

MILK FLAVORS, LIPASE ACTIVITY, AND MILK
PRODUCTION AS RELATED TO SUDDEN
CHANGES OF ENERGY IN
THE COW'S RATION

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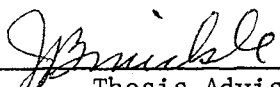
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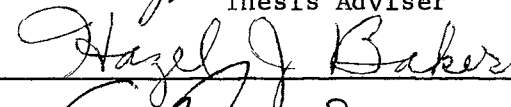
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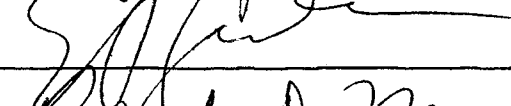
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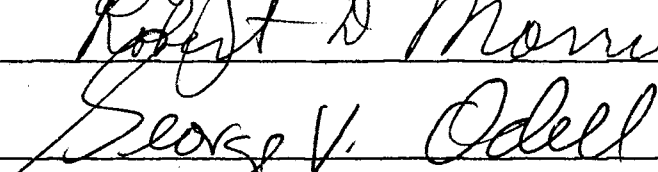
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CHAPTER I

INTRODUCTION

Modern dairy farms employ new techniques of refrigeration and storage that allow the pooling of several milkings prior to shipping to the processor. In the event an off-flavor occurs in fresh milk, and subsequently this milk is pooled into a still larger quantity of milk, the entire lot may become unsalable and result in a sizable loss in revenue. When the intensity of the normal flavor of fresh milk changes or the sudden presence of other flavors becomes noticeable, the consumer objects and sales drop.

Fresh fluid milk is normally bland with a smooth, delicate, and slightly sweet flavor. Fresh milk should impart a pleasant smooth sensation to the mouth and nose. Feed flavor is the most common criticism of fresh milk. While it may be possible to minimize or remove this flavor and some other off-flavors, rancid, oxidized, or cowy flavors often cannot be removed and such milk may be unsalable. Thus, they may be a serious problem to the producing dairyman.

Common practice among dairymen is to group the herd according to milk production or stage of lactation, the fresh cows being on a much higher plane of nutrition to provide them with the energy for high production. As the stage of lactation progresses and production drops correspondingly, the plane of nutrition is decreased. As new fresh cows are put in the high group, some cows must be rotated out of this group

and into a lower group abruptly. One could logically ask if this abrupt change in feeding could cause flavor changes in the milk.

No research has been reported correlating rancid, oxidized, or cowy flavors with abrupt changes in the cow's feed intake. Nor has any work been reported relating lipase activity (the enzyme that causes rancidity) to changes in feed intake. There has been considerable study of oxidized flavors but not in connection with feeding levels. A lowered plane of nutrition can bring about a condition called ketosis which involves acetone bodies in the cow's blood and milk. However, it has never been determined whether the criticism cowy (sometimes called acetone) can be caused by ketosis. It would be desirable to determine if there is a statistical correlation between all of these flavors in milk and abrupt changes in the cow's ration.

Cows are presently changed from one group to another merely by opening a gate between two pens; if this practice should be responsible for off-flavors in the milk, different methods of changing cows between groups could be developed.

The purpose of this study was to simulate such conditions as occur during the abrupt changing of the cow's energy intake 20% downward as if she had been shifted into a lower intake group during her lactation. There is particular interest in the response by the cow in terms of milk flavors.

CHAPTER II

REVIEW OF LITERATURE

Hydrolytic Rancidity

Rancidity in milk is considered to be caused by lipolysis--hydrolysis of the milk fat involving the enzyme lipase (17). One of the results of lipolysis is the accumulation of short-chain fatty acids, and perhaps partial glycerides, in sufficient quantities that they can be detected organoleptically. A recent review by Shahani (35) lists the enzymes in bovine milk. Milk contains several lipases (or esterases); at least one of which, beta-esterase, is capable of hydrolyzing triglycerides, including tributyrin (19, 35). The hydrolysis of tributyrin is often used as a measure of the lipase activity of milk (38).

Lipolysis is a surface reaction with lipase reacting at the surface of the milk fat. In the laboratory, rancidity can be induced in milk by adding the enzyme lipase; thus, early workers labeled milk that became rancid as milk that had a "high lipase content." But rancidity could also be induced simply by increasing the surface area of the substrate (milk fat) by homogenization while milk was in the raw state. Agitation of raw milk in pumps or pipelines also resulted in rancidity. Thus, the causes of rancidity are complex and cannot be measured simply by measuring the lipase activity of a milk sample.

In 1935, Hileman and Courtney (16) reported that variations in the degree of organoleptic rancidity followed a seasonal pattern and tended

to increase as lactation progressed. The conclusions drawn were that the lipase content of milk depended upon the cow's state of lactation and that some additional factor was responsible for less rancidity appearing in the summer and more in the winter. Seasonal fluctuation was again noted by Herrington and Krukovsky (14) in 1939, who reported two types of lipase action, one type that was destroyed by formalin and one that was not. The summary of the article contained a statement that, perhaps unknown at the time to the authors, was to become a recurring theme: "The fact that in milk there are at least two lipases possessing different properties may be responsible for the fact that so little progress has been made in the study of lipolysis in milk."

In 1945, Kelly (22) investigated still another factor that could contribute to the development of rancidity in milk. This work involved milk lipase activity, a method for its determination, and its relationship to the estrous cycle. The lipase activity of the milk was expressed as the ml of 0.1N acid released from the substrate by the equivalent of one ml of the original milk in 24 hours at 37°C. In this article, several other methods used previously by other workers in the study of lipase activity of milk were reviewed and compared. Included in the review was the acid degree method, sometimes referred to as the acid degree value (ADV) or fatty acid degree (FAD) and defined as the ml of 1N KOH required to neutralize the free fatty acids in 100 grams of fat. Recently many other authors have expressed lipase activity as units, where one unit represents the micromoles of free acid produced by one ml of enzyme source at a specified time and temperature (4, 10, 29).

Kelly (22) reported data concerning milk samples collected at frequent intervals from several open (not pregnant) cows. The data showed

a definite relationship between the estrous cycle and lipase activity in the milk. Samples of milk obtained prior to estrous were higher in lipase activity than those obtained during estrous. The few samples taken immediately after estrous showed a slight increase in lipase activity. While the lipase activity of all milk samples was higher just before the heat period, this increase was not sufficient to allow detection by organoleptic testing.

Wells et al. (39) reported that lipase activity varied in the blood of four cows during their lactation. Peak blood plasma lipase values occurred about 24 hours before observed estrous. These peak values were bracketed by lower values occurring two or three days before, and one to three days after, the day of observed heat. Changes in blood lipase activity were reflected and magnified in the milk, although these changes occurred 9 to 15 hours after they were observed in the blood.

Fredeen et al. (11) studied one cow for two successive lactations in connection with milk rancidity and certain management and environmental factors. His data showed that the season of the year and the stage of lactation did appear to have a "cause and effect" relationship on changes in ADV. Moreover, the factors that caused an increase in ADV did not necessarily appear to be responsible for organoleptic rancidity. The conclusion offered was that rancidity did increase in milk as an effect of season or later stage of lactation, but that these effects were not attributable to an increase in lipase alone (when lipase was determined as ADV). While it would appear logical that the feedstuffs available to the cows in the study varied throughout the year, the kinds and amounts consumed by the cows were not reported in the study.

The effect of carotene on lipase activity of milk was studied by

Tarassuk and Regan (37). These workers credit Russian writers in 1909 with noticing that the incidence of rancidity in milk appeared to be minimal when green succulents were included in the dairy ration. The findings of Tarassuk and Regan indicated that the beneficial effect of green feed on milk rancidity was not due to the high carotene content of the feed, nor could rancidity be attributed to low blood levels of carotene.

In 1960, Jensen et al. (18) compared two winter rations--differing only in that one included pasture grasses while the other did not. The milk samples were split three ways; the ADV of one sample was checked as the milk was produced by the cow and labeled "initial ADV." A second sample was cooled immediately to 5°C, held at this temperature for 48 hours, and the test results labeled "spontaneous ADV." The third sample was agitated in a Waring blender prior to being cooled to 5°C and stored for 48 hours--these results were labeled "induced ADV." Their data showed that neither ration alone affected the milk's initial ADV or susceptibility to "induced lipolysis." However, the ADV of the "spontaneous lipolysis" samples were significantly lower ($P < 0.05$), when pasture was included in the ration.

Cannon and Rollins (4) investigated the effect of three planes of nutrition with green chopped alfalfa and three without, upon the ADV of the milk produced. Long-term continuous (seven and nine weeks) and short-term (10 days) "change-over" feeding trials were used. ADV's obtained during the experimental period were adjusted by co-variance to take into account the large variations in ADV among individual cows that existed during the preliminary period. The ADV differences resulting from the various rations, with or without green feed, were not

significant ($P > 0.05$). A total of 15 cows were used in the study; these cows were multiparous, grade Holsteins, 30 to 60 days post-partum. The authors make no mention of estrous cycle observations during the feeding trials; but, since the normal procedure is to breed dairy cows from 60 to 90 days post-partum, the possibility exists that estrous may have occurred during the period of time this study was under way.

Johnson and Von Gunten (21) studied two groups of cows fed either alfalfa hay or sorghum silage as the principle roughage on a "double reversal" feeding trial. Those cows consuming silage produced milk with higher ADV's than did the cows fed alfalfa hay. Unfortunately, none of the data were analyzed statistically and the level of the cow's feed intake was not reported.

As mentioned earlier, a cow may be abruptly shifted from one feeding group to another. Since the ration offered each group is quite different, the cow is forced to adjust to sudden decreases and sometimes increases in the level of energy intake. Satter and Bringe (33) in 1970, reported changes in blood metabolites during such abrupt ration changes but did not report any data concerning lipase activity in the milk produced by these cows. Askew et al. (1) reported that lipase activity differences in the biopsy samples of mammary glands taken from cows shifted from normal to high energy rations were not statistically significant ($P > 0.05$). These two recent publications are the only references found in which the effects of sudden ration changes were studied. Neither of these involved the effects of ration changes on milk lipase activity or milk flavor.

Lipase Activity

There is ample evidence that the principal lipolytic activity of cow's milk is associated with the casein (13, 31, 39). The work of True (38) contains an excellent review of the early work concerning the fractions of milk where the richest sources of lipase activity can be found. That the lipase activity is associated with casein, and more specifically with the alpha-casein complex is well documented.

Several workers (9, 10, 40) have isolated one or more casein fractions from skimmilk that were rich in lipase activity. Fox and Tarassuk (10) used rennet coagulated casein that was first precipitated selectively with ammonium sulfate and fractionated the casein on DEAE-cellulose. The casein separated on Sephadex G-200 into what appeared to be a single homogeneous protein when analyzed using polyacrylamide gel electrophoresis. These authors reported a 10 to 15% recovery, and a 500-fold concentration of the original lipase activity. This resulted in a specific activity of 15,000 units per mg in the isolated protein. When this protein was examined with the ultracentrifuge, the sedimentation coefficient was calculated as 7.5S corresponding to a molecular weight (MW) of 210,000.

Gaffney, Harper, and Gould (12) isolated several fractions containing lipase activity from skimmilk using DEAE-cellulose columns. Those workers used an eight-step gradient of sodium chloride in phosphate buffer to elute the fractions. The eight protein peaks corresponded to the NaCl change in the eluting buffer. When the same workers used a 32-step gradient, they obtained 32 fractions; again each fraction corresponded to a change in salt concentration. While lipase activity was found in all fractions isolated, some of these had much higher

activities than others. In discussing their findings, the questions was raised as to whether the lipase in the different fractions actually represented different lipase entities or merely differences in adsorption of a single lipase to different casein components. These same workers (13), in another article two years later, reported on the results of work with the eluted fraction which had the highest lipase activity. They concluded that milk lipase is a highly surface-active material capable of polymerization in such a way that the active site for lipase activity is oriented toward the surface. The presence in the lipase-rich fractions of sialic acid implicated kappa-casein as being closely associated with lipase activity.

The question as to whether milk lipase is k-casein per se, or only a part of the k-casein complex was still unresolved. True's data (38) showed low correlations between k-casein content (as determined indirectly from the sialic acid content), and the lipase activity of the milk. These findings precluded the possibility of predicting lipase activity from the k-casein content of the milk. True found that the richest lipase activity was again associated with a protein of an estimated 200,000 MW and that this protein would separate into at least two types of sub-units under certain conditions.

While other laboratories were separating the lipase-active fractions on the basis of charge interaction, Downey and Andrews (6) used size as their separation criteria. In this study, lipase activity was first isolated from skim milk by adding NaCl to a final concentration of 0.75M and ultracentrifugation at 80,000 G for one hour. The soluble caseins in the supernatant thus isolated had an estimated MW of 300,000. The supernatant casein contained about 70% of the original tributyrinase

activity of the original milk. With Sephadex chromatography, three fractions were separated with estimated MW of 112,000, 75,000, and 39,000. The MW of a fourth fraction was too small to be calculated by the methods used. These same workers (7) later increased the time in the ultracentrifuge to 2 hours, but otherwise used the same procedure and separated five proteins with lipase activity, that ranged in size from 37,000 to 175,000 MW.

The chromatographic behavior of milk lipase of Sephadex gels in increasing concentrations of phosphate buffer was reported by T. P. Rout (32). When 0.1M phosphate buffer, pH 6.5, was used as eluant, milk lipase separated into two distinct peaks, one being eluted close to the void volume of the column, the other being significantly retarded. When an extract of rennet curd was chromatographed under the same conditions, only the high MW enzyme could be detected. Chromatography of the whey revealed the presence of the low MW enzyme only. Chromatography of the milk lipase preparations in increasing concentrations of NaCl in the absence of phosphate gave results similar to those reported by Downey and Andrews (6) who used 0.02M buffer. However, no traces of the multiple enzymes reported by them could be found when 0.1M phosphate buffer was used as elutant.

Downey and Murphy (8) investigated the relationship between the lipase activity of skimmilk and that of colloidal phosphate-free milk. The casein micelles in milk had a MW larger than 10^8 while the micelles in the colloidal phosphate-free milk were only one fiftieth as large with a MW of approximately 2×10^6 . Evidence that colloidal calcium-phosphate linkages stabilize the larger micellar casein was presented. The model system in this study used pancreatic lipase adsorbed to casein,

and the authors postulated that milk lipases were adsorbed to casein. When the molecular system containing skimmilk casein plus pancreatic lipase was eluted from Sepharose 2-B columns, the skimmilk lipase activity did not parallel the activity of the pancreatic lipase. Apparently the pancreatic lipase was adsorbed to the same protein as the skimmilk lipase but, in addition, was adsorbed to other proteins not associated with the skimmilk lipase. Colloidal phosphate-free milk did not contain the lipase activity found in the milk prior to removal of the phosphate.

Oxidized Flavor

As oxidized flavors develop in milk, the flavor passes through various organoleptic stages which judges have described as flat for slightly oxidized to metallic, paper (cardboard), oily, or tallowy for extremely strong flavors (27).

Major factors that are known to cause oxidized milk have been extensively studied and are well documented in the literature. These factors are cited in a 1964 review (36) wherein several studies are listed covering the various breeds of cows and geographical areas of the United States and several foreign countries, where these flavor criticisms occur in fresh milk. Of the samples tested, 17-21% were found to be oxidized.

The effect of different feeds upon oxidized flavor in milk was also reviewed; but no studies were reported which discussed the effect of plane of nutrition, or a sudden change in plane of nutrition, upon oxidized milk.

A study involving 72 cows over 12 consecutive months was reported by Plowman et al. (31). Milk from these cows was examined

organoleptically, and an attempt was made to correlate the development of oxidized milk to several factors. Correlation coefficients within cows for relative humidity, air temperature, and the type of feed consumed were reported as being "low."

The relationship between sensory and chemical methods for measuring the intensity of oxidized flavors was studied by Lillard and Day (23). Working with 16 samples collected over a 6 month period, the flavor components were identified using column partition chromatography methods. From 97 to 99% of the carbonyl-reactive material in oxidized milk fats was nonvolatile. Concentration of the individual volatile monocarbonyls, at the judges' flavor threshold of the milk fats, was in parts per billion. Statistical analysis indicated that correlation coefficients between all the chemical tests for oxidized flavor intensity and the reciprocal of the judges' flavor threshold were significant at the 1% level, the volatile unsaturated carbonyls giving the highest correlations (0.996).

Feed Flavors

A classical definition for feed flavor can be found in the book entitled Judging Dairy Products by Nelson and Trout (27):

"The feed flavor is characteristic in that it is aromatic, somewhat pleasant and can be readily detected by the sense of smell. The characteristic cleanliness of feed flavors when the sample is rejected from the mouth distinguishes them from the cowy or barny flavors. The feed flavors disappear rather quickly leaving the mouth clean, while cowy or barny flavors persist with an unclean after taste."

