milk constituents of individual cows of three breeds (Davis, 1947)

	Breed					
	Jersey		Guernsey		Holstein	
No. of cows	11		11		12	
No. of samples	160		139		207	
	Max.	Min.	Max.	Min.	Max.	Min.
Percent butterfat	7.8	3.4	6.7	3.1	7.8	2.6

In 1958 Archibald observed that great variations occur in milk and milkfat production within the same individual from year to year as well as from milking to milking. The variation in milk and milkfat production occurs even between quarters of the same udder. Greaves (1940) stated that these variations occurred even though every effort was made to keep physiological and environmental factors constant.

Table 3 shows example data summarizing the variation in milkfat content which can occur from milking to milking. Almost half of the samples varied more than 0.3 percent from the average; however, the test of an individual herd probably remained relatively constant.

Methods of Measuring Milkfat Used in DHIA Testing

Babcock test. For more than 75 years the Babcock test has been the standard method used to determine the milkfat content of milk in the United States.

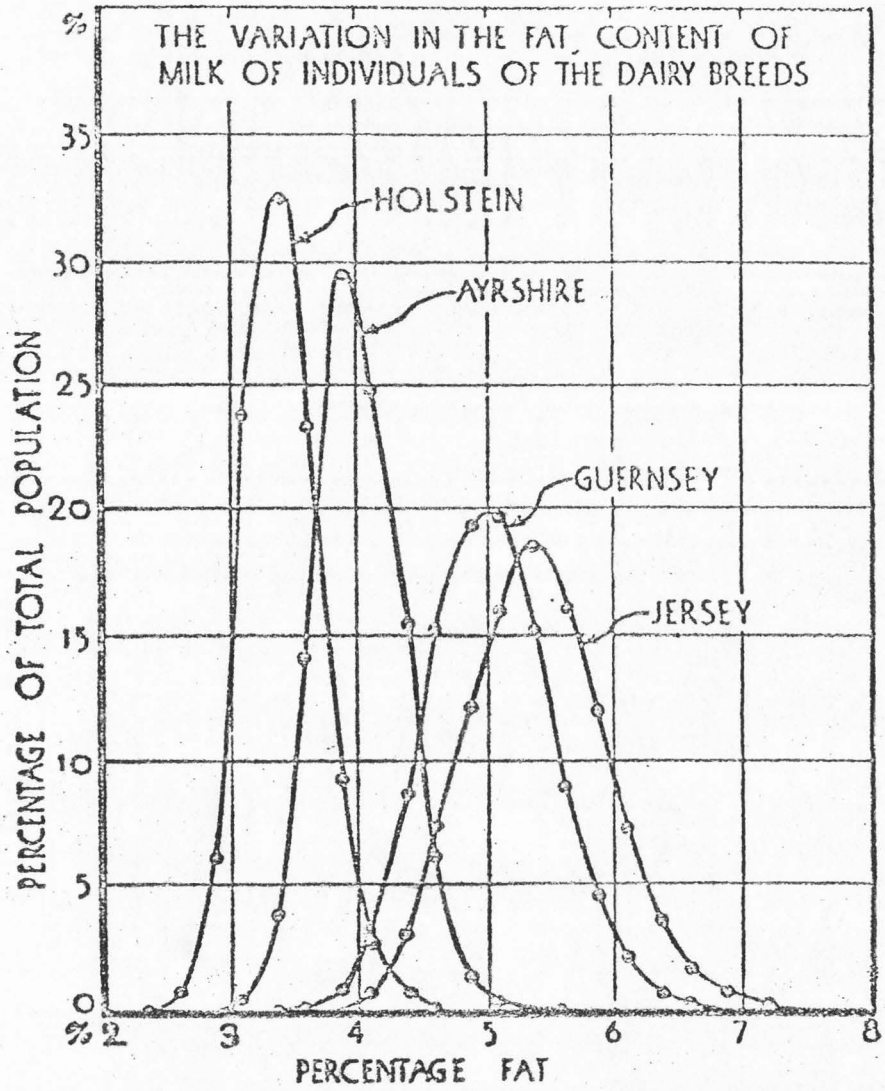

Figure 1. Variation in the average milkfat content of individuals of the dairy breeds. (from Smith, 1959)

Table 3. Average variations in milkfat content from milking to milking (Smith, 1959)

Breed	No. of Milkings	Percentage milkfat content			Percentage variation from average		
		Average	High	Low	0.3	0.3-0.6	0.6-0.9
Aryshire	27	3.93	4.36	3.49	77.7	22.3	---
Holstein	28	3.07	3.79	2.40	39.3	46.4	14.3
Holstein	28	3.18	3.67	2.71	85.7	14.3	---
Jersey	28	5.31	6.31	4.56	50.0	25.0	17.9
Shorthorn	27	4.08	5.29	3.46	63.3	29.6	3.7
Holstein	20	2.98	3.63	2.24	45.0	30.0	25.0

Milk at 17 to 21 C is measured into the test bottle by means of a 17.6 ml pipette. The pipette delivers approximately 17.44 ml or 18 g of milk. Seventeen and one-half milliliters of sulphuric acid (17 to 21 C) with a specific gravity of 1.82 to 1.83 is added into the milk in an 8 percent Babcock test bottle. The addition of the acid into the milk is done slowly but steadily while rotating the bottle. The bottle is shaken cautiously with a gentle rotary motion until the digestion action of the acid appears complete. After the color of the mixture attains a deep mahogany red color, this mixture is centrifuged for 5 min. Hot water (60 C) is then added to raise the mixture to the base of the neck of the bottle. The mixture is again centrifuged for 2 min. Hot water is then added to bring the fat level up into the calibrated neck. The mixture is again centrifuged for 1 min. After tempering the mixture to 69 C in order to adjust the specific gravity of the milkfat to 0.90, volumetric measurement is made by reading from the extreme bottom of the lower meniscus to the extreme top of the upper meniscus.

This is done to compensate for a trace of fat that still remains in the acid mixture and to bring the reading closer to the ether extraction reference fat test method (AOAC. 15001, 1970).

Packard and Ginn (1972) felt this method to be slow, tedious and dangerous; moreover, they observed that the repeatability of the Babcock method on high percentage milkfat products was less than satisfactory. However, most DHIA central test facilities still rely on the Babcock test for maintenance of electronic milk tester calibration.