Feed flavors were extensively reviewed by O. W. Parks (28) who made reference to an eighteenth century work where it was considered that the feed the cow consumed was the contributing cause of abnormal flavors in

the milk. More recent work by Dougherty et al. (5) is cited in this review where the evidence for feed flavors resulting from feed constituents is not as clear cut. Onion slurry was fed to tracheal fistulated cows and the milk produced by these cows analyzed for flavor. If the eructated gases were allowed to enter the lung, a pronounced flavor was detected in the milk as soon as 15 minutes after ingestion. When onion vapors were injected directly into the lung, no flavor developed in the milk. The role of the rumen in liberating flavor substance from onions, which can be transmitted to the milk, became evident when vapors from onion slurry, incubated for 30 minutes at 37°C with rumen ingesta were introduced into the tracheal cannula and a pronounced flavor was found in the milk in 15 minutes. It must be noted that the off-flavor which occurred in the milk was not characteristic of the onion. However, similar experiments on other substances gave rise to off-flavors which were rather typical of the feed involved.

It would appear that the exact cause of feed flavors is still not clearly understood. Nor is the flavor criticism feedy a clear cut defect, rather it is often used in practice as a "catch-all." Because very few samples are scored as perfect, high scoring milk is very often labeled as feedy to differentiate it from perfect milk.

Cow Flavors

This milk flavor is generally attributed to a complex mixture of lower fatty acids, "acetone bodies," and other regularly occurring volatile products (30). In this study, Patton (30) measured the effect of methyl sulfide on the flavor of milk. By taste panel, the threshold for methyl sulfide in distilled water was determined to be 12 parts per

billion. At slightly above this threshold concentration, the compound imparted a milk-like flavor according to the taste observers. When distinctly above the threshold, the flavor was described as malty or cowy. The odor of methyl sulfide was characteristic, not only in the volatiles from milk, but of cow's breath as well. The author went on to speculate that,

"...the occurrence of abnormally high concentrations of methyl sulfide could account for certain 'cowy' and feed-type off-flavors, whereas abnormally low amounts of the compound such as might be met in concentrated or dried milk products may be responsible in part for the lack of so-called 'fresh milk flavor.'"

More often, the criticism cowy is associated with acetone or acetone bodies (27). The relationship of acetone with cowy flavor was reported by Josephson and Keeney (20). When concentrations of acetone were less than 25 parts per million (ppm), most experienced judges described the flavor as feed. These same judges described milk with 50 ppm acetone as slightly cowy, 100 ppm as pronounced cowy, and 150 ppm as very cowy, slightly acetone. Bergman (3) listed the three metabolic pathways involved in the excretion of the ketone bodies by the cow as the breath, the urine, and the milk. In a recent symposium on ketosis in cows, Schultz (34) warned against abrupt changes in plane of nutrition, since this appeared to be a major factor causing ketosis in dairy cattle.

It would be logical, when one considers the work of Dougherty et al. (5), that during ketosis, not only would milk be exposed to the ketones from the blood via the rumen, but also from the lung. Thus, it would appear that even if the ketotic condition were sub-clinical, the production of ketone bodies would have the appropriate pathways to become flavor components of the milk being produced.

No reference is made in any of the literature as to the effect that ketosis, at any level of severity, may have on the milk produced during the period the cow is ketotic.

CHAPTER III

METHODS AND MATERIALS

Uniformity Trial

A three-year-old Holstein cow (No. 102), known to be free of disease or other abnormalities, was selected from the Oklahoma State University experimental dairy herd. The cow was assigned an individual stall where her feed intake could be controlled, and she could be fed and handled under uniform conditions. Body weights of the cow were determined by weighing before each milking for two days (four milkings). These weights were then used in calculating the National Research Council (NRC) (26) energy requirements. Water was available at all times, and salt blocks were available in an adjacent exercise lot. She was also assigned to an individual stall in a stanchion barn where the concentrate could be fed on an individual basis. Her daily ration consisted of a 50:50 ratio of hay to concentrate on a weight/weight basis. The alfalfa hay was selected from a single lot that had been analyzed and found to contain 11% digestible protein (DP) and 49 megacalories per hundred weight. The concentrate fed had a "guaranteed analysis" of 11% DP and 86 megacalories per hundred weight.

During the experimental period, the cow was milked twice daily starting at 6:00 in the morning and 5:00 in the evening. The milk was sampled for 12 consecutive days. The sample was cooled immediately in a water bath to 5°C and stored at this temperature until analyzed. The

milk was protected against exposure to light at all times.

Milk samples were collected during the evening milking, and the weight of all milk produced by the cow was recorded. The whole milk was analyzed for milk fat and total solids content by the Mojonnier method (25), the solids-not-fat were found by difference. A portion of each sample was skimmed and analyzed for lipase activity units by the method of True (38), where a lipase unit is the micromoles of butyric acid liberated per minute of reaction time per ml of skimmilk at 37°C. Ash and calcium content in the skimmilk was determined by the method of Jenness (16). A portion of the skimmilk was dialyzed and its lipase activity, ash, and calcium content again determined using the same analytical techniques.

The methods of Barr and Goodnight, Statistical Analysis System (2), were used. Linear, quadratic, cubic, and quartic effects of each of the ten variables as related to time were determined and correlation coefficients between time and each variable also were calculated. Multiple regression coefficients of the ten dependent variables were calculated with the independent variable being the day the milk was produced.

Preliminary Work

Temperature, wind direction, and wind velocity were recorded, and the correlation coefficients between each of the three variables and lipase activity were calculated.

Possible stress on the cow from the changing day-to-day conditions in the herd was studied. The daily routine of the cow was upset by forcing her to come into the milking parlor at unusual times during the milking period. The cow was then held back in the holding stall while

other cows were allowed to by-pass her to be fed and milked while she fretted. The effect of strenuous exercise, involving running until the heart beat and respiration rate had doubled, was studied. Milk samples were collected during these periods; the milk was analyzed for lipase activity, and also tasted for flavor.

Trial I

For trial I, a double reversal or "switch back" design as developed by H. L. Lucas (24) was used. Twelve cows were used: eight Ayrshires, two Holsteins, and two Jerseys. These were chosen such that six pairs of animals were obtained, based on breed, stage of lactation, and initial milk production. The pairs were divided at random into two groups with one member of each pair in each group. The groups were assigned at random to the design shown in Figure 1.

A two week standardization period was used to adjust the cows to the experimental procedures.

The treatment (low) consisted of reducing the concentrate intake so that only 80% of the NRC energy requirements were being met. When the cow was on full feed (normal), 100% of the NRC energy requirements were being met. These twelve cows were handled, their milk sampled and analyzed in the same manner as was the single Holstein (No. 102) used in the uniformity trial. In addition, a portion of each sample was assigned a flavor score by a panel of one to four judges after 12 hours of storage. Milk was weighed and recorded each milking and a sample collected from each cow at the evening milking from Sunday through Friday. During the course of this trial, three cows had to be removed from the experiment for various reasons.

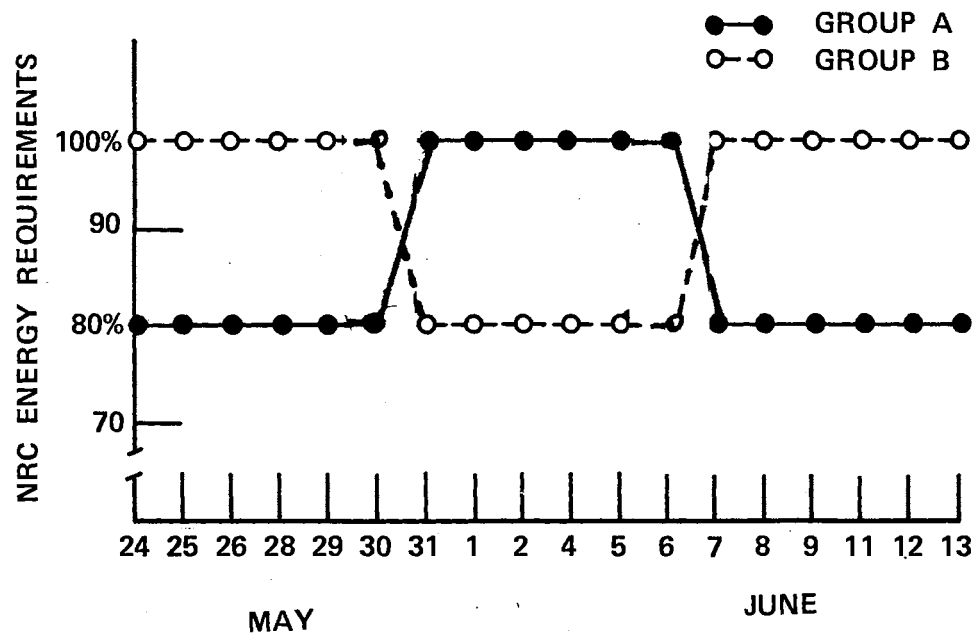


Figure 1. The Double Reversal Design Used in Trial I Showing % NRC Requirements Plotted Over Days

Trial II

At the completion of trial I, the cows were given a three week standardization period to minimize any possible carry-over effect of the previous trial. Two of the cows eliminated from the previous trial due to mastitis had recovered and were included in the experiment. A new cow (No. 003) was substituted into the Holstein pair, replacing the cow lost during the previous trial. Samples were again collected from Sunday through Friday and handled in the same manner as in the previous trials.

The portion of the milk sample to be flavor scored was assigned a letter code, at random. The tasting of the samples then proceeded according to the alphabetical order of the samples. In addition, three of the samples were chosen at random to be presented to the judges as unidentified duplicates within the alphabetical order of all samples. This was done to allow for measurements within judge, as well as among the judges' flavor scores.

The experimental period chosen was 28 days long, June 25 to July 21, 1972. This time period can be divided into the three day segments as shown in Figure 2. All cows received the treatment (the low energy ration) for one week and the normal ration for the remainder of the time. The two groups did not receive the low ration at the same time--group A was treated June 28 to July 5 (Period one), and group B from July 12 to July 18 (Period two).

Analysis of the Data

The Statistical Analysis System (SAS) was used to calculate correlation between the flavor scores (mean of all judges) vs milk production

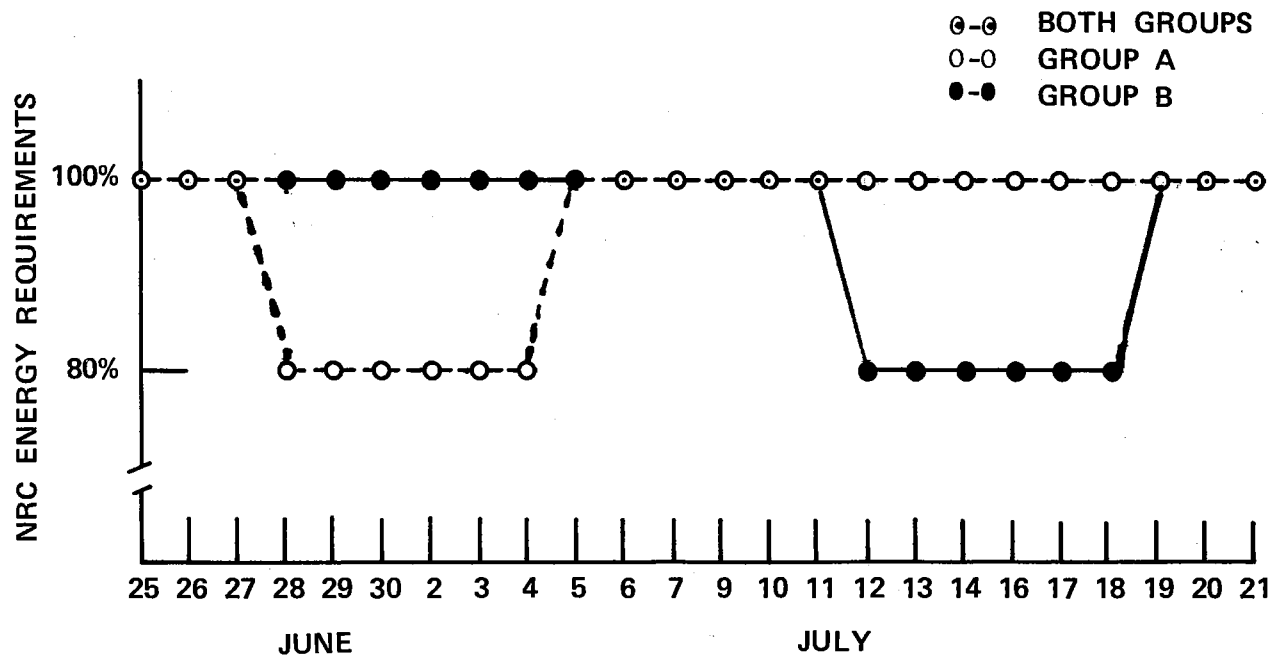


Figure 2. The Experimental Design of Trial II Showing the % NRC Requirements Plotted Over Days

and lipase activity. Multiple regression coefficients of lipase on milk production and flavor scores were also calculated. Analysis of variance was used to isolate the effects of treatment differences, day in treatment group, cow in treatment group, as well as the interactions between these variables. Differences between the judges, among the judges, interactions between judge and day, as well as between the flavor criticisms, and among the flavor criticisms were calculated.

CHAPTER IV

RESULTS AND DISCUSSION

Uniformity Trial

The multiple regression coefficients and sequential sums of squares associated with the linear, quadratic, cubic, and quartic effects upon the ten dependent variables in this trial were calculated using SAS and are shown in Table I.

It is apparent from this table that the quartic regressions fitted are insufficient to explain a significant part of the variability in any of the variables except the amount of ash in the skimmilk after dialysis (dialysate). The relative sizes of the sums of squares explained by the linear, quadratic, cubic, and quartic effects indicate a cyclic variation in the levels of the variables in the milk from day to day. This variation is more complicated than a quartic relation, but the data is insufficient to attempt any further model.

Preliminary Work

Data collected on weather and compared to lipase activity in the cow's milk during the corresponding period of time, showed that lipase activity varied independently of the weather. The calculated correlation coefficients (Table II) between lipase activity and mean daily temperature, wind velocity and direction, are very low.

The effects of any stress caused by upsetting the cow's routine by

TABLE I

MULTIPLE REGRESSION OF TEN DEPENDENT VARIABLES
ON TIME, SEQUENTIAL SUM OF SQUARES FOR
LINEAR, QUADRATIC, CUBIC, AND
QUARTIC EFFECTS FOR COW NO.
102 DURING TWELVE DAY
UNIFORMITY TRIAL

Variable	Linear	Quadratic	Cubic	Quartic	EMS ^a
Lipase Units ^b in Skimmilk	0.21 ^c -0.96 ^d	0.04 0.27	0.12 -0.03	0.18 0.00	0.55
Lipase Units ^e in Dialysate	0.16 -0.82	0.04 0.30	0.16 -0.04	0.47 0.00	0.27
Kg Daily Milk Production	304.30 0.64	34.77 -0.35	34.50 0.01	0.32 0.00	94.44
mg Ash per ml Whole Milk	7050.00 128.00	5682.00 -44.38	140.80 5.24	8848.00 -0.21	2355.20
mg Ash per ml Dialysate	1132.00 ^f -58.55	55.05 15.38	1210.00 ^f -1.63	548.50 0.05	142.85
mg Calcium per ml Whole Milk	2.02 26.79	41.69 -7.77	14.78 0.86	191.50 -0.03	50.90
mg Calcium per ml Dialysate	106.50 -25.70	79.39 9.49	135.50 -1.22	468.80 0.05	92.27
% Fat in Whole Milk	1.12 6.62	2.64 -1.94	0.64 0.22	4.80 -0.01	0.83
% Total Solids in Whole Milk	0.82 -3.53	3.88 1.04	0.09 -0.11	1.21 00.00	1.69
% Solids-not-fat in Whole Milk	5.89 11.99	0.76 -3.24	4.92 0.35	6.68 -0.01	0.64

^aError Mean Square.

^b μ moles of butyric acid liberated per minute per ml of skimmilk at 37°C.

^cThe top line is the sequential sum of squares.

^dThe bottom line is the regression coefficient.

^e μ moles of butyric acid liberated per minute per ml of dialyzed skimmilk at 37°C.

^f $P < 0.05$.

TABLE II
CORRELATION BETWEEN LIPASE ACTIVITY AND
THREE WEATHER VARIABLES OF
32 OBSERVATIONS

Weather Variable	Correlation Coefficients
Mean Daily Temperature	0.08 ^a
Mean Wind Direction	-0.29 ^a
Mean Wind Velocity	-0.31 ^a

^ap > 0.05.

exercise or timing changes, could not be observed to cause lipase variation in the milk, i.e., milk lipase activity varied independently of these conditions when they were imposed upon the cow.

Milk collected from the cow during the time when she was fed the 80% NRC energy ration had an objectionable odor and could be criticized as being cowy when tasted. This same flavor was again found in the milk when the cow abruptly changed from the 80% to the 100% ration. However, by the time the cow had been on adequate feed intake for a few days, this flavor disappeared.

Trial I

Data from this trial were collected from May 24 through July 13, 1972. Two cows developed mastitis and could not be included in the trial results; one cow had a recurrence of a previous electrolyte imbalance and was also removed. The remaining nine cows appeared to be normal and were observed through the entire experimental period.

Means of milk production, lipase activities, and flavors scores (assigned to each sample by a panel of judges) were recorded, and the means over six samples are shown in Table XII in the Appendix.

The analyses of variance (24) for trial I are shown in Table III. Milk production was significantly lower ($P < 0.01$) during the treatment periods when the cows were on the low ration. Lipase activity also was significantly lower ($P < 0.05$) during the low treatment periods. The flavor scores appeared to be lower during the treatment period, but these differences were not statistically significant ($P > 0.05$).

TABLE III
ANALYSES OF VARIANCE OF MILK PRODUCTION,
LIPASE ACTIVITY, AND MEAN FLAVOR
SCORES FOR TRIAL I

Source	df	SS	MS	F
Milk Production				
Treatment Group	1	77.85	77.85	26.57 ^a
Error	7	20.48	2.93	
Lipase Activity				
Treatment Group	1	0.08	0.08	7.36 ^b
Error	7	0.07	0.01	
Mean Flavor Score				
Treatment Group	1	0.04	0.04	^c
Error	7	1.54	0.22	

^a $p < 0.01$.

^b $p < 0.05$.

^c $p > 0.05$.

Trial II

Milk Production

The data for milk production, lipase activity, and flavor scores were recorded and are shown in Tables XIII - XVI in the Appendix.

The mean for daily milk production was plotted over time for the means of both treatment groups, Figure 3. The mean of daily milk production was less for the treated groups than for that of the controls, but this difference was not statistically significant ($P > 0.05$) when tested using an analysis of variance technique.

The difference of each individual cow from the mean of the group was then plotted, Figures 5 - 14 in the Appendix. It would appear that the wide variation among cows would make the detection of even large group differences difficult.

Lipase Activity

The mean of lipase activity for each treatment group was plotted (Figure 4). From these data it would appear that the treated animals were lower in lipase activity than the controls. But these differences also were not statistically significant ($P > 0.05$) for the groups as a whole.

It was observed in trial I that the greatest amount of change in flavor score would occur immediately after the feed was changed. Thus, the data for trial II can be arranged in a series of observations expressed as the mean of the three-day results. These means are shown in Tables IV - VII.

It can be seen in Table IV that the variance among cows is

relatively large compared to the variance among days. This also is indicated by analyses of variance in Tables XVII - XXIV in the Appendix. The scores for the 72 hour samples, shown in Table VII, appear to drop for group A (the treated group in period one, trial II), but so do the scores for the controls (group B). In period two, trial II, the scores appear to improve during the treatment of group B, while the controls (group A) decreased.

The lipase activity of each cow was plotted as a difference from the group mean after these means had been adjusted to zero, Figures 15 - 24 in the Appendix. One cause of variation can be seen when the day of estrous (heat) is noted, where the graph is marked with an "H." From the work of Wells et al., it is known that lipase activity in blood and milk varies considerably immediately prior to, and a day or two after estrous. An example of this can be seen in the plot of cows No. 094, 426, 773, 789, and 841. Further, it would appear that in the case of cow No. 841 that a "silent heat" (estrous without any outward signs) occurred on the 18th of July. This day was well within the expected norms of a recurring cycle had the cow not conceived at the previous insemination and this cow did cycle again on a later date.

It would appear that cow No. 789 "held up" her milk on the day of estrous (July 18) only to have a sizable increase in the amount produced the following day, Figure 22 in the Appendix; a corresponding decrease in lipase activity is also noticed for the same three-day period.

The rhythmical up and down nature of the plotted lipase data agrees with the high variance for quartic effects noted in the preliminary work with cow No. 102. This effect is more pronounced in some cows, i.e., cow No. 042, and not as pronounced in some others, i.e., cow No. 773.

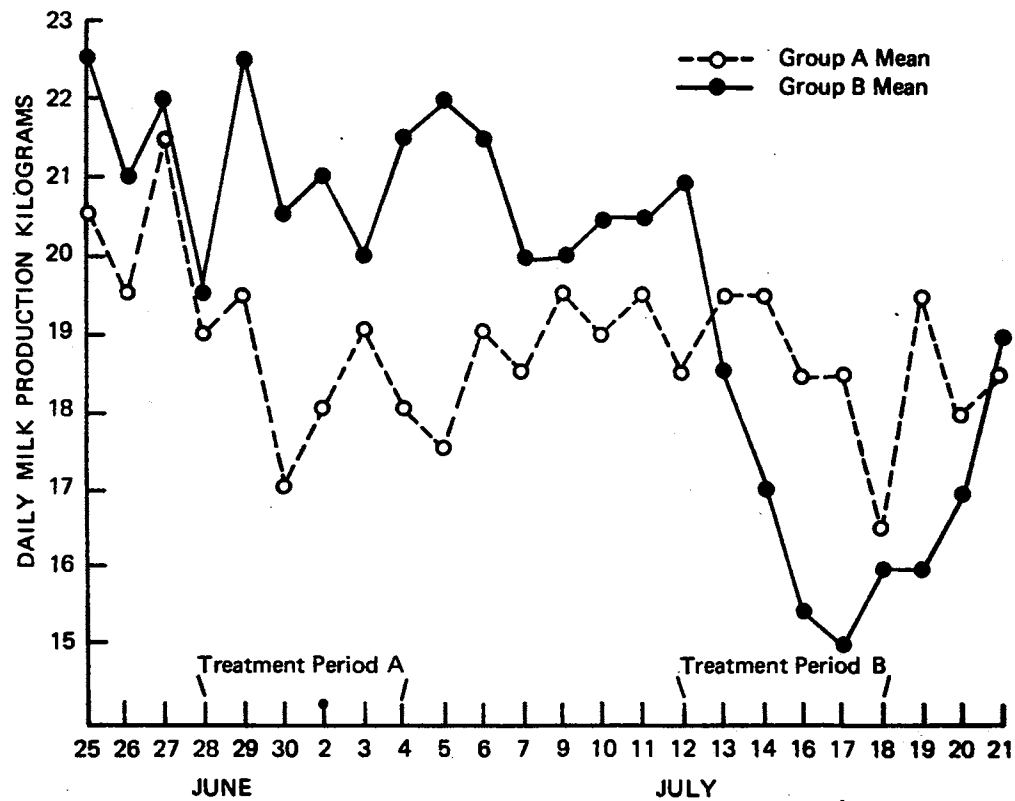


Figure 3. Mean Daily Milk Production of Each Group in Trial II

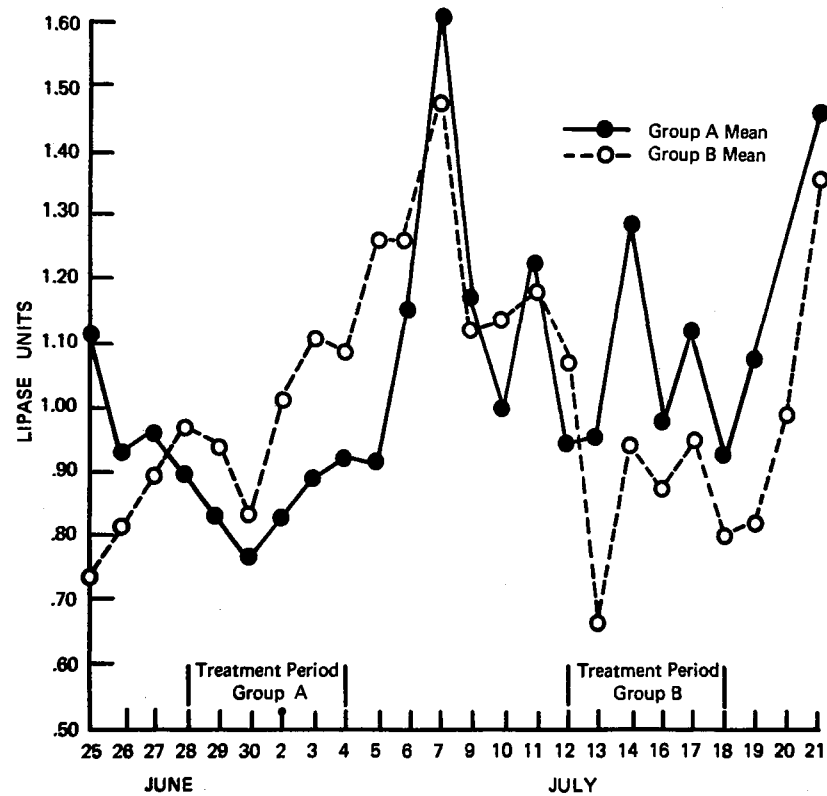


Figure 4. Mean Daily Lipase Activity in the Milk of the Two Groups. A Lipase Unit is Equal to the μ moles of Butyric Acid Liberated per Minute per ml of Skimmilk @ 37°C

TABLE IV

KILOGRAMS OF MILK PRODUCED BY INDIVIDUAL COWS,
EXPRESSED AS A MEAN OVER THREE
DAY PERIODS, TRIAL II

Cow No.	6/25-27	6/28-30	7/2-4	7/5-7
	Normal ^a	Low ^b	Low	Normal
003	14	13	12	12
042	23	20	18	19
070	24	19	19	20
090	13	12	11	11
773	24	24	25	25
789	<u>23</u>	<u>22</u>	<u>22</u>	<u>23</u>
Total	121	110	107	110
	Normal	Normal	Normal	Normal
094	22	22	21	21
426	24	24	23	15
841	23	22	22	23
976	<u>15</u>	<u>14</u>	<u>15</u>	<u>15</u>
Total	84	82	81	74
	7/9-11	7/12-14	7/16-18	7/19-21
	Normal	Normal	Normal	Normal
003	13	13	13	13
042	21	20	19	20
070	22	22	21	21
090	12	12	11	11
773	25	26	24	23
789	<u>22</u>	<u>21</u>	<u>19</u>	<u>24</u>
Total	115	114	107	112
	Normal	Low	Low	Normal
094	20	20	17	19
426	22	21	18	17
841	23	20	17	19
976	<u>15</u>	<u>13</u>	<u>10</u>	<u>10</u>
Total	80	74	62	65

^aNormal ration meets 100% of the NRC requirements.

^bLow ration meets 80% of the NRC requirements.

TABLE V
LIPASE ACTIVITY^a OF INDIVIDUAL COWS; EXPRESSED
AS A MEAN OVER THREE DAY PERIODS, TRIAL II

Cow No.	6/25-27	6/28-30	7/2-4	7/5-7
	Normal ^b	Low ^c	Low	Normal
003	0.95	0.75	0.60	0.77
042	1.15	1.07	1.12	1.37
070	1.04	0.84	1.09	1.43
090	0.69	0.46	0.53	0.76
773	0.59	0.23	0.27	0.76
789	<u>1.07</u>	<u>1.06</u>	<u>1.31</u>	<u>1.72</u>
Total	5.49	4.41	4.92	6.81
	Normal	Normal	Normal	Normal
094	0.99	1.20	1.39	1.43
426	0.68	0.60	0.61	0.94
841	0.77	1.09	1.33	1.89
976	<u>0.48</u>	<u>0.40</u>	<u>0.57</u>	<u>0.70</u>
Total	2.92	3.29	3.90	4.96
	7/9-11	7/12-14	7/16-18	7/19-21
	Normal	Normal	Normal	Normal
003	0.99	0.85	0.75	1.14
042	1.41	1.26	1.18	1.45
070	1.30	1.38	1.27	1.40
090	0.67	0.66	0.69	0.71
773	0.40	0.40	0.33	0.28
789	<u>1.45</u>	<u>1.25</u>	<u>1.29</u>	<u>1.51</u>
Total	6.22	5.80	5.51	6.49
	Normal	Low	Low	Normal
094	1.37	1.09	1.05	1.39
426	0.81	0.66	0.56	0.73
841	1.51	1.05	1.18	1.46
976	<u>0.54</u>	<u>0.40</u>	<u>0.34</u>	<u>0.31</u>
Total	4.23	3.20	3.13	3.89

^aLipase unit = μ moles of butyric acid liberated per minute per ml of skimmilk @ 37°C.

^bNormal ration meets 100% of the NRC requirements.

^cLow ration meets 80% of the NRC requirements.

TABLE VI

FLAVOR SCORES OF THE MILK FROM INDIVIDUAL COWS
 SCORED AFTER 12 HOURS STORAGE, EXPRESSED AS
 A MEAN OVER THREE DAY PERIODS, TRIAL II

Cow No.	6/25-27	6/28-30	7/2-4	7/5-7
	<u>Normal^a</u>	<u>Low^b</u>	<u>Low</u>	<u>Normal</u>
003	36.5	37.4	36.7	37.2
042	36.6	36.9	36.1	37.2
070	36.6	36.9	36.4	36.2
090	37.0	37.0	36.3	36.9
773	36.8	36.8	36.8	37.1
789	<u>36.9</u>	<u>37.3</u>	<u>36.8</u>	<u>37.5</u>
Total	220.4	222.3	219.1	222.1
	<u>Normal</u>	<u>Normal</u>	<u>Normal</u>	<u>Normal</u>
094	36.7	37.3	36.5	37.1
426	37.0	37.0	37.2	36.7
841	37.4	36.7	37.2	36.7
976	<u>37.4</u>	<u>36.1</u>	<u>36.2</u>	<u>37.1</u>
Total	149.4	147.1	147.1	147.6
	<u>Normal</u>	<u>Normal</u>	<u>Normal</u>	<u>Normal</u>
	7/9-11	7/12-14	7/16-18	7/19-21
	<u>Normal</u>	<u>Normal</u>	<u>Normal</u>	<u>Normal</u>
003	37.2	36.4	36.9	36.8
042	35.4	36.1	36.0	35.9
070	36.7	36.6	36.5	36.9
090	36.9	37.0	37.0	36.7
773	36.7	36.8	36.3	36.3
789	<u>37.5</u>	<u>37.4</u>	<u>36.9</u>	<u>37.0</u>
Total	220.4	220.3	219.6	219.6
	<u>Normal</u>	<u>Low</u>	<u>Low</u>	<u>Normal</u>
094	36.8	37.7	37.3	37.2
426	37.0	36.7	36.8	37.4
841	37.2	37.2	36.9	37.0
976	<u>36.8</u>	<u>37.0</u>	<u>36.4</u>	<u>36.9</u>
Total	147.8	148.6	147.4	148.5

^aNormal ration meets 100% of the NRC requirements.

^bLow ration meets 80% of the NRC requirements.

TABLE VII

FLAVOR SCORES OF THE MILK FROM INDIVIDUAL COWS
 SCORED AFTER 72 HOURS STORAGE, EXPRESSED AS
 A MEAN OVER THREE DAY PERIODS, TRIAL II

Cow No.	6/25-27	6/28-30	7/2-4	7/5-7
	<u>Normal^a</u>	<u>Low^b</u>	<u>Low</u>	<u>Normal</u>
003	36.3	35.7	36.3	36.8
042	36.0	35.5	33.9	35.6
070	36.4	35.6	36.6	36.2
090	36.8	36.5	36.0	37.0
773	36.6	36.9	36.4	36.6
789	<u>33.5</u>	<u>33.4</u>	<u>34.2</u>	<u>35.2</u>
Total	215.6	213.6	213.4	217.4
	<u>Normal</u>	<u>Normal</u>	<u>Normal</u>	<u>Normal</u>
094	36.6	36.5	36.3	36.0
426	36.6	35.8	36.0	36.0
841	36.4	36.5	36.9	36.7
976	<u>37.4</u>	<u>36.9</u>	<u>36.1</u>	<u>36.8</u>
Total	147.0	145.7	145.3	145.5
	<u>7/9-11</u>	<u>7/12-14</u>	<u>7/16-18</u>	<u>7/19-21</u>
003	36.8	35.7	36.0	36.8
042	35.1	35.2	35.1	35.6
070	36.7	36.4	36.2	36.8
090	36.7	36.1	36.6	35.6
773	36.8	35.9	35.9	36.8
789	<u>34.0</u>	<u>35.8</u>	<u>35.8</u>	<u>34.8</u>
Total	216.1	215.1	215.6	216.4
	<u>Normal</u>	<u>Low</u>	<u>Low</u>	<u>Normal</u>
094	35.8	36.7	36.7	37.0
426	35.3	36.5	36.5	37.0
841	36.9	36.4	36.8	37.0
976	<u>36.1</u>	<u>36.9</u>	<u>35.9</u>	<u>37.2</u>
Total	144.1	146.5	145.9	148.2

^aNormal ration meets 100% of the NRC requirements.