Milko-Tester (M-T). In 1959 a Danish scientist, Haugaard, published a photometric method for fat determination. In 1962 a photometric instrument for milkfat determination, the Milko-Tester (M-T), was on the market. The M-T measures milkfat by measuring the light scattered by milkfat globules in a diluted milk sample. Shipe (1972) reported that if the size of milkfat globules is the same, the amount of light reaching the photocell is inversely proportional to the amount of milkfat. There are currently three models of M-T: the Mark II, the Mark III, and the Automatic. The Mark III differs from the Mark II in that it has a digital readout, it homogenizes milk and diluent, it uses less sample per test, and it is semi-automatic. For the Mark III (Anon, Foss Electric), 1.8 ml of milk sample is mixed with 15 ml of diluent which is made up of Triton 100, Antifoam Y30, distilled water, and EDTA (ethylenediamine tetraacetate). This mixture is then homogenized and the reading is made. Several studies, Shipe (1972), Shannon, Cardwell and True (1972), Pachard, Ginn and Rosenau (1973), Minzner and Kroger (1974), and Sherbon, Shipe, Tyrrell and Burke (1967), compared

the M-T with the Babcock, Mojonnier, Rose-Gottlieb and Gerber methods. The results indicated that the M-T was comparable to the above mentioned methods. The M-T was approved for the determination of fat in raw and unhomogenized milk on the basis of the results of a collaborative study conducted by Shipe in 1969. The development of this instrument is significant because it allows large-scale testing of milkfat such as in DHIA laboratories.

It is essential that the M-T be calibrated most carefully. In another collaborative study conducted by Shipe (1973), it was found that each instrument gave repeatable results, but the results between instruments were different. The major source of error with the M-T was the failure of the operator to recalibrate or even to make a random verification of performance. Consequently, detailed methods for routine calibration and verification of the M-T are needed.

The A.O.A.C. (1970) method for calibration calls for comparing results by the M-T and a reference method on at least 20 samples, and that the standard deviation between the M-T and reference should not differ more than 0.10 for individual cow samples or 0.06 for herd samples.

Dairy Herd Improvement Association

Purpose. The DHIA program was organized by the DHIA to improve milk production of dairy cows by supervising the recording of milk weights and by conducting tests for milkfat content. The DHIA reports this information to its members and provides recommendations on breeding, feeding and management.

Low milkfat test problems associated with the centralization of milkfat testing. Several investigations including those of AL-Omar et al. (1973), Dill et al. (1975), Harding et al. (1973), Kroger et al. (1968, 1971), Packard et al. (1973), and Moyes et al. (1975), were conducted after receiving complaints from dairy farmers that the tests from the centralized laboratories were lower than those previously obtained.

Kroger et al. (1968) observed that the churning of milk samples during shipment was the main cause of lower milkfat readings. In 1969 Kroger demonstrated that no churning was observed when sample bottles were completely filled, or when the temperature was below 10 C, even with partially filled containers. Churning increased with increase in time and with increase in head space and was partially reduced through incorporation of certain phosphates.

Moyes et al. (1975) observed that the problem of low readings persisted in Utah and several other states. These states followed the recommendations of shipping samples in styrofoam insulated shippers, loaded with milk samples in rigid plastic containers. These containers were supposed to keep the temperature of the milk below the critical churning range (under 10 C) until it arrived at the central laboratory. However, Moyes et al. (1975) demonstrated that this method of shipment allowed churning of the samples, and consequently lower milkfat readings. Within a few hours of delivery at the post office, the samples were in the churning temperature range (10 to 26 C).

Chemical methods to prevent lower milkfat tests. Kroger et al. (1968) tried to prevent churning by the use of neutral and alkaline

mineral salts such as calcium hydroxide, calcium oxide, magnesium oxide, sodium citrate, dibasic sodium phosphate, sodium pyrophosphate and sodium metaphosphate. These chemical additives are used to delay churning in soft serve frozen desserts. Kroger found only sodium metaphosphate to be effective in prevention of churning, but it did not eliminate churning completely.

In 1973 Minzner and Kroger felt that high initial bacterial count was the main cause of reduced M-T reading in the samples which were kept at room temperature. With 0.397 percent potassium dichromate, the bacterial count was reduced from 100,000 spc to almost zero in 12 days and the reading was stable through 20 days.

In 1973 Harding and Morris examined the reconstitution of churned samples. Lissapol, a surface active agent which is used for dispersal of oil slicks, was tried but resulted in lower tests due to the over-homogenization of fat in the M-T homogenizer. Reemulsification using a high-speed homogenizer restored much of the fat test. However, Moyes et al. (1975) felt that this was impracticable since homogenization affects M-T readings (Shipe, 1972a) and is impractical with small samples. Moyes et al. (1975) confirmed that churning was insignificant in samples when shipped below 10 C in containers free of entrapped air. Churning was reduced by the presence of sodium hexametaphosphate and potassium dichromate. The current practice by the Utah DHIA involves collection of milk samples in 64 ml Nasco Whirl-Pak plastic bags (B679N) containing potassium dichromate (Unek Products Laboratories). These bags are sealed to eliminate air space. They

are arranged in a shallow compartmentalized cardboard box (American Packing Company, Salt Lake City, Utah) and mailed or shipped Wycoff Parcel Service without refrigeration.

Milkfat Churning

Mechanism. According to Judkin (1963) the physical chemistry involved in churning is not entirely understood. Churning was defined by Webb et al. (1974) as destabilization of the lipid-phase emulsion by means of mechanical agitation. The contemporary theory of churning is the "Auto-floatation Theory." The foundation of this theory was laid by Pockels in 1902. According to this theory, churning is initiated by agitation and the incorporation of numerous small air bubbles, the basic concept being that the fat globules are brought to agglomeration at the air-plasma interface.

The fat globule membrane material spreads out on the surface of air bubbles, causing partial denuding of the globules of their protective layer. The partially denuded fat globules gather and form small clumps around liquid fat exuded from the fat globules and at the interface causing a more hydrophobic condition. When enough free fat has spread on the surface of the air bubbles, the air bubbles collapse. The globules then gather into clusters. These clusters of globules will be refloated on other bubbles and the aggregation of fat globules continues. King (1952) observed that the optimum temperature range of churning is between 17 to 21 C, and churning at low temperatures could not occur because only a small amount of fat is liquified and the hardness of the fat globules hinders the squeezing out of liquid fat. Thus the layer of free liquid fat cannot form on

the surface of the globules. At temperatures above the milkfat melting point range, globules are destabilized by churning but do not stick together to form a cluster, because the fat is entirely liquified.