^bLow ration meets 80% of the NRC requirements.

Flavor Scores

A total of 89 samples for each cow at 12 hours and 88 at 72 hours was tasted. Flavor score means for both the 12 and 72 hour samples were calculated over all judges and plotted in Figures 15 - 24 in the Appendix. This allows a comparison of the two scores on a day-to-day basis. One would logically expect lower scores in the older milk (72 hour), but this was not always the case. In fact, only one cow (No. 789) had a consistently lower 72 hour score. Her milk was criticized a total of 84 out of 88 times as being oxidized at 72 hours; the only day the milk was not oxidized after 72 hours was on July 18, the day she was in heat. The opposite can be seen in the scores of cow No. 773 which normally were quite high but her lowest score for 72 hour milk appeared the day after estrous, Figure 21 in the Appendix. With this cow the 12 hour milk was feedy, but three of the four judges criticized the 72 hour sample as oxidized. The cow with the lowest flavor scores was No. 042 who had 50 criticisms for cowy and 28 for rancid in the 12 hour samples, 6 cowy, 30 rancid, and 34 oxidized in the 72 hour samples. A comparison between the lipase activity in milk and the flavor score, whether the sample was cowy, oxidized, or rancid, would probably show that the scores varied independently of the lipase activity as most of these samples had relatively low lipase activities.

Correlation and Regression Calculations

A preliminary run was made on these data for milk production, lipase activity, and mean flavor scores, using an SAS program to calculate multiple regression coefficients and correlation. Four cows (two from each group) that would appear to bear the most promising

correlation between these three variables, were selected for a preliminary run on the computer. The results of the calculations are shown in Table VIII. The correlation between time and any of the three variables was very low (-0.32 or less) and the regression coefficients were close to zero indicating the prediction line was almost horizontal.

Analysis of Variance

For statistical analysis, the treatment period from June 28 to July 4 was designated as period one, and from July 12 to July 18 as period two. Lipase activity, milk production, and the two tasting times, 12 and 72 hours, were analyzed within each period. This resulted in four variables for analysis. To balance the design, only those days with all judges were used in the analysis, except for variable four (period two, 72 hours) where three judges scores were used since one judge had missed three days. The calculations are shown in Tables XXV - XXIX in the Appendix. For all four variables, a total of 670 samples was used. The percentages of samples with each flavor criticism are shown in Table IX. The only one of the four variables tested in which the treated cows were significantly different ($P < 0.05$) from the controls was during period two for the 72 hour samples when the treated samples scored higher than the controls, Table XXIX.

In one out of the four variables (period one, 12 hour samples), the judges differed significantly from each other ($P < 0.05$), Table XXV.

The judges varied between themselves on the fresh (12 hour) samples, but the most variation was between themselves on a day-to-day basis if the samples were 72 hours old.

TABLE VIII
CORRELATION AND REGRESSION COEFFICIENTS OF FOUR
COWS FOR THREE VARIABLES: MILK PRODUCTION,
LIPASE ACTIVITY, AND MEAN FLAVOR
SCORE

Cow No.		Milk x Lipase	Milk x Flavor	Flavor x Lipase	Milk x Lipase	Milk x Flavor	Flavor x Lipase
		No Treatment			Treatment		
003	B ^a	0.01	-0.08 ^b	0.04	0.06 ^c	-0.20	0.02
	r ^d	0.19	-0.23 ^b	0.21	0.60 ^c	-0.27	0.18
070	B	0.00	0.02	0.02	0.01	0.19	0.03
	r	-0.06	0.14	0.05	0.17	0.27	0.25
426	B	0.01 ^c	-0.02	0.00	0.01 ^b	-0.01	0.04
	r	0.32 ^c	-0.10	0.00	0.44 ^b	-0.07	0.31
841	B	-0.01	-0.02	0.15 ^b	-0.01	-0.05	0.13 ^c
	r	-0.08	-0.08	0.26 ^b	-0.12	-0.24	0.58 ^c

^aThe regression of lipase on milk, flavor on milk, lipase on flavor where flavor is the flavor score.

^bStatistically significant $P < 0.05$.

^cStatistically significant $P < 0.01$.

^dThe correlation coefficient.

TABLE IX
FLAVOR CRITICISM FREQUENCIES IN 670 SAMPLES
USED FOR ANALYSIS OF VARIANCE

Flavor Criticism	Number of Observations	Percentage of Total
Feed	290	43.3
Feed and Oxidized	21	3.1
Feed and Cowy	70	10.4
Feed and Rancid	1	0.1
Oxidized	137	20.1
Cowy	103	15.4
Cowy and Oxidized	4	0.6
Cowy and Rancid	1	0.1
Rancid	27	3.9
No Criticism	16	2.4

Intensity of Flavors

The four flavors: feed, cowy, rancid, and oxidized, were tabulated by assigning a value to the intensity of the flavor in the sample. Pronounced intensity was assigned a value of three, slight a two, and if the flavor was not present, a value of one. The summary of the 670 samples used in the analysis are tabulated and shown in Table X. Feed criticisms were more common to 12 hour samples, while oxidized was the most common criticism in the 72 hour samples. Cowy criticisms were about equally divided between the 12 and 72 hour samples, and between the treated and not treated periods.

TABLE X
COMPARISON OF THE SAMPLE MEANS FOR ALL JUDGES OF
THE INTENSITY OF FLAVOR CRITICISMS FOR ALL
PERIODS AFTER STORAGE AT 12 AND 72 HOURS

No. of Samples		Intensity of Flavor Criticism ^a			
		Feed	Cow	Rancid	Oxidized
		12 Hour, Period One			
Treatment	96	1.78	1.44	1.00	1.13
No Treatment	64	1.80	1.42	1.01	1.09
		72 Hour, Period One			
Treatment	120	1.43	1.33	1.12	1.78
No Treatment	80	1.71	1.29	1.00	1.34
		12 Hour, Period Two			
Treatment	64	1.63	1.37	1.01	1.05
No Treatment	96	1.68	1.29	1.13	1.09
		72 Hour, Period Two			
Treatment	60	1.50	1.28	1.00	1.35
No Treatment	90	1.25	1.22	1.18	1.69

^a1.00 = no criticism; 2.00 = slight criticism; 3.00 = pronounced criticism.

Duplicated Samples

All the complete data, where all judges and all duplicates were available, for the 72 hour samples were analyzed in a randomized block design technique. The analysis of variance of this design was calculated and is shown in Table XI. The differences between judges as well as the differences for judges between duplicates were not statistically significant ($P > 0.05$).

TABLE XI
ANALYSIS OF VARIANCE FOR DIFFERENCES BETWEEN
JUDGES AND BETWEEN DUPLICATES FOR 72 HOUR
SAMPLES, TRIAL II

Source	df	SS	MS	F
Total	23	240.95		
Duplicate	1	12.04	12.04	
Judge	3	38.29	12.76	
Error	19	190.62	10.03	

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this research was to investigate the relationships among several variables that could cause a change in the flavor of milk. A uniformity trial was conducted to observe a cow under "ideal conditions" and to measure several components in her milk during this period. Considerable day-to-day variation was noted. Preliminary work was conducted to measure weather, stress, and feed intake as possible causes of variation. Trial I was conducted using a double reversal design where the treatment consisted of feeding the cow at 80% of her calculated NRC energy requirement during six-day treatment periods. Trial II was designed to allow measurements over shorter periods of time.

During the 12 days that cow No. 102 was observed in the uniformity trial, lipase activity, fat, solids-not-fat, total solids, calcium, and ash were found to have a quartic relationship over time; but the amount of milk produced had a linear effect over time. Weather (i.e., wind velocity, wind direction, and daily temperature), stress, and exercise appeared to vary independently of the lipase activity in the milk. The feed intake, however, appeared to be related to undesirable flavor criticisms in milk produced during periods of reduced feed intake. Trial I indicated that, during those periods when the cows were treated at 80% NRC energy requirements, milk production was significantly lower ($P < 0.01$), and lipase activity was also significantly lower ($P < 0.05$);

but the flavor differences were not significant ($P > 0.05$). Trial II data show the wide variation between cows, but it also shows one cow responded with rancid milk while another responded with oxidized milk. It would appear that the effect of sudden decreases in meeting the cow's energy requirements could cause some cows to produce milk with undesirable flavor and that these cows would require different kinds of handling than is now commonly used.

Four judges tasted a total of 2,496 milk samples over a 24-day period and these data, indicating that different judges tasted different flavors in the same sample, were available for analysis. There was no statistically significant difference between the judges when they all tasted the same sample, nor within the judges when they were required to taste samples in duplicate; however, not all samples were scored identically, nor did all four judges agree exactly on certain flavor criticisms. Slight flavors caused more differences between judges than did distinct flavors. Since there is no information in the literature concerning how different judges score the same sample, nor comparisons between flavor thresholds of different judges, this large body of data will be further analyzed at a later date.

LITERATURE CITED

1. Askew, E.W., J.D. Benson, J.W. Thomas, and R.S. Emery. 1971. Metabolism of fatty acids by mammary glands of cows fed normal, restricted roughage, or magnesium oxide supplemented rations. J. Dairy Sci., 54:854.
2. Barr, A.J., and J.H. Goodnight. 1972. In a user's guide to the statistical analysis system. Students Supply Stores, North Carolina Univ., Raleigh.
3. Bergman, E.N. 1971. Hyperketonemia-ketogenesis and ketone body metabolism. J. Dairy Sci., 54:936.
4. Cannon, R.Y., and G.H. Rollins. 1964. Effect of source of nutrients and nutritional level of the cow on development of hydrolytic rancidity in the milk. J. Dairy Sci., 47:41.
5. Dougherty, R.W., W.F. Shipe, G.U. Gudnason, R.A. Ledford, R.D. Peterson, and R. Scarpellino. 1962. Physiological mechanisms involved in transmitting flavors and odors to milk. I. Contribution of eructated gasses to milk flavor. J. Dairy Sci., 45:472.
6. Downey, W.K., and P. Andrews. 1965. Gel filtration applied to the study of lipases and other estrases. Biochem. J., 94:642.
7. Downey, W.K., and P. Andrews. 1969. Evidence for the presence of several lipases in cow's milk. Biochem. J., 112:559.
8. Downey, W.K., and R. F. Murphy. 1970. Association of lipases with micellar and soluble casein complexes. J. Dairy Res., 37:47.
9. Fox, P.F., M. Yaguchi, and N.P. Tarassuk. 1967. Distribution of lipase in milk proteins. II. Dissociation from k-casein with dimethylformamide. J. Dairy Sci., 50:307.
10. Fox, P.F., and N.P. Tarassuk. 1968. Bovine milk lipases. I. Isolation from skimmilk. J. Dairy Sci., 51:826.
11. Fredeen, H., J.E. Bowstead, W.L. Dunkley, and L.M. Smith. 1951. Hydrolytic rancidity in milk. II. Some management and environmental factors influencing lipolysis. J. Dairy Sci., 34:521.
12. Gaffney, P.J., Jr., W.J. Harper, and I.A. Gould. 1966. Distribution of lipase among components of a water extract of rennet casein. J. Dairy Sci., 49:921.

13. Gaffney, P.J., Jr., W.J. Harper, and I.A. Gould. 1968. Characteristics of lipase rich fractions of milk protein. *J. Dairy Sci.*, 51:1161.
14. Herrington, B.C., and V.N. Krukovsky. 1939. Studies on lipase action. III. Lipase action in the milk of the individual cow. *J. Dairy Sci.*, 22:149.
15. Hileman, J.L., and E. Courtney. 1935. Seasonal variations in the lipase content of milk. *J. Dairy Sci.*, 18:247.
16. Jenness, R. 1953. Titration of calcium and magnesium in milk and milk fractions with ethylenediamine tetraacetate. *Anal. Chem.*, 25:966.
17. Jensen, R.G. 1964. Lipolysis. *J. Dairy Sci.*, 47:210.
18. Jensen, R.G., G.W. Gander, and A.H. Duthie. 1960. Spontaneous and induced lipolysis in milk during alternate feeding of two winter rations and a winter ration and pasture. *J. Dairy Sci.*, 43:762.
19. Jensen, R.G., J. Sampugna, R.M. Parry, Jr., and T.L. Forster. 1963. Absence of fatty acid specificity during lipolysis of some synthetic triglycerides by beta-esterase preparations from milk. *J. Dairy Sci.*, 46:842.
20. Josephson, D.V., and D.G. Keeney. 1947. Relationship of acetone bodies to "cowy" flavor in milk. *Milk Dealer*, 36:40.
21. Johnson, P.E., and R.L. Von Gunten. 1962. A study of factors involved in the development of rancid flavor in milk. *Okla. Exp. Sta., Stillwater. Bull.*, B-593.
22. Kelly, P.L. 1945. Milk lipase activity: A method for its determination and its relationship to the estrual cycle. *J. Dairy Sci.*, 28:803.
23. Lillard, D.A., and E.A. Day. 1961. Autoxidation of milk lipids. II. The relationship of sensory to chemical methods for measuring the oxidized flavor of milk fats. *J. Dairy Sci.*, 44:623.
24. Lucas, H.L. 1955. Switchback trials for more than two treatments. *North Carolina Agr. Exp. Sta. Paper No. 662*, p. 146.
25. Mojonnier, T., and H.C. Troy. 1925. The technical control of dairy products. 2nd Ed. Mojonnier Bros. Co., Chicago.
26. National Research Council, U.S. 1966. Nutrient requirements of dairy cattle. Publ. 1349. Washington, D.C.
27. Nelson, J.A., and G.M. Trout. 1965. Judging dairy products. The Olsen Publ. Co., Milwaukee, Wisc.

28. Parks, O.W. 1967. Milk flavors. The chemistry and physiology of flavors: A symposium. H.W. Schultz, E.A. Day, and L.M. Libby, eds. p. 296. AVI Publ. Co., Westport, Conn.
29. Patel, C.V., P.F. Fox, and N.P. Tarassuk. 1968. Bovine milk lipase. II. Characterization. J. Dairy Sci., 51:1879.
30. Patton, S., D.A. Forss, and E.A. Day. 1956. Methyl sulfide and the flavor of milk. J. Dairy Sci., 39:1469.
31. Plowman, R.D., J.W. Smith, and K.E. Nelson. 1963. Factors affecting flavor in milk from individual cows. J. Dairy Sci., 46:630.
32. Rout, T.P. 1970. Investigations into the molecular weight of milk lipase. Proc. Aust. Biochem. Soc., 3:15.
33. Satter, L.D., and A.N. Bringe. 1970. Effect of abrupt ration changes on milk and blood components. J. Dairy Sci., 52:1776.
34. Schultz, L.H. 1971. Management and nutritional aspects of ketosis. J. Dairy Sci., 54:962.
35. Shahani, K.M., W.J. Harper, R.G. Jensen, R.M. Parry, and C.A. Zittle. 1973. Enzymes in bovine milk: A review. J. Dairy Sci., 56:531.
36. Shipe, W.F. 1964. Oxidations in the dark. J. Dairy Sci., 44:221.
37. Tarassuk, N.P., and W.M. Regan. 1943. A study of the blood carotene in relationship to lipolytic activity of milk. J. Dairy Sci., 26:987.
38. True, L.C. 1969. Studies on bovine skimmilk lipase and its relationship to certain milk proteins. Ph.D. Thesis. Okla. State Univ., Stillwater.
39. Wells, M.E., O.P. Pryor, D.M. Haggerty, H.C. Pickett, and J.B. Mickle. 1969. Effects of estrous cycle and lactation on lipase activity in bovine milk and blood. J. Dairy Sci., 52:1110.
40. Yaguchi, M., N.P. Tarassuk, and N. Abe. 1964. Distribution of lipase in milk protein. I. DEAE-cellulose column chromatography. J. Dairy Sci., 47:1167.

APPENDIXES

TABLE XII

MEANS OF MILK PRODUCTION, LIPASE ACTIVITY^a, AND
FLAVOR SCORES OF INDIVIDUAL COWS DURING
THREE ONE WEEK PERIODS, TRIAL I

Period	042	070	<u>Group 1: Cow No.</u>		
			090	773	789
<u>Kilograms Milk Production</u>					
1. Low ^a	24.7	23.7	14.3	23.7	23.0
2. Normal ^b	24.4	22.8	14.9	26.2	25.3
3. Low	21.1	19.7	14.3	25.0	21.9
<u>Lipase Activity Units</u>					
1. Low	0.81	1.02	0.38	0.21	0.96
2. Normal	1.22	1.22	0.55	0.25	1.13
3. Low	0.89	0.88	0.40	0.33	1.00
<u>Flavor Scores</u>					
1. Low	35.2	36.3	37.0	37.3	37.3
2. Normal	36.6	35.3	36.8	37.3	37.0
3. Low	35.8	35.0	36.7	36.5	36.6
<u>Group 2: Cow No.</u>					
	094	426	941	976	
<u>Kilograms Milk Production</u>					
1. Normal	25.7	19.1	17.7	20.1	
2. Low	24.2	16.4	18.1	16.1	
3. Normal	24.7	18.5	18.3	15.9	
<u>Lipase Activity Units</u>					
1. Normal	1.11	0.48	0.62	0.32	
2. Low	1.25	0.49	0.67	0.25	
3. Normal	1.13	0.62	0.55	0.27	
<u>Flavor Scores</u>					
1. Normal	37.3	36.5	35.7	35.8	
2. Low	36.7	36.7	36.1	37.1	
3. Normal	36.3	36.4	37.4	36.5	

^aLipase unit = μ moles of butyric acid liberated per minute per ml of skimmilk @ 37°C.