Davies (1936) reported that large fat globules churn much more easily; therefore, the milk from breeds which secrete large fat globules (Jersey, Guernsey) churns readily, and milk from breeds which secrete small fat globules (Holstein) and milk from cows in advanced lactation churns to a lesser degree.

Methods of measuring churning. There are several methods of measuring churning.

1. Measurement of the free fat in a milk sample: Since churning causes damage to fat globules resulting in liberation of some free fat, free fat is measured as the degree of churning. This measurement can be done by Gas-Liquid Chromatography (Pomeranz and Meloan, 1971). However, Stannard (1975) felt that the measurement of free fat for determination of degree of churning is relatively insensitive since it indicates the final stage of churning and agglomeration; and it does not measure damage to the fat globule membrane, which in turn does not result in release of free fat.

2. The use of marker enzymes to measure churning: Two enzymes, alkaline phosphatase and xanthine oxidase, are known to be associated with the fat globule membrane (Zittle et al., 1956). In 1975 Stannard assayed the degree of churning by measuring these two enzymes in skim-milk and cream layers before and after churning, and he found that this method is sensitive and reproducible for detection of milk churning.

3. Measurement of percent milkfat reduction by the M-T: In 1975 Moyes et al. reported the ratio of Churned divided by Initial in order to measure the degree of churning. A lower reading is obtained with both the M-T and the Babcock because of inability to obtain a representative sample when samples have been churned (Harding and Morris, 1973). The sampling device draws the sample from below the surface where the majority of the churned fat is located, thus introducing a negative sampling bias. This bias, in badly churned samples, has only been significantly reduced through reemulsification. The method of sampling before and after churning should thus give a good indication of the degree of milkfat destabilization for use in this study.

METHODS AND PROCEDURES

Milk Samples

Milk samples were obtained from both Jersey and Holstein animals. Three herds were sampled from each breed, representing high, medium and low milkfat herd averages on records of the previous year (Table 4).

Table 4. Average milkfat test and numbers of animals tested in churning studies

Breed	Herd	Average Percentage Milkfat	Numbers of Cows	Herd Number	Owner, Location
Jersey	Low	4.2	39	1	Aaron Richards Farmington, Utah
	Medium	4.8	36	2	Arthur Anderson, Wellsville, Utah
	High	5.0	30	3	Jan Turner, Morgan, Utah
Holstein	Low	3.6	36	4	Utah State Univ., Logan, Utah
	Medium	3.7	33	5	Ropeletto Brothers, Taylor, Utah
	High	3.8	36	6	Russell Wayment, West Warren, Utah

Each herd was sampled four times from September, 1975 to March, 1976. Some individual cows were not sampled on all four visits because the cow was sold, had mastitis, or there were not enough metering devices available.

The milk samples were collected at the morning milking using a modified Surge Tru-Test meter (Babson Brothers). Each sample of milk (140 ml) was preserved with two tablets of potassium dichromate (UNEK Product Laboratories). All samples were transported to the laboratory by automobile immediately after milking, a distance of 1.6 to 129 km. Samples were taken when temperatures were approximately -9.4 to -1.1 C and were transported unrefrigerated in the trunk of a car. After the samples arrived at the USU Central Milk Testing Laboratory, they were kept at room temperature (20.0 to 22.2 C) until analyzed. All samples were churned and tested within nine hours of sampling.

After thorough mixing of each individual sample by pouring back and forth between two containers (A.O.A.C. 15001), 65 ml of milk was poured into a 140 ml wide mouth glass jar (with screw cap metal lid). The sample was then shaken on a horizontal two-speed oscillating shaker (Eberbach Corporation, Ann Arbor, Michigan) for 3 hrs at 180 oscillations per min at room temperature. The samples which were shaken were referred to as the Churned samples and the unshaken samples as the Initial samples.

Measurement of Milkfat

All samples were tested on a Milko-Tester Mark III after tempering to 38 C and mixing by pouring back and forth between two containers (140 ml glass containers). Thorough mixing of milk samples was essential prior to the measurement. The M-T was operated and standardized in accordance with A.O.A.C. 16.058.

For the Holstein samples, duplicate measurements were taken for both the Churned and Initial samples. Duplicate measurements were made for the Initial Jersey samples, and triplicate measurements were made for the Churned Jersey samples because they were less reproducible. The two most similar tests were averaged for the average Churned test for Jersey samples.

Measurement of Degree of Churning

In this study churning is defined as the process of damaging or destroying milkfat globules, thereby causing a lower reading by the M-T.

The degree of churning was calculated by the following equation:

$$\frac{\text{Initial} - \text{Churned}}{\text{Initial}} \quad X \quad \text{Degree of Churning (percentage)}$$

Statistical Analysis

The following parameters were recorded and punched on cards for statistical analysis: breed, herd, cow identity, Initial (duplicate), average of Initial tests, Churned (duplicate for Holstein, triplicate for Jersey), average of Churned samples, and Difference between mean Initial and Churned. The average Churned for Jerseys was calculated from the two most similar tests from triplicates. Example data are tabulated in the Appendix.

Data were prepared for regression analysis using the formula:

$$Y_{ijkl} = \mu + b_i + h{:}b_{ij} + c{:}hb_{ijk} + e_{ijkl}, \text{ where}$$

Y_{ijkl} = lth sample from kth cow in the jth herd of the ith breed

μ = the population mean

b_i = the effect of the ith breed

$h:b_{ij}$ = the effect of the jth herd with the ith breed

$c:hb_{ijk}$ = the effect of the kth cow in the jth herd in the ith breed

e_{ijkl} = a random error associated with lth sample of the kth cow in the jth herd in the ith breed

The mean, standard error of mean, coefficient of variation, repeatability, regression curves, standard deviation, and analysis of variance were computed electronically on the Burroughs 6700 computer at Utah State University, utilizing the STATPAC program series developed by Dr. R. L. Hurst.

The standard error of mean (S.E.) was calculated as the standard deviation divided by the square root of the number of observations.

The coefficient of variation (C.V.) was calculated as the standard deviation divided by the mean, and multiplied by 100.