^bLow ration meets 80% of the NRC requirements.

^cNormal ration meets 100% of the NRC requirements.

TABLE XIII

MILK PRODUCTION, LIPASE ACTIVITY^a, AND 12 HOUR FLAVOR SCORES
OF ALL COWS, ALL JUDGES OVER ALL DAYS, TRIAL II.
RANCID, COWY, FEED, AND OXIDIZED FLAVORS
ARE NOTED WITH THE FIRST INITIAL

Cow (No.)	Milk (Kg.)	Lipase (Units)	Judges				Milk (Kg.)	Lipase (Units)	Judges				Milk (Kg.)	Lipase (Units)	Judges																																									
			1 ^b	2	3	4			1	2	3	4			1	2	3	4																																						
Date: 6-25-72																			Date: 6-26-72																			Date: 6-27-72																		
003	13	0.82	37F	37C	37F	35C	15	0.91	36FC	37F	36F	36FC	15	1.11	36C	37F	36C	36FC																																						
042	24	1.29	38F	36C	37F	35C	24	1.15	36FC	36C	38	37F	22	1.00	38F	35C	36F	37F																																						
070	24	1.10	37FC	37F	37F	37F	24	1.02	36FC	37F	36FC	35C	27	0.99	36F	37F	37F	37F																																						
090	13	0.70	37F	36F	38F	36FC	14	0.66	38F	38F	37FC	36FC	13	0.71	37FC	38F	36C	37F																																						
773	27	1.16	37F	38F	37F	37F	21	0.29	36F	38F	37FC	36F	25	0.31	37F	37FC	35C	36FC																																						
789	23	1.07	37F	38F	37F	37F	21	1.02	38F	37F	36F	37F	24	1.11	36C	36FC	37F	37F																																						
Date: 6-28-72																			Date: 6-29-72																			Date: 6-30-72																		
003	14	1.02	37F		37F	38F	14	0.59	38F	38F	37F	38F	12	0.64		36C	38F	38F																																						
042	21	1.18	36FC		37F	37F	19	0.94	37F	37F	37F	38F	20	1.08		35C	37C	38F																																						
070	18	0.75	38F		36FC	37F	20	0.93	37F	37F	36FC	37F	20	0.85		37F	37F	37F																																						
090	13	0.64	36FC		37F	38F	12	0.36	38F	38F	38F	36FC	12	0.39		37C	36C	36C																																						
773	26	0.32	36F		37F	37F	28	0.26	36F	38F	36FC	38F	19	0.11		37F	36C	37F																																						
789	21	0.91	38F		38F	37F	22	1.34	37	38F	37F	38F	22	0.93		36C	38F	38F																																						
094	22	1.21	36FO		38F	37F	23	1.35	37F	38F	37F	37F	20	1.04		37F	37F	38F																																						
426	25	0.69	37F		36F	37F	26	0.64	37	38F	38F	38F	23	0.48		37F	37F	38F																																						
841	22	1.22	360		37F	36FC	22	1.00	36?	38F	36C	37F	22	1.04		37F	36	38F																																						
976	14	0.39	38F		37F	35FC	15	0.41	36C	37F	36FC	36FC	14	0.41		36C	35C	35C																																						

TABLE XIII (Continued)

Cow (No.)	Milk (Kg.)	Lipase (Units)	Judges				Milk (Kg.)	Lipase (Units)	Judges				Milk (Kg.)	Lipase (Units)	Judges					
			1	2	3	4			1	2	3	4			1	2	3	4		
Date: 7-2-72							Date: 7-3-72							Date: 7-4-72						
003	12	0.38	37F	37C	36FC	37F	12	0.79	36FC	37F	38C	38F	13	0.64	36C	38F	36F	36FC		
042	18	0.99	36C	34C	36C	36FC	19	1.34	36FO	360	36C	36FC	18	1.02	37F	35C	36FC	38F		
070	20	0.93	36F	36C	36C	36FC	20	1.13	360	37F	37F	37F	18	1.20	36C	36C	37F	38F		
090	11	0.52	360	37F	35C	36FC	11	0.54	35C	38F	38	38F	12	0.54	36C	37C	35C	35C		
773	24	0.27	36F	37F	35C	37F	27	0.21	36FC	37F	36C	38F	25	0.32	37F	38F	35C	38F		
789	21	1.37	360	36F	38F	38F	22	0.80	37F	360	37F	36FO	21	1.25	37F	37F	37F	38F		
Date: 7-2-72							Date: 7-3-72							Date: 7-4-72						
094	21	1.42	34FC	37C	37F	36FC	21	1.45	350	36C	36FO	37F	20	1.29	36F	38F	38	38F		
426	23	0.41	37F	38F	38F	38F	21	0.61	37F	37F	37F	38F	25	0.80	37F	37FC	36FC	37F		
841	21	1.33	38F	38F	36FC	38F	21	1.03	37F	38F	37F	38F	24	1.30	36FC	37F	35C	38F		
976	15	0.54	37C	37C	36FC	36FC	15	1.03	36FC	36R	36FC	36FC	15	0.60	360	36C	36FC	36FC		
Date: 7-5-72							Date: 7-6-72							Date: 7-7-72						
003	11	0.46	37F	37F	37F	36FC	11	0.76	38	38F	37F	37F	13	1.08		37F	37F	38F		
042	18	0.92	37F	36C	37F	36FC	20	1.45	38	35C	36R	36R	21	1.75		35CR	37F	35CF		
070	19	1.00	37F	37F	37F	36FC	20	1.50	36C	37F	36C	36FC	21	1.78		36CF	36C	35CF		
090	11	0.67	36C	37F	38F	38F	12	0.69	36C	37F	37FC	36FC	11	0.92		38F	37F	36CF		
773	24	0.34	38	37F	35C	38F	27	0.43	36F	37F	37C	36FC	23	1.52		37F	38F	38F		
789	22	1.52	37F	38F	38F	38F	23	1.57	36F	37F	37F	38F	23	2.06		38F	37F	37F		
Date: 7-5-72							Date: 7-6-72							Date: 7-7-72						
094	21	1.31	36F	37F	38	37F	22	1.54	36F	37F	38F	37F	19	1.44		38F	37F	38F		
426	25	0.88	37F	37F	35C	36FC	24	0.87	36C	36C	37F	36FC	23	1.08		37F	38F	37F		
841	23	1.82	360	37C	35C	36FC	23	1.61	36F	37F	37F	38F	23	2.25		37F	37CF	36FC		
976	16	0.67	350	37F	38F	38F	15	0.64	36F	37F	37F	38F	14	0.80		38F	38F	37F		

TABLE XIII (Continued)

Cow (No.)	Milk (Kg.)	Lipase (Units)	Judges				Milk (Kg.)	Lipase (Units)	Judges				Milk (Kg.)	Lipase (Units)	Judges					
			1 ^D	2	3	4			1	2	3	4			1	2	3	4		
Date: 7-9-72							Date: 7-10-72							Date: 7-11-72						
003	13	1.02	37F	38F	38F		12	0.91	37F	37F	37	35FC	14	1.05	37F	37C	37F	38FC		
042	22	1.49	36C	36C	37C	35R	21	1.23	36R	35R	36R	34R	21	1.50	36R	35RC	34R	34RC		
070	23	1.37	36F	37F	37F	37F	20	1.10	37F	36FC	38F	36FC	23	1.44	36F	37F	38F	35FC		
090	12	0.72	36C	36C	37F	36CF	12	0.49	36C	36FC	38F	37F	11	0.70	38	37C	38F	38F		
773	24	0.39	37F	37F	37C	38F	27	0.39	360	37F	38F	36FC	24	0.43	360	36F	36F	36FC		
789	22	1.48	37F	37F	37FO	38F	21	1.24	38	37F	37F	38F	23	1.64	38	37F	38F	38F		
Date: 7-9-72							Date: 7-10-72							Date: 7-11-72						
094	21	1.36	37	37F	36FC	37FO	21	1.37	36C	37F	38F	36FC	20	1.37	37F	37C	37F	37F		
426	21	0.77	37F	38F	38	36FC	20	0.80	37F	360	37F	36FC	24	0.85	37F	37F	38F	36FC		
841	22	1.52	37F	37F	38	37F	24	1.55	37F	36FC	38F	36FC	23	1.46	38	38F	38F	37F		
976	15	0.50	37F	37FC	37F	38F	14	0.47	36C	35C	37C	36FC	15	0.66	37	37C	36F	38F		
Date: 7-12-72							Date: 7-13-72							Date: 7-14-72						
003	16	0.79	37F	37F	38	37F	14	0.71		37FC	37F	36R	14	1.06		36F	37F	38F		
042	21	1.22	37F	35R	38	36RC	20	1.25		35R	37F	36FC	20	1.30		36FC	33C	35R		
070	22	1.17	360	36C	38	35FC	22	1.29		37F	38	38F	22	1.68		37F	35C	36FC		
090	10	0.57	36FC	38F	38	37F	14	0.56		37F	37F	36R	13	0.86		36C	36C	38F		
773	26	0.36	38F	36C	37F	35FC	25	0.34		38F	37F	38F	26	0.51		37F	34C	38F		
789	21	0.97	36FC	37F	370	38F	21	1.00		37F	38	38F	21	1.77		38F	37F	38F		
Date: 7-12-72							Date: 7-13-72							Date: 7-14-72						
094	21	1.27	37	38F	38	37F	19	0.80		37F	38	38F	18	1.21		38F	38F	38F		
426	25	0.75	37F	37F	38	37F	20	0.46		36C	37F	36FC	20	0.76		37F	36C	38F		
841	21	1.27	37F	37F	38	38F	22	0.81		36C	38	35C	18	1.06		37F	38F	38F		
976	16	0.63	37F	37F	38	37F	12	0.21		37F	38	36FC	12	0.35		37F	37F	36C		

TABLE XIII (Continued)

Cow (No.)	Milk (Kg.)	Lipase (Units)	Judges				Milk (Kg.)	Lipase (Units)	Judges				Milk (Kg.)	Lipase (Units)	Judges					
			1 ^b	2	3	4			1	2	3	4			1	2	3	4		
Date: 7-16-72							Date: 7-17-72							Date: 7-18-72						
003	11	0.72	37F	37F	35C	37F	12	0.85	37F	37F	37F	38F	15	0.66	360	37F	37F	38F		
042	17	0.95	37F	36C	38F	38F	19	1.56	37F	35R	35R	34R	21	1.03	36C	35R	37F	34R		
070	20	1.20	36C	37F	35C	36FC	21	1.29	360	37F	38F	37FC	21	1.33	360	37F	36F	37F		
090	12	0.70	36F	37F	37F	38F	11	0.68	37F	37F	38	38FR	11	0.69	36C	37F	37F	36FC		
773	25	0.31	37F	37F	34C	36FC	24	0.34	37F	37F	37C	37F	22	0.33	37F	36FC	35C	37F		
789	21	1.48	37F	37F	38F	38F	21	1.44	37C	37F	38F	38F	13	0.95	37F	36R	360	34R		
094	18	1.07	37F	37F	38	38F	18	1.05	37F	37F	38F	38F	17	1.04	36C	36C	38F	38F		
426	17	0.59	37F	37C	37F	36FC	17	0.66	37F	37F	38F	38F	19	0.42	360	36C	35C	38F		
841	16	1.05	37F	37FC	36C	38FC	18	1.45	38	38F	37C	38F	17	1.04	37C	35C	36C	36FC		
976	11	0.41	36C	37F	38F	38F	8	0.29	370	37C	35C	36FC	11	0.33	36C	36C	35C	38FR		
Date: 7-19-72							Date: 7-20-72							Date: 7-21-72						
003	13	1.11	36C	36C	37F		13	0.80	37	37F	37C		13	1.52	360	38F	37F			
042	20	1.34	36R	35R	35R		20	1.13	36R	35R	35R		20	1.88	360	38F	38F			
070	21	1.44	37F	37F	38F		21	1.16	36CO	37F	38F		22	1.60	36C	37F	36F			
090	11	0.57	360	37F	38F		11	0.59	36C	36C	36C		10	0.97	37F	38F	37F			
773	24	0.29	370	37F	36C		23	0.28	37F	36FC	36C		22	0.26	37F	36C	35C			
789	26	1.19	360	37F	37F		22	1.38	37F	37F	37F		25	1.97	37F	38F	37F			
094	18	1.11	36S	37F	38F		20	1.40	37	37F	38F		20	1.66	37F	37F	38F			
426	22	0.56	37	37F	38F		17	0.55	37F	38F	38F		25	1.07	360	38F	38F			
841	16	1.01	36FC	37F	38F		21	1.36	37F	36C	38F		19	2.00	37F	37F	37F			
976	9	0.23	370	36FC	37F		10	0.33	37F	37F	37C		11	0.36	37F	37C	37F			

^aLipase unit = μ moles of butyric acid liberated per minute per ml of skimmilk at 37°C.

^bJudges are identified by number.

TABLE XIV

FLAVOR SCORES AFTER 72 HOURS FOR ALL COWS, ALL
JUDGES OVER ALL DAYS, TRIAL II

Cow (No.)	Judges				Judges				Judges			
	1 ^a	2	3	4	1	2	3	4	1	2	3	4
	Date: 6-25-72 ^b				Date: 6-26-72				Date: 6-27-72			
003	36F	36C	37F	36FC	350		38F	36FC	36C	35C	36C	360
042	360	35CR	360	360	37F		360	360	360	35CR	360	360
070	37FC	37FC	360	37F	350		37F	37F	37	37F	360	360
090	37FC	37F	36C	37F	35C		38F	360	37F	38F	36F	38F
773	36F	38F	36FC	37F	36F		37FC	38F	36C	36C	36C	36C
789	350	320	340	330	340		330	340	350	320	330	330
094	36F	37C	37F	37F	37F		350	38F	37	37F	37F	37
426	36F	37F	36FC	37F	36F		36F	38F	37	36C	36F	38
841	37F	36CO	38F	37F	37F		36FO	360	36F	360	37F	38F
976	38F	37F	36FC	37F	38F		37FC	38F	38F	37F	37F	38F
	Date: 6-28-72				Date: 6-29-72				Date: 6-30-72			
003		37F	37F	37F	350	350	320	340	360	360	37FO	340
042		37F	360	38F	350	340	350	340	360	350	350	350
070		36CO	34FO	350	36C	340	360	340	36FC	37F	37FO	37F
090		37C	37F	38F	35CO	37F	37F	350	35C	37F	37F	36FC
773		36C	35C	37F	37F	37F	37F	38F	37F	38F	36FC	38F
789		330	330	340	350	330	320	330	340	330	340	340
094		36C	36F	37F	36F	37F	37F	360	37F	37F	37FO	36FC
426		35C	35C	35C	36F	37F	360	350	37F	36F	36F	380
841		37F	35C	37F	37F	37C	36C	35FC	37F	37F	36FC	38F
976		37F	37F	37F	360	38F	36F	38F	36FC	36C	38F	36FC
	Date: 7-2-72				Date: 7-3-72				Date: 7-4-72			
003	36FO	360	35FC	360	36	37C	36C	35FC	360	36C	37C	38F
042	35R	33R	33R	34R	340	32R	33R	34R	340	340	36FO	35FO
070	36FC	37F	37F	38F	37F	36C	37F	37F	340	35FO	37C	38F
090	35C	37FC	36C	35FC	36C	37F	37F	36FC	36F	35C	38F	35FC
773	36F	37F	35C	38F	36	37F	35C	36FC	36C	37F	37F	36FC
789	350	330	340	350	340	350	330	340	350	340	350	330
094	36FC	360	38F	37F	360	360	36FO	360	35	37F	37F	37F
426	37F	360	36FC	360	37F	37FC	360	36FO	360	350	36FO	340
841	37F	37F	37F	38F	37F	37F	36C	38F	37F	36C	37F	36FC
976	37F	35C	35FC	36FC	350	36C	36FC	38F	360	36C	37F	36FC
	Date: 7-5-72				Date: 7-6-72				Date: 7-7-72			
003		37FO	36C	35C	36F	37F	37F	38F	36F	37F	38	38F
042		36FO	360	360	350	370	36R	35R	35R	35R	35R	35R
070		35F	36C	35C	36F	37F	37F	37F	36FO	37F	36F	37F
090		36C	38F	37F	37F	37F	37F	36FC	36FO	38F	37F	38F
773		38F	36C	36FC	37F	37F	36C	38F	360	37F	350	360
789		350	370	340	350	350	360	350	350	350	350	350
094		35CO	370	360	36F	350	350	350	36	38F	37F	37F
426		340	350	350	360	360	360	350	37F	38F	37F	38F
841		36FC	36C	37FC	37F	37F	370	360	36F	37F	38	38F
976		360	37F	37F	36F	37C	37F	37F	36F	37F	37F	38F

TABLE XLV (Continued)

Cow (No.)	Judges				Judges				Judges			
	1 ^a	2	3	4	1	2	3	4	1	2	3	4
	Date: 7-9-72 ^b				Date: 7-10-72				Date: 7-11-72			
003	36	36C	38F	38F	36	37F	38F	37F	36CO	36F	37F	
042	36R	36CR	34R	35CR	36F	33R	34R	36C	34R	36R	36CR	
070	36C	36C	36C	38F	360	360	37F	37F	370	37F	37F	
090	360	36C	38	38F	360	36C	37F	36FO	37F	370	37F	
773	37	37F	37F	38F	37F	37F	36C	37F	36FC	36C	38F	
789	350	330	340	330	340	340	350	330	340	36C	330	
094	360	360	340	350	350	340	36FO	350	37F	37F	37F	
426	350	340	340	350	360	360	360	36FO	350	360	340	
841	37F	37F	38F	38F	36	37F	37F	38F	37F	36F	350	
976	36C	36C	37C	36FC	36	36C	36C	36FC	37F	35C	36FC	
	Date: 7-12-72				Date: 7-13-72				Date: 7-14-72			
003		36R	37FO	350	360	350	360	350	36C	360	360	350
042		35R	360	350	37F	35R	36R	35R	36R	35R	34R	34R
070		37F	37F	37F	360	36C	37C	37F	360	36C	360	35FR
090		37F	350	38F	37	340	360	330	36F	37F	360	37F
773		36C	35C	36FC	36C	36C	34C	36FC	37F	37F	35C	38F
789		340	340	330	350	340	340	330	350	360	350	330
094		37F	38F	37F	36C	350	360	350	37F	37FC	37F	38F
426		35C	37F	36FC	36C	360	37F	36CO	37F	37F	37F	38F
841		37F	38F	38F	360	350	360	340	37F	37F	37F	36FO
976		37F	37F	37F	37F	37F	37F	37F	36C	37C	37F	37F
	Date: 7-16-72				Date: 7-17-72				Date: 7-18-72			
003	360	360	35R	360	37F	360	37F		350	350	360	
042	350	350	350	340	36R	35R	35R		350	35R	36R	
070	360	37F	36F	36FC	350	37F	37F		350	36CO	360	
090	36C	37F	37F	37FR	36FO	36C	37F		360	37F	38F	
773	37F	36C	35C	36FC	37F	36FO	35CF		36CO	36C	35C	
789	350	350	350	340	360	350	350		37F	37F	38F	
094	360	360	38F	38F	360	36C	37F		36C	36CO	38F	
426	360	350	35C	350	38	37F	38F		36C	36C	37F	
841	37F	37F	37F	36FC	360	37F	37F		360	360	37F	
976	36C	37F	37F	35R	350	36C	36C		37F	360F	35C	
	Date: 7-19-72				Date: 7-20-72				Date: 7-21-72			
003	37F	36R	37F		37F	37F	38F	36FC	340	38F	38F	37F
042	37F	35R	35R		360	35R	36R	340	35R	35R	37F	36R
070	37F	37F	37F		37F	360	38F	37FO	360	36FO	38F	37FC
090	360	37F	38F		350	360	360	330	350	360	360	330
773	37F	37F	37F		37F	37F	36C	38F	36FO	37FC	37F	37FC
789	350	340	350		350	340	360	330	350	350	360	350
094	37F	37F	38F		36F	37F	38F	38F	36FO	36C	38F	36FC
426	37	37FC	36C		37F	37F	37F	38F	37F	37F	37F	37F
841	360	36FO	36C		37F	38F	38F	38F	37F	37F	37F	38F
976	360	37F	37F		370	38F	38F	37F	37F	38F	38F	38F

^aJudges are identified by number.^bDate collected, tasted 3 days later.

TABLE XV

FLAVOR SCORES OF DUPLICATE SAMPLES TASTED
AFTER 12 HOURS STORAGE, TRIAL II.

Cow (No.)	Judges				Cow (No.)	Judges				Cow (No.)	Judges			
	1 ^a	2	3	4		1	2	3	4		1	2	3	4
Date: 6-25-72					Date: 6-26-72					Date: 6-27-72				
042	38F	36C	37F	35C	094	38F	38F	38F	38F	003	36C	37F	36C	36FC
	38F	35C	37F	36FC		37F	38F	38F	38F		36C	37FO	37F	38F
					841	36F	37F	38F	38F	070	36F	37F	37F	37F
						36	36C	38	38F		37FC	37F	36FC	37F
										841	37F	38F	37F	38F
											37F	37F	38F	36FC
Date: 6-28-72					Date: 6-29-72					Date: 6-30-72				
090	36FC		37F	38F	094	37F	38F	37F	37F	042		35C	37C	38F
	36FC		38F	38F		37F	38F	37F	37F			35C	37FC	38F
426	37F		36F	37F	976	36C	37F	36FC	36FC	094		37F	37F	38F
	350		37F	35FC		36C	37FC	36FC	36FC			37F	38F	38F
773	36F		37F	37F						426		37F	37F	38F
	36FO		37F	37F								38F	37C	38F
Date: 7-2-72					Date: 7-3-72					Date: 7-4-72				
042	36C	34C	36C	36FC	003	36FC	37F	38F	38F	042	37F	35C	36FC	38F
	37FC	34CR	36FC	36FC		36FC	37F	36FC	38F		37F	35C	36FC	38F
426	37F	38F	38F	38F	042	36FO	360	36C	36FC	070	36C	36C	37F	38F
	36F	38F	37F	38F		360	37F	37F	36FC		36FC	35C	36F	38F
773	36F	37F	35C	37F	976	36FC	36R	36FC	36FC	789	37F	37F	37F	38F
	37FC	38F	35C	38F		36FC	35C	37F	36FC		37F	37F	37F	36FC
Date: 7-5-72					Date: 7-6-72					Date: 7-7-72				
789	37F	38F	38F	38F	094	36F	37F	38F	37F	094		38F	37F	38F
	38	37F	36FC	38F		36F	37F	37F	38F			37F	37F	37F
976	350	37F	38F	38F	789	36F	37F	37F	38F	426		37F	38F	37F
	36FC	36C	36C	36FC		37	38F	37F	38F			37F	38F	38F
					976	36F	37F	37F	38F	841		37F	37FC	36FC
						37F	37F	37F	38F			37F	37FC	37FC
Date: 7-9-72					Date: 7-10-72					Date: 7-11-72				
042	36C	36C	37C	35R	070	37F	36FC	38F	36CF	042	36R	35RC	34R	34RC
	37C	36C	35FR	35R		37F	37FC	38F	36CF		36R	37R	34R	34RC
773	37F	37F	37C	38F	090	36C	36FC	38F	37F	094	37F	37F	37F	37F
	37F	37F	37C	38F		36C	36FC	38F	37F		37F	37CF	37F	38F
789	37F	37F	37FO	38F	426	37F	360	37F	36CF					
	37F	37F	37FO	38F		37F	37F	38F	36CF					
Date: 7-12-72					Date: 7-13-72					Date: 7-14-72				
003	37F	37F	38	37F	090		37F	37F	36R	003		36F	37F	38F
	36F	38F	38	36FC			38F	37F	37F			36C	36C	36FR
070	36	36C	38	35CF	789		37F	38	38F	042		36FC	35C	35R
	36	36C	37F	36FC			37F	37F	38F			36FC	35C	38FR
094	37	38F	38	37F						070		37F	35C	36FC
	36	37F	38	37F								37F	35C	36FC
Date: 7-16-72					Date: 7-17-72					Date: 7-18-72				
003	37F	37F	35C	37F	773	37F	37F	37C	37F	426	360	36C	35C	38F
	37	38F	34C	37F		37F	37F	35C	37F		37F	36C	35C	37F
094	37F	37F	38	38F	789	37C	37F	38F	38F	841	37C	35C	36C	36FC
	37F	37F	38	37F		38	37F	38F	38F		37FC	35C	35C	36FC
976	36C	37F	38F	38F										
	36C	36C	37F	38F										
Date: 7-19-72					Date: 7-20-72					Date: 7-21-72				
090	360	37F	38F		094	37	37F	38F		003	360	38F	37F	
	36	37F	38F			36C	37CF	38F			360	38F	38F	
426	37	37F	38F		976	37F	37F	37C		042	360	38F	38F	
	36	37F	38F			37F	37F	37C			37F	35R	37F	
841	36FC	37F	38F							090	37F	38F	37F	
	37FC	37F	37F								36C	36C	38F	

^aJudges identified by number.

TABLE XVI
FLAVOR SCORES OF DUPLICATE SAMPLES TASTED
AFTER 72 HOURS STORAGE, TRIAL II

Cow (No.)	Judges				Cow (No.)	Judges				Cow (No.)	Judges			
	1 ^a	2	3	4		1	2	3	4		1	2	3	4
Date: 6-25-72					Date: 6-26-72					Date: 6-27-72				
070	37FC	37FC	360	37F	003	350		38F	36FC	094	37	37F	37F	37
	36C	36C	36F	37F		360		38F	38F		360	350	350	360
841	37F	36CO	38F	37F						426	37	36C	36F	38F
	36FC	35CO	360	350							36F	37F	37F	37F
										773	36C	36C	36C	36C
											36C	37FC	36C	36FC
Date: 6-28-72					Date: 6-29-72					Date: 6-30-72				
070		36CO	34FO	350	003	350	350	320	340	042	360	350	350	350
		35FO	350	350		340	350	350	340		350	350	350	340
789		330	330	340	426	36F	37F	360	350	094	37F	37F	37FO	36FC
		330	320	340		360	350	360	340		37F	37F	36F	36FC
976		37F	37F	37F	841	37F	37C	36C	35FC	426	37F	36F	36F	380
		37F	37F	38F		36C	36C	36FC	36FC		37F	36FC	37F	38F
Date: 7-2-72					Date: 7-3-72					Date: 7-4-72				
003	36FO	360	35FC	360	090	36C	37F	37F	36FC	426	360	350	36FO	340
	35C	38F	35C	38F		36	37F	38	36FC		36	360	37C	350
090	35C	37FC	36C	35FC	094	360	360	36FO	360	773	36C	37F	37F	36FC
	35C	37F	36C	34R		350	360	360	360		36C	37F	37F	37F
094	36FC	360	38F	37F	426	37F	37FC	360	36FO					
	36FC	360	37FC	36FC		350	36FC	360	360					
Date: 7-5-72					Date: 7-6-72					Date: 7-7-72				
094		35CO	370	360	003	36F	37F	37F	38F	042	35R	35R	35R	35R
		360	350	350		36F	37F	38F	38F		35R	35R	35R	35R
773		38F	36C	36FC	773	37F	37F	36C	38F	070	36FO	37F	36F	37F
		36F	36FC	38F		36F	37F	38F	38F		36FO	37F	36F	37F
841		36FC	36C	37FC						976	36F	37F	37F	38F
		35C	36C	36FC							37F	37F	35C	38F
Date: 7-9-72					Date: 7-10-72					Date: 7-11-72				
090	360	36C	38	38F	003	36	37F	38F	37F	003		36CO	36F	37F
	350	37FC	38F	37F		36	37F	370	37F			350	370	38F
094	360	360	340	350	773	37F	37F	36C	37F	070		370	37F	38F
	360	360	340	350		36FO	37F	37C	37F			360	37F	38F
426	350	340	340	350	841	36	37F	37F	38F	773		36FC	36C	38F
	360	350	340	350		37F	37F	37F	38F			36C	36CF	37F
Date: 7-12-72					Date: 7-13-72					Date: 7-14-72				
426		35C	37F	36FC	003	360	350	360	350	094	37F	37F	37F	38F
		36C	37F	36FC		360	350	37F	340		37F	37F	38F	37F
773		36C	35C	36FC	070	360	36C	37C	37F	773	37F	37F	35C	38F
		36C	35C	35F		360	36C	37F	37F		37F	37F	35C	37F
841		37F	38F	38F	426	36C	360	37F	36CO	976	36C	37C	37F	37F
		37F	37FO	350		360	360	360	350		36C	36C	37F	37F
Date: 7-16-72					Date: 7-17-72					Date: 7-18-72				
070	360	37F	37F	36FC	090	36FO	36C	37F		003	350	350	360	
	360	37F	360	37F		360	36C	37FR			360	350	360	
426	360	350	35C	350	841	360	37F	37F		042	350	35R	36R	
	360	360	35C	360		37F	38F	38F			360	35R	35R	
773	37F	36C	35C	36FC	976	350	36C	36C		090	360	37F	38F	
	37F	36C	35C	36FC		340	36C	36C			360	36CF	38F	
Date: 7-19-72					Date: 7-20-72					Date: 7-21-72				
094	37F	37F	38F		070	37F	360	38F	37FO	426	37F	37F	37F	37F
	37F	37F	37F			360	360	38F	350		37F	37F	37F	38F
773	37F	37F	37F		090	350	350	360	330	773	36FO	37FC	37F	37FC
	360	37F	37F			360	350	350	330		37F	37F	35C	36FC
976	360	37F	37F		841	37F	38F	38F	38F					
	350	36F	37F			37F	38F	38F	38F					

^aJudges identified by number.

TABLE XVII
ANALYSIS OF VARIANCE FOR DAILY MILK PRODUCTION
DIFFERENCES OF INDIVIDUAL COWS DURING
PERIOD ONE, TRIAL II

Source	df	SS	MS	F
Total	59	5928.33		
Treatment Group (T-G)	1	370.07	370.07	a
Cow in (T-G) ^b	8	5050.60	632.57	
Day	5	102.73	20.55	a
(T-G) x Day	5	24.78	4.96	a
Cow x Day in (T-G) ^c	40	370.15	9.25	

The mean daily milk production was 19.12 Kg and the C.V. was 7.21%.

^a $p > 0.05$.

^bError term for treatment group.

^cError term for day.

TABLE XVIII
ANALYSIS OF VARIANCE FOR DAILY MILK PRODUCTION
DIFFERENCES OF INDIVIDUAL COWS DURING
PERIOD TWO, TRIAL II

Source	df	SS	MS	F
Total	59	7.75		
Treatment Group (T-G)	1	0.24	0.24	a
Cow in (T-G) ^b	8	6.10	0.76	
Day	5	0.29	0.06	a
(T-G) x Day	5	0.04	0.01	a
Cow x Day in (T-G) ^c	40	1.06	0.03	

The mean daily milk production was 17.7 Kg and the C.V. was 0.4%.

^a $P > 0.05$.

^bError term for treatment group.

^cError term for day.

TABLE XIX
ANALYSIS OF VARIANCE FOR LIPASE ACTIVITY^a
DIFFERENCES IN THE MILK OF INDIVIDUAL
COWS DURING PERIOD ONE, TRIAL II

Source	df	SS	MS	F
Total	59	7.75		
Treatment Group (T-G)	1	0.24	0.24	b
Cow in (T-G) ^c	8	6.10	0.76	
Day	5	0.30	0.06	b
(T-G) x Day	5	0.04	0.01	b
Cow x Day in (T-G) ^d	40	1.06	0.03	

The mean was 0.83 lipase units and the C.V. was 19.7%.

^aLipase units = μ moles of butyric acid liberated per minute per ml of skimmilk at 37°C.

^b $p > 0.05$.

^cError term for treatment group.

^dError term for day.

TABLE XX
ANALYSIS OF VARIANCE FOR LIPASE ACTIVITY^a
DIFFERENCES IN THE MILK OF INDIVIDUAL
COWS DURING PERIOD TWO, TRIAL II

Source	df	SS	MS	F
Total	59	9.01		
Treatment Group (T-G)	1	0.34	0.34	b
Cow in (T-G) ^c	8	6.94	0.87	
Day	5	0.66	0.13	6.50 ^d
(T-G) x Day	5	0.35	0.07	3.50 ^e
Cow x Day in (T-G) ^f	40	0.73	0.02	

The mean was 0.88 lipase units and the C.V. was 16.1%.

^aLipase units = μ moles of butyric acid liberated per minute per ml of skimmilk at 37°C.

^b $P > 0.05$.

^cError term for treatment group.

^d $P < 0.01$.

^e $P < 0.05$.

^fError term for day.