RESULTS AND DISCUSSION

Mean Estimate of Initial and Churned Milkfat Test

The mean estimate for Initial and Churned was calculated from data for the three Jersey and three Holstein herds on December, 1975 and January, February and March, 1976. (See Table 4)

Table 5. Means, standard error of means, and coefficient of variation for Initial and Churned of Jerseys and Holsteins

Breed	Herd	Percentage		
		Mean	S.E.	C.V.
		Mean estimate of initial		
Jerseys	1	4.83	0.043	9.20
	2	5.47	0.052	9.73
	3	5.10	0.075	11.87
	Combined*	5.14	0.045	14.34
Holstein	4	3.52	0.068	19.24
	5	3.52	0.034	18.56
	6	3.32	0.042	11.39
	Combined*	3.43	0.036	17.73
		Mean estimate of churned		
Jerseys	1	2.85	0.063	22.48
	2	2.80	0.067	24.67
	3	2.62	0.099	30.74
	Combined*	2.77	0.059	34.76
Holsteins	4	2.78	0.070	24.99
	5	2.10	0.049	21.10
	6	2.06	0.046	20.34
	Combined*	2.33	0.042	30.34

*Combined is obtained from data of all observations within breeds.

Mean reduction in milkfat reading in Jerseys was greater than in Holsteins (5.14 - 2.77 = 2.37; 3.43 - 2.33 = 1.10 respectively). The significance of this will be discussed later.

Values of coefficient of variation (C.V.) for Initial and Churned for Jerseys were 14.34 and 34.76 respectively. Since the C.V. measures the variability of individual observations from the mean, these figures indicate that there is greater variability in Churned than Initial. Holsteins show the same trend of lower C.V. values for Initial than for Churned (17.73 and 30.34 respectively), but to a lesser degree. However, since the C.V.'s for Jerseys for Churned were obtained from triplicate samples, and the C.V.'s for Holsteins for Churned were obtained from duplicate samples, the difference between them would be reduced if both means were obtained from an equal number of samples (i.e., the more samples included in a mean, the less variable, lower C.V. one would expect it to be).

Values of standard error of mean (S.E.) for combined herd data for Jerseys and combined herd data for Holsteins (for both Initial and Churned) are less than values obtained from each individual herd. This is because S.E. values for the combined data were obtained from a larger number of observations than those of each individual herd. Since S.E. measures the reliability of the mean, the values for combined data are more reliable than those for individual herds. On the other hand, C.V. values obtained for combined data are larger than those of each individual herd for both Initial and Churned for both Jerseys and Holsteins. This is because the environment is essentially the same for all the cows in a herd, whereas the values for combined data were obtained from three different environmental conditions.

The average milkfat percentage obtained in this study was different than expected from DHI test data as shown in Table 6.

Table 6. Comparison of the milkfat percentage between data obtained from DHI and data obtained in this study

Breed	Herd	Milkfat percentage	
		DHI	Current study
Jerseys	1	Lowest (4.2)	Lowest (4.83)
	2	Medium (4.8)	Highest (5.4)
	3	Highest (5.0)	Medium (5.10)
Holsteins	4	Lowest (3.6)	Highest (3.52)
	5	Medium (3.7)	Highest (3.52)
	6	Highest (3.8)	Lowest (3.32)

This difference can be attributed to the following factors: The DHIA data were annual summary figures obtained from the combined samples of morning and evening milkings throughout the four seasons of the year. In contrast, in this study data was obtained only from the morning milking and only during the Winter, and it was taken only from selected cows which were in the second through eighth month of lactation. The animals would be expected to produce a mean higher fat test during the time interval tested; however, this was not true for the Holsteins. No explanation for this is possible. The possible M-T instrument bias in favor of high fat breeds was considered; however, the calibration checks revealed bias, if any, in favor of low fat breeds. This was counter to that expected (Sheppard, op cit.).

Degree of Churning

The degrees of churning for Jersey and Holstein milk samples are summarized in Table 7.

Table 7. The degree of churning among the six herds in Jersey and Holstein breeds

Breed	Herd	Degree of churning		
		Mean	S.E.	C.V.
		Percentages		
Jerseys	1	40.75	1.03	25.85
	2	48.92	1.00	20.96
	3	48.43	1.60	26.90
	Combined	45.76	1.02	36.10
Holsteins	4	21.27	0.93	43.35
	5	39.96	1.13	25.52
	6	35.43	1.53	39.66
	Combined	31.64	0.98	52.11

The degree of Churning among Jerseys was almost one-third greater than that of Holsteins. Since the Jersey milk contains greater amounts of larger milkfat globules than the Holstein milk, as shown in Table 1, the data in Table 8 are in consonance with Davies' (1936) statement, " . . . has shown that cream with larger fat globules churns easiest, . . . milk from breeds giving large fat globules is the easiest to churn. . . ."

The data also correlates with Moyes et al. (1975) who reported that, "The Jersey milk samples ultimately lost a higher proportion of fat than Holstein."

Difference Between Initial and Churned Milkfat Test

The mean difference between Initial and Churned was calculated by subtraction of mean Churned from mean Initial. The results are shown in Table 8.

Table 8. Mean differences between Initial and Churned

Breed	Herd	Percentage Difference		
		Mean	S.E.	C.V.
Jerseys	1	1.95	0.049	25.74
	2	2.67	0.054	20.87
	3	2.48	0.091	29.69
	Combined	2.36	0.058	40.15
Holsteins	4	0.73	0.035	47.07
	5	1.41	0.041	26.26
	6	1.17	0.060	46.54
	Combined	1.09	0.038	58.26

The mean differences for Jerseys and Holsteins were 2.36 and 1.09 respectively, which indicates that reduction in milkfat absolute readings after churning was more than twice as great in Jerseys as it was in Holsteins. However, the C.V. values indicate that variability is smaller for the Jerseys. This fact, together with the findings from the section, "Mean Estimate of Initial and Churned Milkfat Test" (the C.V. values of the mean estimate of Initial of Jerseys is smaller than that of the Holsteins) and from the next section, "Degree of Churning" (C.V. values of the degree of churning of the Jerseys is smaller than that of the Holsteins) as shown in Tables 4 and 7, suggest that: (1) the Jersey cows were more homogeneous than Holstein cows in genetic

backgrounds, or (2) the feed and environmental conditions were relatively similar among the three Jersey herds in comparison with the three Holstein herds.

Breed Differences

Variance components for breed effects on the degree of churning and on Initial, Churned and Differences were derived from the analysis of variance and were tested using the F-test. The F-ratio was found by dividing the breed mean square by the herd within-breed mean square. The results are shown in Table 9.