TABLE XXI
ANALYSIS OF VARIANCE FOR FLAVOR SCORE DIFFERENCES
OF MILK FROM INDIVIDUAL COWS AFTER 12 HOURS
STORAGE DURING PERIOD ONE, TRIAL II

Source	df	SS	MS	F
Total	59	48.18		
Treatment Group (T-G)	1	0.04	0.04	a
Cow in (T-G) ^b	8	15.64	1.95	
Day	5	5.88	1.18	a
(T-G) x Day	5	3.42	0.68	a
Cow x Day in (T-G) ^c	40	23.19	0.58	

The mean flavor score was 36.62 and the C.V. was 2.07%.

^a $P > 0.05$.

^bError term for treatment group.

^cError term for day.

TABLE XXII
ANALYSIS OF VARIANCE FOR FLAVOR SCORE DIFFERENCES
OF MILK FROM INDIVIDUAL COWS AFTER 72 HOURS
STORAGE DURING PERIOD ONE, TRIAL II

Source	df	SS	MS	F
Total	59	120.33		
Treatment Group (T-G)	1	10.00	10.00	a
Cow in (T-G) ^b	8	50.00	6.25	
Day	5	14.33	2.87	2.70 ^c
(T-G) x Day	5	3.67	0.73	a
Cow x Day in (T-G) ^d	40	42.33	1.06	

The mean flavor score was 35.83 and the C.V. was 2.87%.

^a $P > 0.05$.

^bError term for treatment group.

^c $P < 0.05$.

^dError term for day.

TABLE XXIII
 ANALYSIS OF VARIANCE FOR FLAVOR SCORE DIFFERENCES
 OF MILK FROM INDIVIDUAL COWS AFTER 12 HOURS
 STORAGE DURING PERIOD TWO, TRIAL II

Source	df	SS	MS	F
Total	59	84.58		
Treatment Group (T-G)	1	3.40	3.40	a
Cow in (T-G) ^b	8	15.68	1.96	
Day	5	21.48	4.30	4.56 ^c
(T-G) x Day	5	6.45	1.29	a
Cow x Day in (T-G) ^d	40	37.57	0.94	

The mean flavor score was 36.92 and the C.V. was 2.63%.

^aP > 0.05.

^bError term for treatment group.

^cP < 0.01.

^dError term for day.

TABLE XXIV
ANALYSIS OF VARIANCE FOR FLAVOR SCORE DIFFERENCES
OF MILK FROM INDIVIDUAL COWS AFTER 72 HOURS
STORAGE DURING PERIOD TWO, TRIAL II

Source	df	SS	MS	F
Total	59	76.73		
Treatment Group (T-G)	1	18.68	18.68	7.59 ^a
Cow in (T-G) ^b	8	19.72	2.47	
Day	5	3.73	0.75	^c
(T-G) x Day	5	3.82	0.76	^c
Cow x Day in (T-G) ^d	40	30.78	0.77	

The mean flavor score was 36.23 and the C.V. was 2.42%.

^a $p < 0.05$.

^bError term for treatment group.

^c $p > 0.05$.

^dError term for day.

TABLE XXV
ANALYSIS OF VARIANCE FOR PERIOD ONE,
TRIAL II, 12 HOUR FLAVOR SCORES

Source	df	SS	MS	F
Total	159	144.44		
Treatment Group (T-G)	1	0.38	0.38	a
Cow in (T-G) ^b	8	19.51	2.44	
Day (D)	3	9.62	3.21	3.12 ^c
(T-G) x D	3	5.05	1.68	a
Cow x D in (T-G) ^d	24	24.64	1.03	
Judge (J)	3	15.32	5.11	4.26 ^c
(T-G) x J	3	1.14	0.38	a
Cow x J in (T-G) ^e	24	28.85	1.20	
D x J	9	5.41	0.60	a
(T-G) x D x J	9	2.44	0.27	a
Cow x D x J in (T-G) ^f	72	32.09	0.45	

The mean flavor score was 36.77 and the C.V. was 2.59%.

^a $p > 0.05$.

^bError term for treatment group.

^c $p < 0.05$.

^dError term for day.

^eError term for judge.

^fError term for D x J.

TABLE XXVI
ANALYSIS OF VARIANCE FOR PERIOD ONE,
TRIAL II, 72 HOUR FLAVOR SCORES

Source	df	SS	MS	F
Total	199	360.62		
Treatment Group (T-G)	1	44.85	44.85	a
Cow in (T-G) ^b	8	132.17	16.52	
Day (D)	4	8.07	2.02	a
(T-G) x D	4	5.15	1.29	a
Cow x D in (T-G) ^c	32	44.87	1.40	
Judge (J)	3	0.22	0.07	a
(T-G) x J	3	0.05	0.01	a
Cow x J in (T-G) ^d	24	37.33	1.55	
D x J	12	17.73	1.48	2.27 ^e
(T-G) x D x J	12	8.05	0.67	a
Cow x D x J in (T-G) ^f	96	62.13	0.65	

The mean flavor score was 35.87 and the C.V. was 1.78%.

^a $P > 0.05$.

^bError term for treatment group.

^cError term for day.

^dError term for judge.

^e $P < 0.05$.

^fError term for D x J.

TABLE XXVII
ANALYSIS OF VARIANCE FOR PERIOD TWO,
TRIAL II, 12 HOUR FLAVOR SCORES

Source	df	SS	MS	F
Total	159	154.99		
Treatment Group (T-G)	1	4.68	4.68	a
Cow in (T-G) ^b	8	15.75	1.97	
Day (D)	3	15.77	5.26	4.82 ^c
(T-G) x D	3	1.36	0.45	a
Cow x D in (T-G) ^d	24	26.18	1.09	
Judge (J)	3	2.27	0.76	a
(T-G) x J	3	2.07	0.69	a
Cow x J in (T-G) ^e	24	24.47	1.02	
D x J	9	10.06	1.12	a
(T-G) x D x J	9	5.71	0.63	a
Cow x D x J in (T-G) ^f	72	46.67	0.65	

The mean flavor score was 36.80 and the C.V. was 2.19%.

^a $p > 0.05$.

^bError term for treatment group.

^c $p < 0.01$.

^dError term for day.

^eError term for judge.

^fError term for D x J.

TABLE XXVIII
ANALYSIS OF VARIANCE FOR PERIOD TWO,
TRIAL II, 72 HOUR FLAVOR SCORES

Source	df	SS	MS	F
Total	149	122.29		
Treatment Group (T-G)	1	14.19	14.19	8.91 ^a
Cow in (T-G) ^b	8	12.77	1.60	
Day (D)	4	4.23	1.06	^c
(T-G) x D	4	2.41	0.60	^c
Cow x D in (T-G) ^d	32	39.37	1.23	
Judge (J)	2	1.01	0.51	^c
(T-G) x J	2	2.57	1.29	^c
Cow x J in (T-G) ^e	16	17.74	1.11	
D x J	8	7.85	0.98	3.50 ^f
(T-G) x D x J	8	2.11	0.26	^c
Cow x D x J in (T-G) ^g	64	18.03	0.28	

The mean flavor score was 36.11 and the C.V. was 1.46%.

^a $P < 0.05$.

^bError term for treatment group.

^c $P > 0.05$.

^dError term for day.

^eError term for judge.

^f $P < 0.01$.

^gError term for D x J.

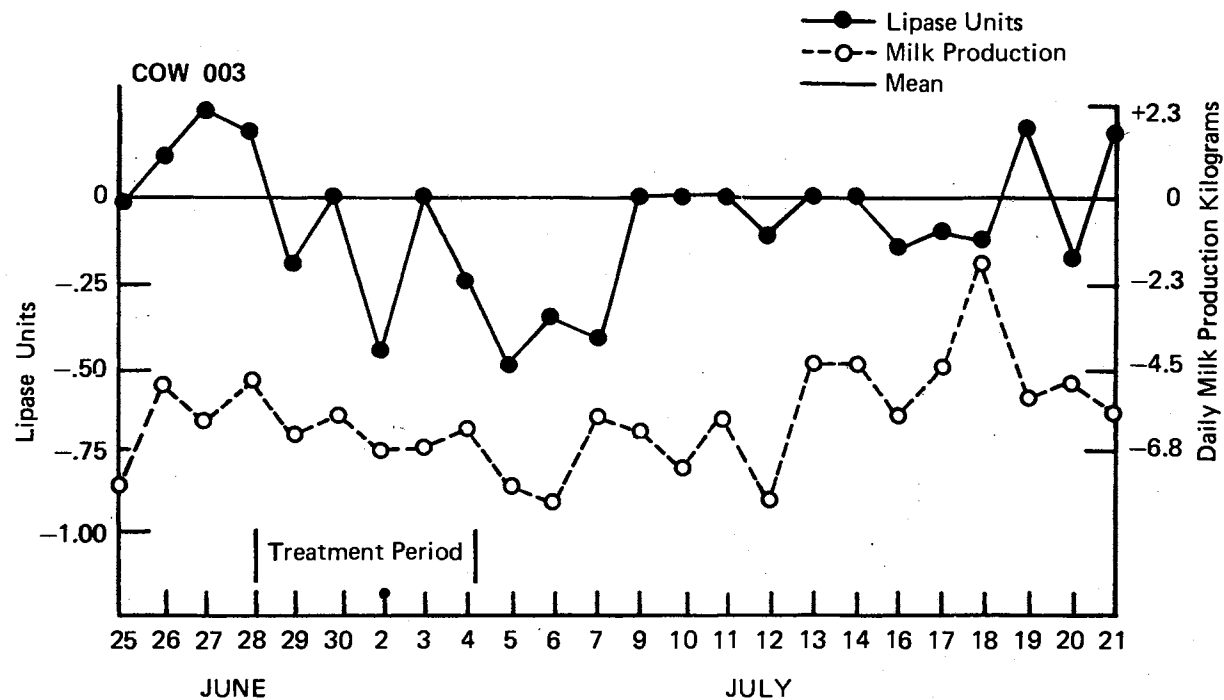


Figure 5. Difference from the Mean, Adjusted to Zero, of Lipase Units (the Amount of Enzyme Required to Liberate One μ mole of Butyric Acid per Minute per ml of Reaction Time @ 37°C) and Milk Production for Cow No. 003 When Plotted Over Days

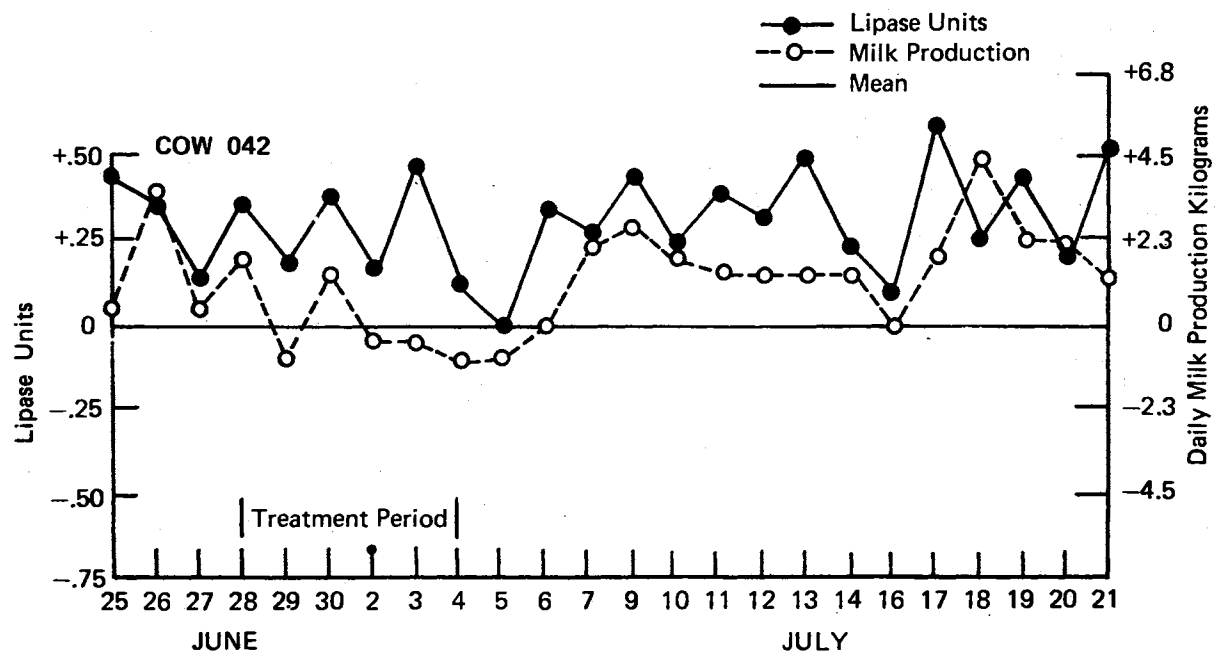


Figure 6. Difference from the Mean, Adjusted to Zero, of Lipase Units (the Amount of Enzyme Required to Liberate One μ mole of Butyric Acid per Minute per ml of Reaction Time @ 37°C) and Milk Production for Cow No. 042 When Plotted Over Days

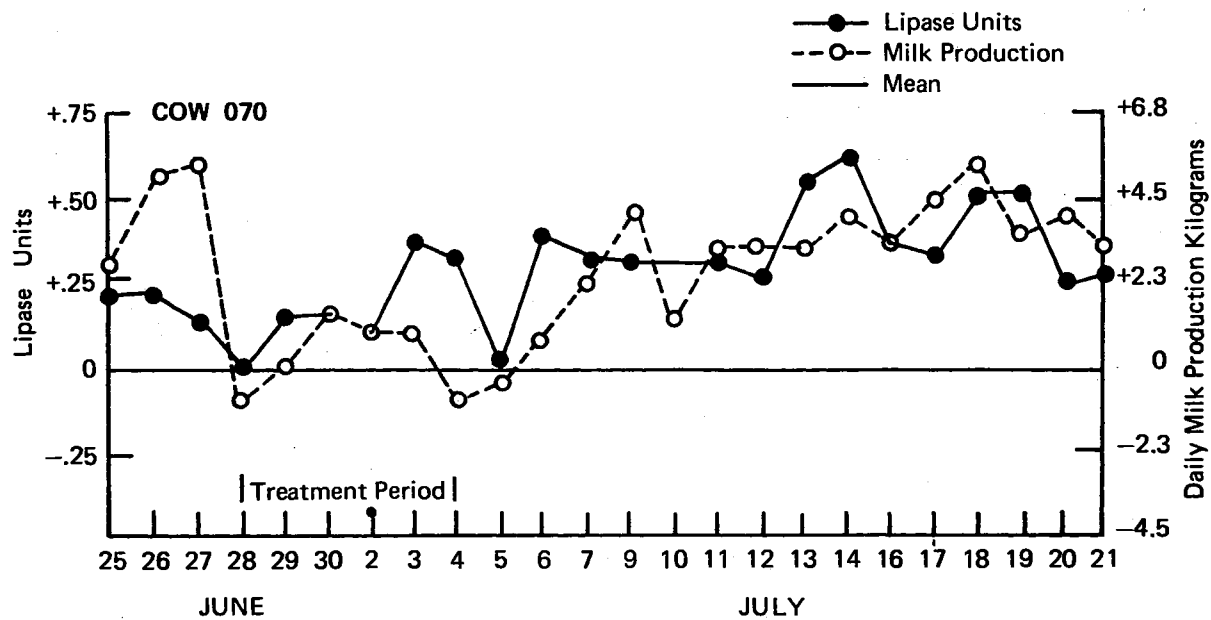


Figure 7. Difference from the Mean, Adjusted to Zero, of Lipase Units (the Amount of Enzyme Required to Liberate One μ mole of Butyric Acid per Minute per ml of Reaction Time @ 37°C) and Milk Production for Cow No. 070 When Plotted Over Days