Table 9. Analysis of variance of breed effects on degree of churning, Initial, Churned and Difference

Model	Dependent variable	Degrees of freedom	MS	F Value
1	Degree of churning	1	33824.3	3.837
	Initial	1	551.5	55.981*
	Churned	1	40.2	3.224
	Difference	1	293.2	15.138*
2	Degree of churning	1	7005.5	0.816
	Churned	1	16.1	1.180
	Difference	1	17.6	2.264

F values which are significant at the 1 percent level are indiciated by*

In this analysis breed effects were analyzed under two different models. In Model One four variables were examined to determine if differences in these traits existed between the Holstein and Jersey breeds. The results show that there are significant differences at the 1 percent level in the Initial and Difference. In Model Two the Initial was treated as an independent variable along with the breed, herd and

cows within herds. This measure should show any breed differences in the remaining three variables, allowing for the fact that there is a difference in Initial milkfat test. The results show that these three variables do not differ statistically; therefore, we can attribute any apparent differences between the breeds in these three traits to the fact that these breeds are inherently different in the mean level of milkfat they produce. It should be pointed out that due to the limited numbers of breeds and herds studied (two and six respectively), these results may not be conclusive. There may be differences between breeds other than due to Initial which would show up if a larger number of breeds and herds (e.g. 5 and 40) were studied.

Herd Differences

Variance components for herd effects on Initial, Churned, Difference and degree of churning were derived from the analysis of variance and were tested using the F-test. The F-ratio was determined by dividing the herd mean square by the between cows within herd mean square. The results are shown in Table 10.

Herd effects among Jerseys were significant at the 1 percent level for the Initial and Difference and at the 5 percent level for the degree of churning; but the Churned was not significantly different. Among Holsteins, herd effects were significant for all four variables. The data shows that there are differences among individual herds within the breed. These differences might be due to the fact that the selection of six herds was based on the milkfat content differences within a breed. Thus it is not surprising to find a significant difference in most of these traits among herds.

Table 10. Analysis of variance of herd effects on Initial, Churned, Difference and degree of churning

Breed	Variable	Degrees of freedom	MS	F Value
Jersey	Initial	2	15.09	12.103**
	Churned	2	1.67	0.812
	Difference	2	19.59	8.444**
	Degree of churning	2	2876.57	4.378*
Holstein	Initial	2	3.33	5.05**
	Churned	2	21.13	20.92**
	Difference	2	15.04	17.32**
	Degree of churning	2	12152.27	20.74**

[a] values which are significant at the 1 percent and 5 percent levels are indicated by ** and * respectively.

Differences Among Cows

Variance components for cow effects on the Initial, Churned, Difference and degree of churning were derived from the analysis of variance and were tested using the F-test. The F-ratio was derived by dividing the between cow mean square by the within cow mean square. The results are shown in Table 11.

Differences between cows were significant at the 1 percent level for both Jerseys and Holsteins for all four variables.

These results indicate that within each breed there are not only differences between individual cows in Initial, but also in how milk from individual cows responds to churning. These results indicate that cows which produce milk with higher tendency for churning are more apt to consistently receive less reliable readings when milk samples are subjected to adverse shipment conditions than other cows which produce milk with less tendency for churning.

Table 11. Analysis of variance of cow effects on Initial, Churned, Difference and degrees of churning

Breed	Variable	Degrees of freedom	MS	F Value
Jerseys	Initial	107	1.25	4.605*
	Churned	107	2.06	4.176*
	Difference	107	2.32	6.736*
	Degree of churning	107	661.57	5.390*
Holsteins	Initial	105	0.66	2.740*
	Churned	105	1.01	3.443*
	Difference	105	0.87	4.816*
	Degree of churning	105	585.94	4.582*

F values which are significant at the 1 percent level are indicated by*.

Within Cow Repeatability

Within cow repeatability was measured as the correlation among the four separate samples of milk taken from each cow. Table 12 presents the repeatability of the Initial, Churned, Difference and the degree of churning.

Table 12. Intra-cow repeatability from month to month for the Initial, Churned, Difference and degree of churning

	Intra-cow Repeatability			
Breed	Initial	Churned	Difference	Degree of churning
Jerseys	0.49 + 0.05	0.46 + 0.05	0.60 + 0.05	0.54 + 0.05
Holsteins	0.33 + 0.06	0.41 + 0.06	0.52 + 0.05	0.51 + 0.05

Note: 1.0 would be perfect repeatability

The repeatability of each of the four traits is greater for Jerseys than for Holsteins. The repeatability of Difference and degree

of churning in both breeds is higher than Initial or Churned. This shows that the degree of churning of a sample (since degree of churning is calculated from Difference), can be predicted in future samples from the same cow more accurately than can the Initial or Churned. The Churned of the Jerseys was probably biased downward in relation to Holsteins because of the difference in procedures in obtaining the mean Churned for Jerseys. The average of the two closest measurements out of three measurements of churned samples were used for Jerseys, while the average of both was calculated for churned samples for Holsteins and for Initial for both breeds.

It can be concluded that some animals will consistently be biased against when their milk is subjected to churning while other animals in the same herd will not be so severely biased against from month to month. These samples were assayed during the period of lactation when milk was least susceptable to churning. Later in lactation samples would be more churnable. It is not known whether these individual cow biases would be consistent during those times. However, it would be suspected to follow the pattern found during middle lactation.

Repeatability of Multiple Determinations of Initial and Churned

Comparison of the precision of nonchurned and churned samples tested by the M-T is reported in this section. The objective was to determine if the measurement of milkfat of churned samples could have the same precision as the measurement of nonchurned samples.

In these data each sample was counted separately; i.e., if a cow was tested four times, the samples from this cow were counted four times. Theoretically, if the technique were perfect and the samples and instru-

ments were in perfect condition, the measurements in duplicate or triplicate would be exactly the same and repeatability would be 1.00. These data show a high repeatability value for the Initial for both breeds. Thus the precision of testing the nonchurned samples was excellent. The repeatability of Churned for both Jerseys and Holsteins was lower than that of the Initial, and especially for Jerseys. Thus churned samples, particularly those for Jerseys, cannot be tested with precision, which means that Jersey cows will receive less accurate tests.

Table 13. Repeatability of multiple determinations of Initial and Churned

Breed	Test	Repeatability
Jerseys	Initial	0.98 + 0.002
	Churned	0.88 + 0.001
Holsteins	Initial	0.998 + 0.002
	Churned	0.96 + 0.004

Note: 1.0 would be perfect repeatability

The fact that the repeatability of Churned was lower than that of Initial in both Jerseys and Holsteins can be explained as follows: When milkfat is churned, the fat globules are destabilized and form clumps of milkfat globules in larger numbers. When analytical samples are taken into the M-T pipette, there may not be a representative quantity of milkfat from the churned and melted samples (See Figure 2b).

The repeatability value for the Churned was lower for Jerseys than for Holsteins. This was due to the fact that the higher test

(a) A churned sample with white clumps of milkfat at room temperature.

(b) A churned sample after heating to 37.7 C, showing liquified milkfat on the surface.

Figure 2. The churned sample with milkfat before (a), and after heating to 37.7 C (b).

Jersey milk churns more readily (Table 8) than the lower test Holstein milk, and a greater variance in sample pickup occurs because of the sample tube inability to pick up uniform quantities of the oiled off fat droplets.

Photographs of churned samples were taken before and after warming to 37.7 C (Figure 2), which clearly shows churned milkfat. The liquified churned milkfat can be seen on the surface. The sampler of the M-T draws a constant volumetric sample and doesn't start to draw the sample until only after immersion of the sample tube well below this surface. Therefore, only well suspended, submerged oil droplets or fat globules are picked up.

Regression

Regression curves were computed for the Churned, Difference and degree of churning, and were plotted against the Initial for both Jerseys and Holsteins (Figures 3, 4 and 5).

Regression lines for Initial versus degree of churning were polynomial curves for both Jerseys and Holsteins. The degree of churning was greater for Jerseys than Holsteins. However, the reliability of a regression line depends upon the number of observations at each point. For instance, the Initial of less than 3.50 percent of Jerseys on Figure 3, 4 and 5 was represented by only seven observations, while the number of observations occurred in the area of 4.50 to 5.99 percent for Jerseys and 2.50 to 3.99 percent for Holsteins, those areas of the regression curves are the most reliable. (See Table 14.) Therefore, even though there is one curve for each breed in Figures 3, 4 and 5, there may be ultimately only one curve in Figures 3, 4 and

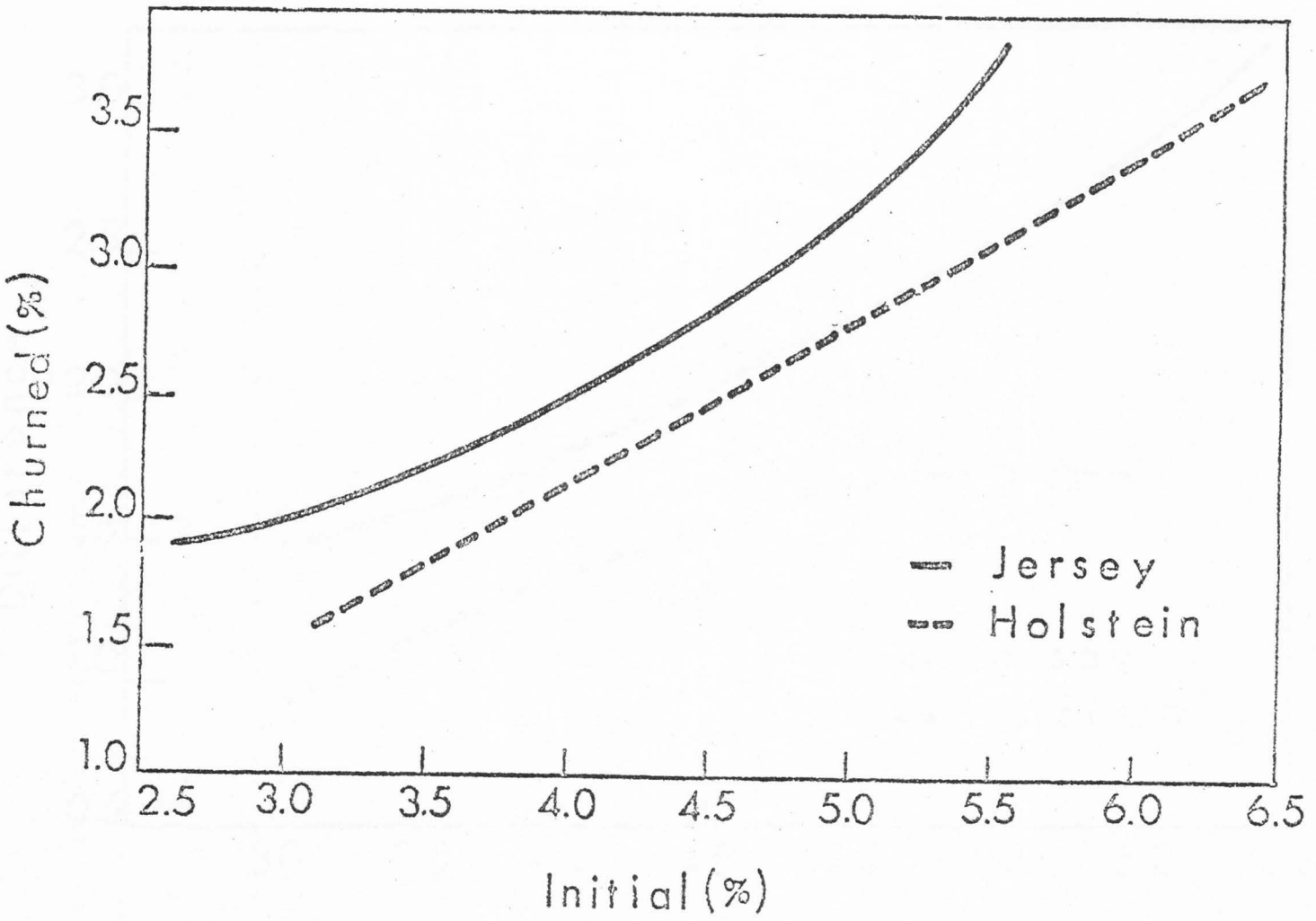

Figure 3. Regression curves of the Churned (%) on the Initial (%) for Jerseys and Holsteins.

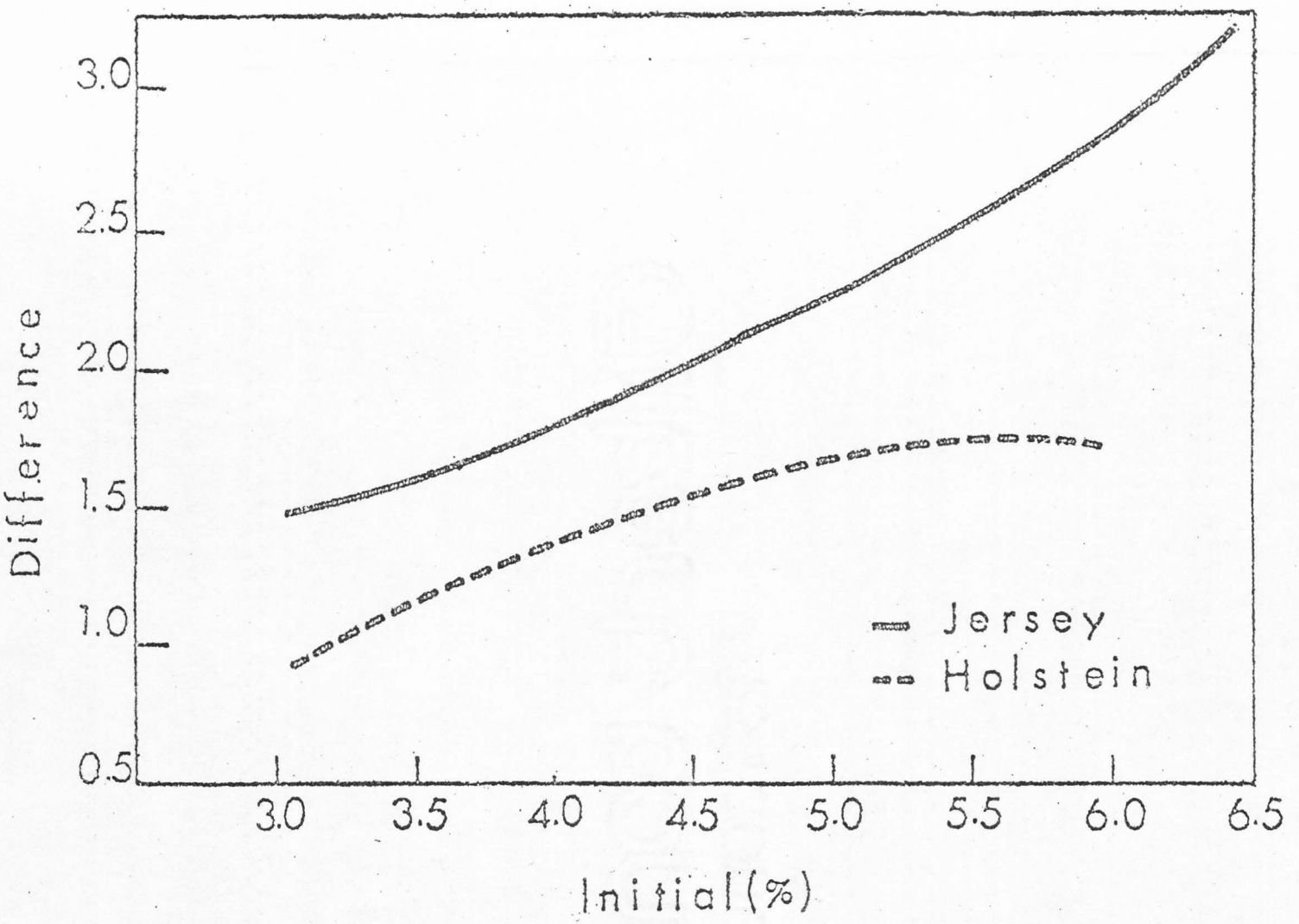

Figure 4. Regression curves of the Difference (%) and the Initial (%) for Jerseys and Holsteins.

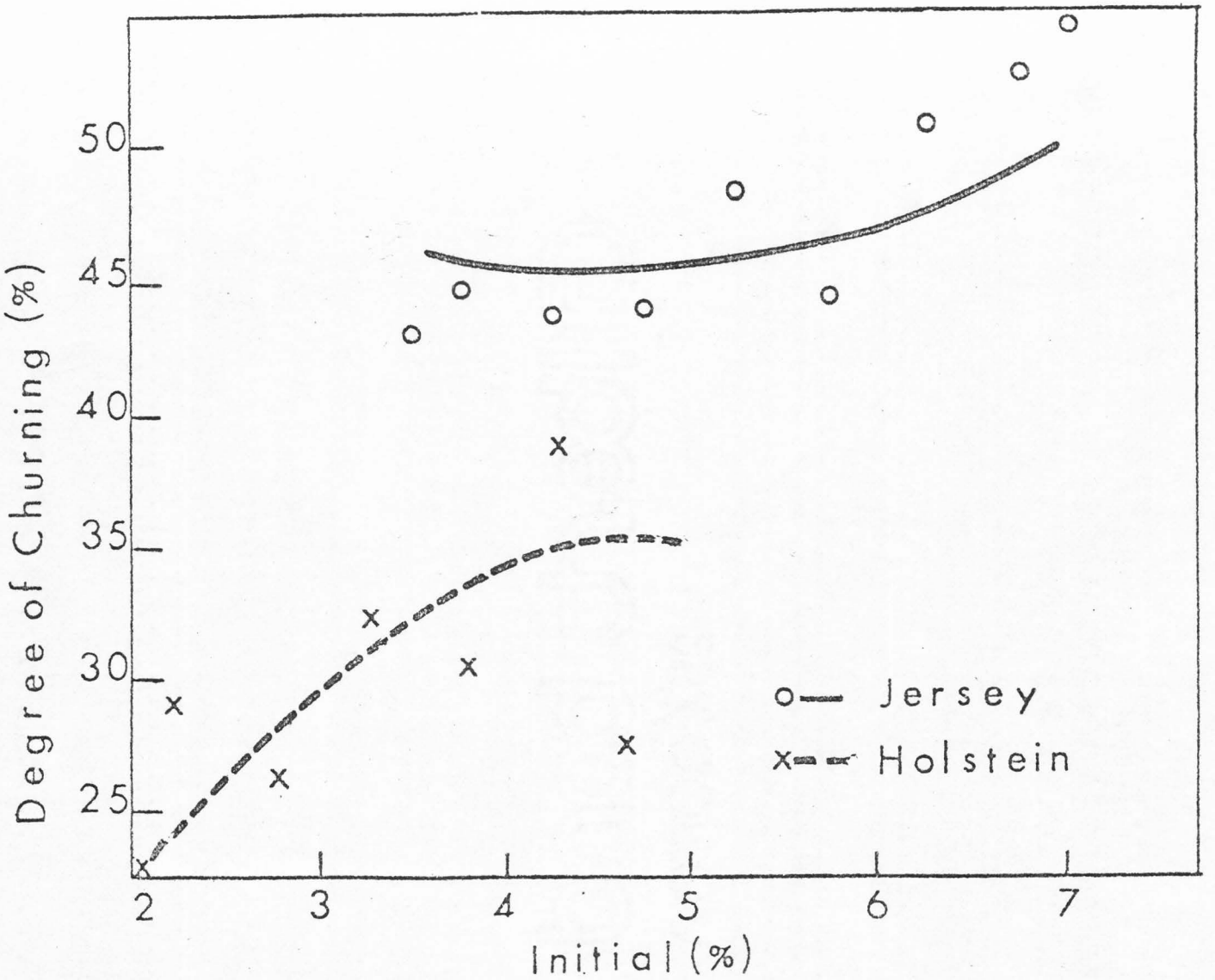

Figure 5. Regression curves of the degree of churning (%) on the Initial (%) for Jerseys and Holsteins.

5 regardless of breed in the total population. For example, the curves of Figure 5 might become one curve starting from the lowest point on the left corner and rising gradually to a higher degree of churning as the Initial rises. Therefore, it seems that the degree of churning is proportional to the Initial, and it does not depend on the breed which is in accord with the section, "Breed Differences" on page 26.

Table 14. Number of observations for Initial range and mean degree of churning

Breed	Initial range (%)	Number of observations	Mean initial (%)	Mean degree of churning (%)
Jerseys	Less than 3.5	7	3.28	42.43
	3.50 - 3.99	22	3.77	44.65
	4.00 - 4.49	47	4.29	43.69
	4.50 - 4.99	86	4.74	43.75
	5.00 - 5.49	95	5.22	47.82
	5.50 - 5.99	80	5.70	44.31
	6.00 - 6.49	35	6.21	49.43
	6.50 - 6.99	11	6.71	51.31
	More than 7.0	4	7.58	53.10
Holsteins	2.00 - 2.49	15	2.36	29.47
	2.50 - 2.99	67	2.78	27.92
	3.00 - 3.49	133	3.23	32.50
	3.50 - 3.99	101	3.71	30.80
	4.00 - 4.49	50	4.20	38.99
	4.50 - 4.99	6	4.74	28.10
	More than 5.0	2	7.62	10.30

CONCLUSIONS

1. Both Jersey and Holstein milk samples experienced greater variability in milkfat observations in Churned than in Initial.

2. Jersey milk samples had greater values for the Difference, consequently, a higher degree of churning than Holstein milk samples.

3. Jerseys had a smaller variability of observations in Initial, Difference and degree of churning than Holsteins.

4. Jerseys had a greater repeatability in Initial, Churned, Difference and degree of churning than Holsteins.

5. Conclusions 3 and 4 suggest that the Jerseys studied were more homogeneous in genetic background or feed and environmental conditions than the Holsteins.

6. Statistically, the Initial was inherently different between Jerseys and Holsteins.

7. Six herds were different from each other in Initial, Churned, Difference and degree of churning. The only exception to this was that the Churned of the three Jersey herds were not significantly different from each other.

8. Cows within a herd were different from each other in mean Initial, mean Churned, and degree of churning. This suggested that cows which produce milk with a higher tendency for churning are apt to receive less reliable readings.

9. The repeatability of multi-determinations of Churned is lower than that of Initial for both breeds, especially to a greater degree for the Jerseys. This indicated that the Churned sample could not be measured for its milkfat content as accurately as the nonchurned sample.

10. Regression lines for Initial versus degree of churning were polynomial curves for both Jerseys and Holsteins. The degree of churning was proportional to the Initial.

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APPENDIX

Table 15. Example data obtained from Jersey Herd 3, March 3, 1976

Cow No.	I	I	AVE	CH	CH	CH	AVE	DIF
66	444	448	446	152	143	113	149	297
169								
198	560	561	560	253	264	265	265	295
211	430	433	432	243	224	247	245	187
325	525	525	525	160	196	193	195	330
333	510	515	513	235	241	220	238	275
344	499	498	498	173	175	179	174	324
354	529	535	538	174	195	189	192	346
355								
360	462	471	467	180	207	209	208	259
361	539	539	539	157	183	228	170	369
367	498	496	497	309	311	327	310	187
368	477	478	478	225	263	287	275	203
383	550	560	555	194	217	215	216	339
386	500	504	502	175	179	207	177	325
390	519	523	521	158	184	185	185	336
392								
393	464	465	465			Coagulated		
396								
406	693	504	499	177	220	222	221	278
377	516	515	515	197	197	241	197	318
ELLA	463	464	464	177	168	193	173	291
407	464	463	464	295	296	310	296	168
408	379	384	382	207	186	220	214	168
409								
411	561	564	563		241	240	241	322
412	482	488	485	209	216	236	213	272
413								
414								
426								
MELBA	555	555	555	242	260	272	266	289
TINKERBELL	406	400	403	261	260	291	261	142
ROSEALICE	544	549	547	223	235	249	229	318
HENRIE	480	484	482	188	190	210	189	293

Table 16. Example data obtained from Holstein Herd 6, March 1, 1976

Cow no.	I	I	AVE	CH	CH	AVE	DIF
187	273	275	274	153	160	157	117
234	268	266	267	149	143	146	121
254	286	287	287	291	291	291	004
275	319	320	320	168	169	169	151
285	348	350	349	175	173	174	175
293	256	256	256	154	153	154	102
310	287	287	287	190	193	192	095
313							
317	308	306	307	188	190	189	118
319	297	300	299	151	151	151	148
321	271	273	272	179	179	179	093
363	324	324	324	224	229	227	097
370	291	292	292	229	229	229	063
386	276	276	276	253	246	250	026
392	288	289	289	168	172	170	119
395	248	247	248	155	160	158	090
396	283	284	284	180	202	194	090
397							
398	269	267	268	155	159	157	111
401	280	278	279	225	224	225	054
404							
431	302	302	302	176	177	177	125
432	367	367	367	252	249	250	117
433							
434	359	359	359	237	236	237	122
435	352	354	353	296	296	296	057
436							
437	350	353	352	204	204	204	148
438	259	258	259	154	161	158	101
439	359	361	360	207	208	208	152
440							
441	384	385	385	219	211	215	170
442							
443	292	293	293	129	125	127	166
444							
445							

VITA

Kazuko Monobe

Candidate for the Degree of

Master of Science

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