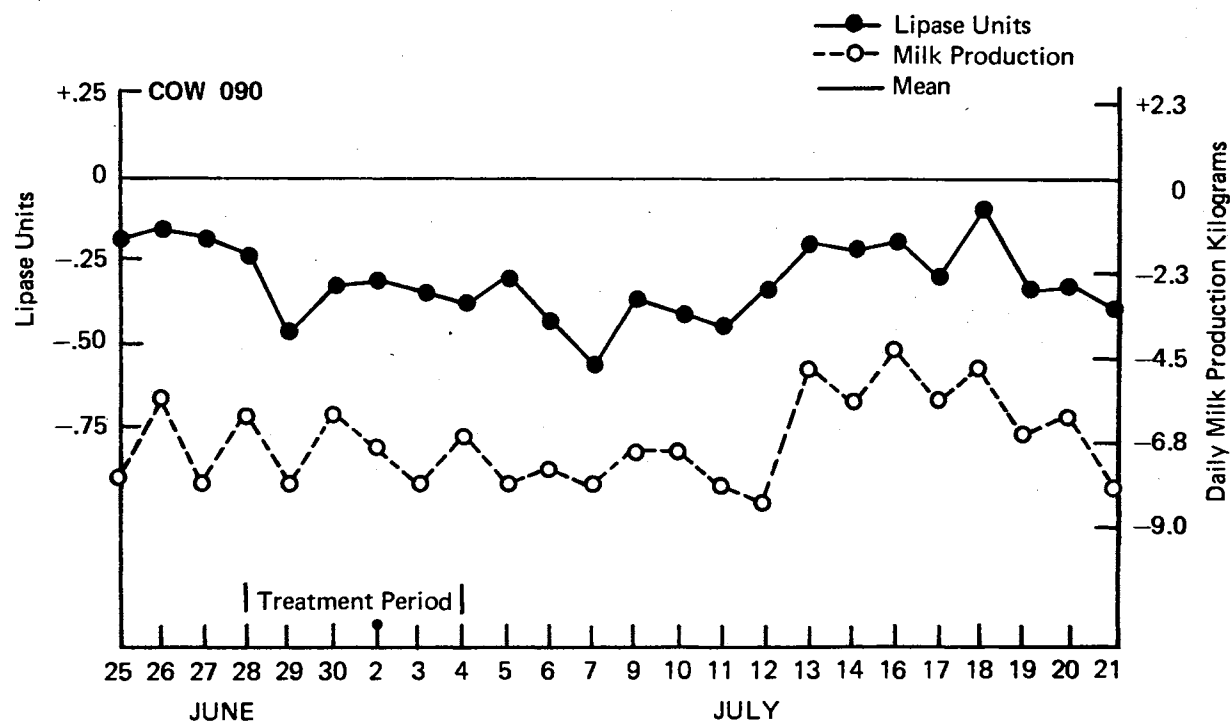


Figure 8. Difference from the Mean, Adjusted to Zero, of Lipase Units (the Amount of Enzyme Required to Liberate One μ mole of Butyric Acid per Minute per ml of Reaction Time @ 37°C) and Milk Production for Cow No. 090 When Plotted Over Days

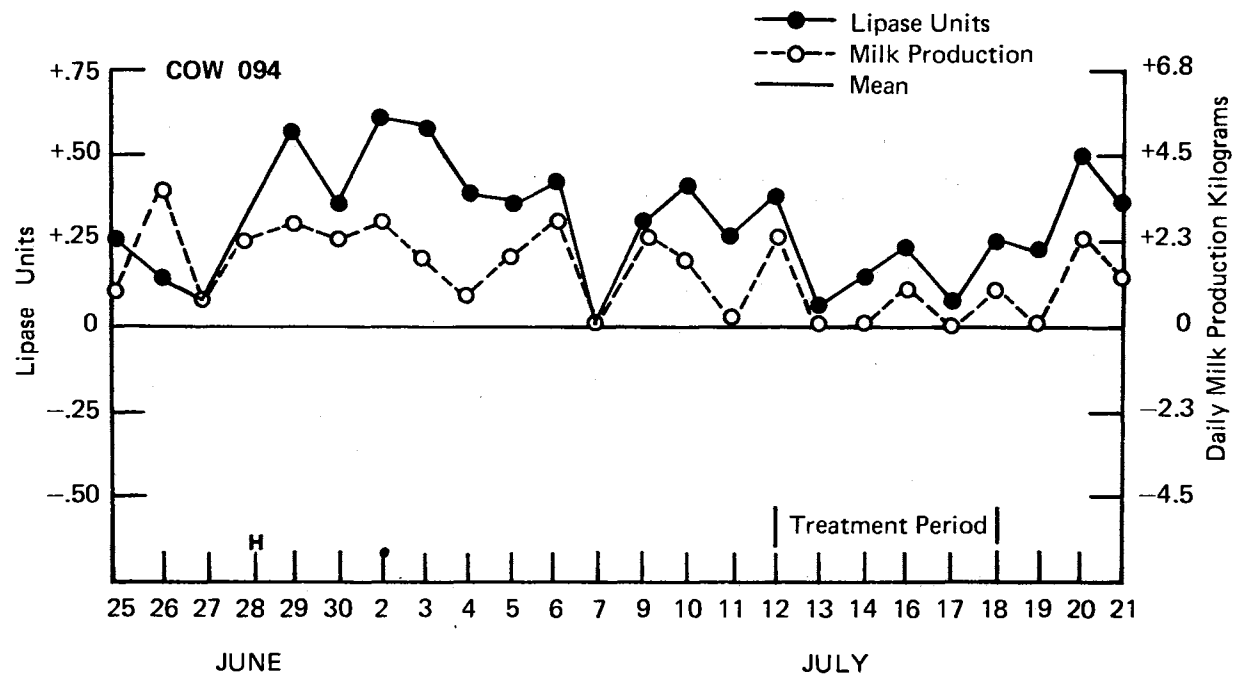


Figure 9. Difference from the Mean, Adjusted to Zero, of Lipase Units (the Amount of Enzyme Required to Liberate One μ mole of Butyric Acid per Minute per ml of Reaction Time @ 37°C) and Milk Production for Cow No. 094 When Plotted Over Days

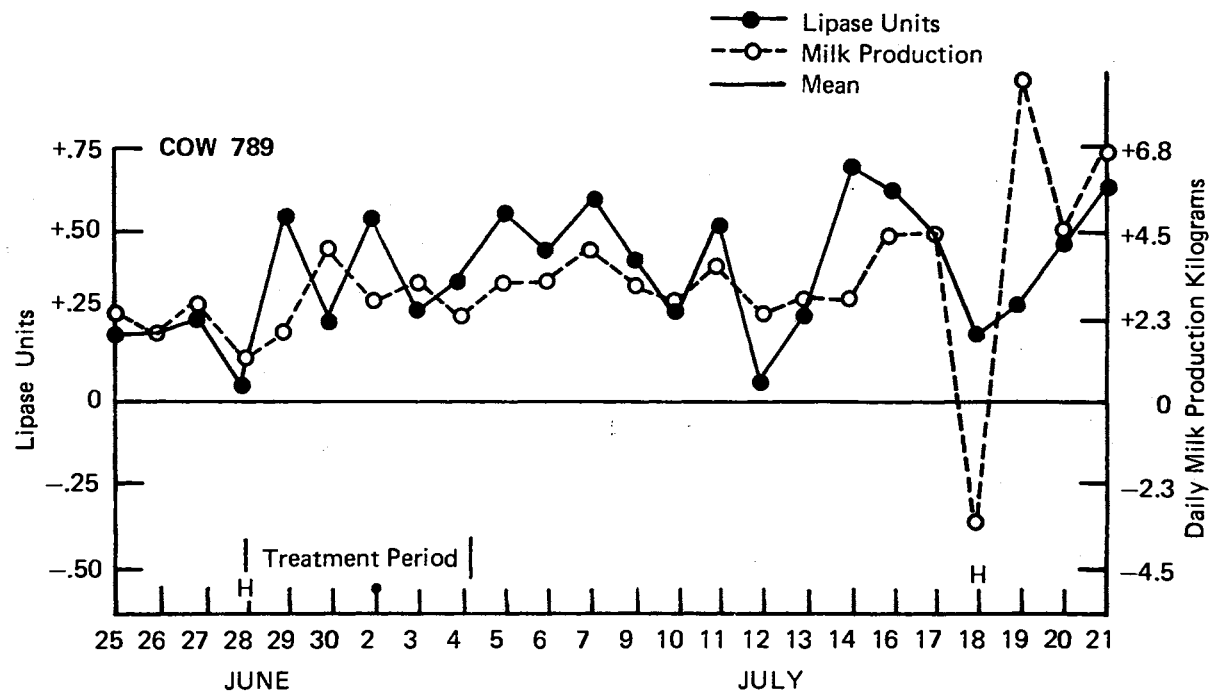


Figure 12. Difference from the Mean, Adjusted to Zero, of Lipase Units (the Amount of Enzyme Required to Liberate One μ mole of Butyric Acid per Minute per ml of Reaction Time @ 37°C) and Milk Production for Cow No. 789 When Plotted Over Days

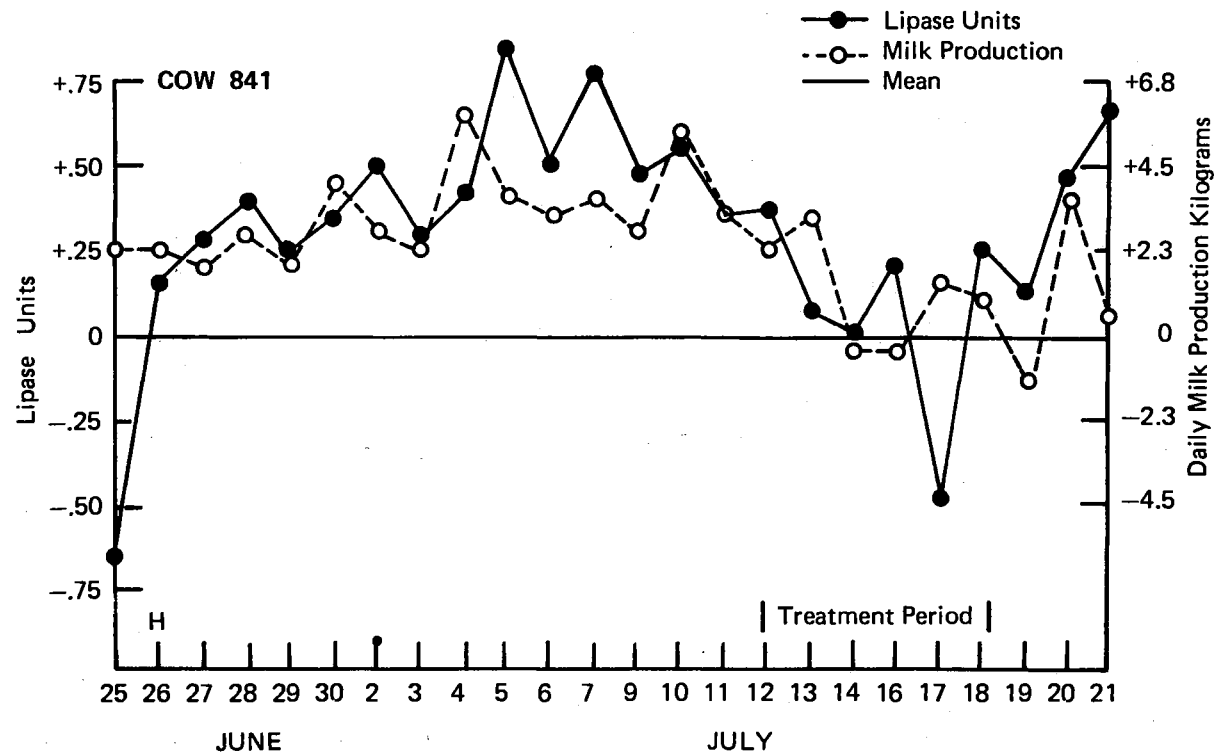


Figure 13. Difference from the Mean, Adjusted to Zero, of Lipase Units (the Amount of Enzyme Required to Liberate One μ mole of Butyric Acid per Minute per ml of Reaction Time @ 37°C) and Milk Production for Cow No. 841 When Plotted Over Days

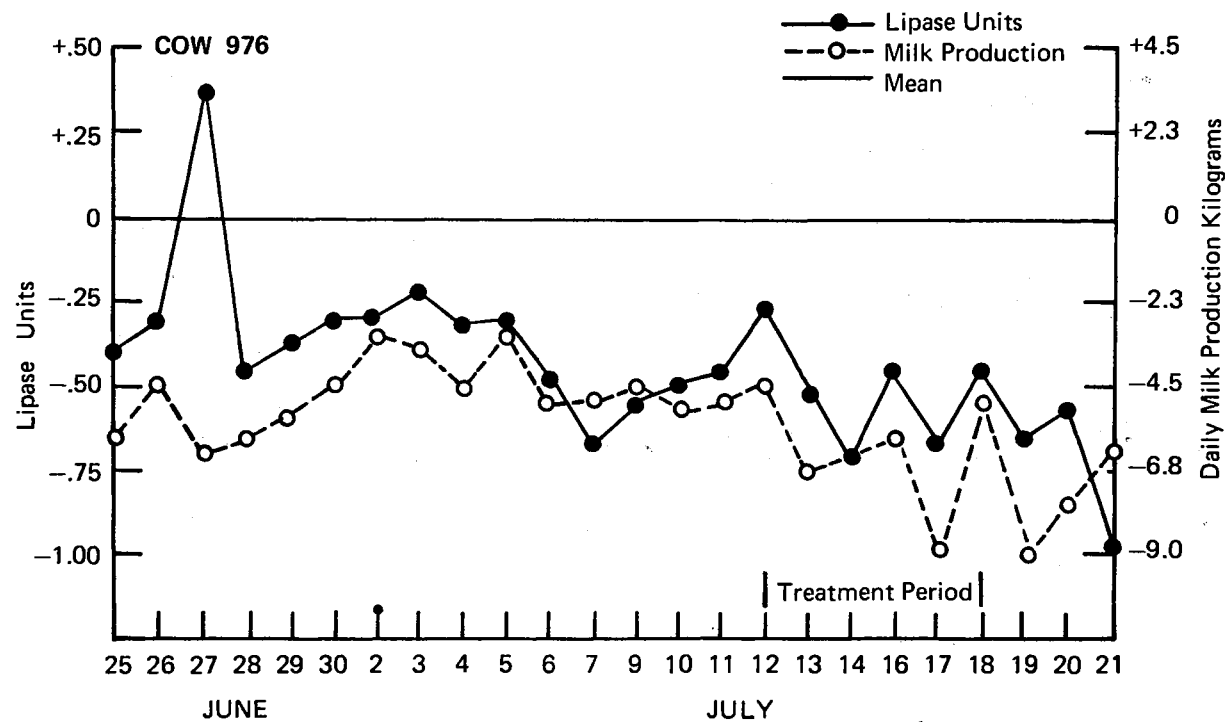


Figure 14. Difference from the Mean, Adjusted to Zero, of Lipase Units (the Amount of Enzyme Required to Liberate One μ mole of Butyric Acid per Minute per ml of Reaction Time @ 37°C) and Milk Production for Cow No. 976 When Plotted Over Days

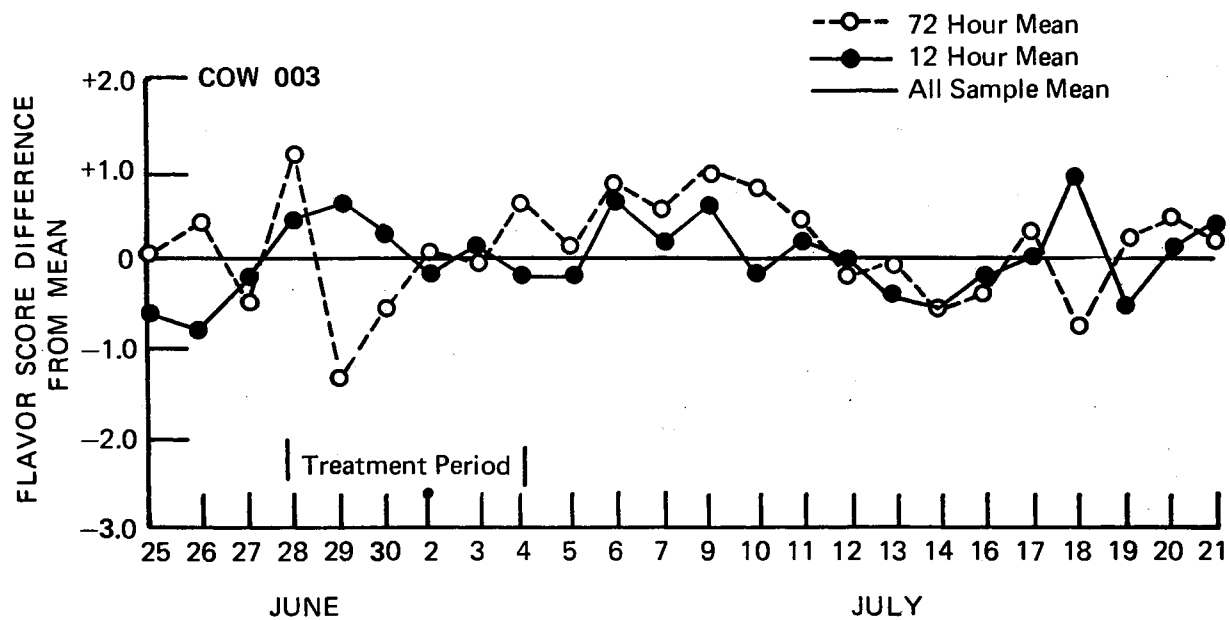


Figure 15. Difference from the Mean, Adjusted to Zero, of Daily Mean Flavor Scores for Milk From Cow No. 003, Tasted After 12 and 72 Hours and Plotted Over Days

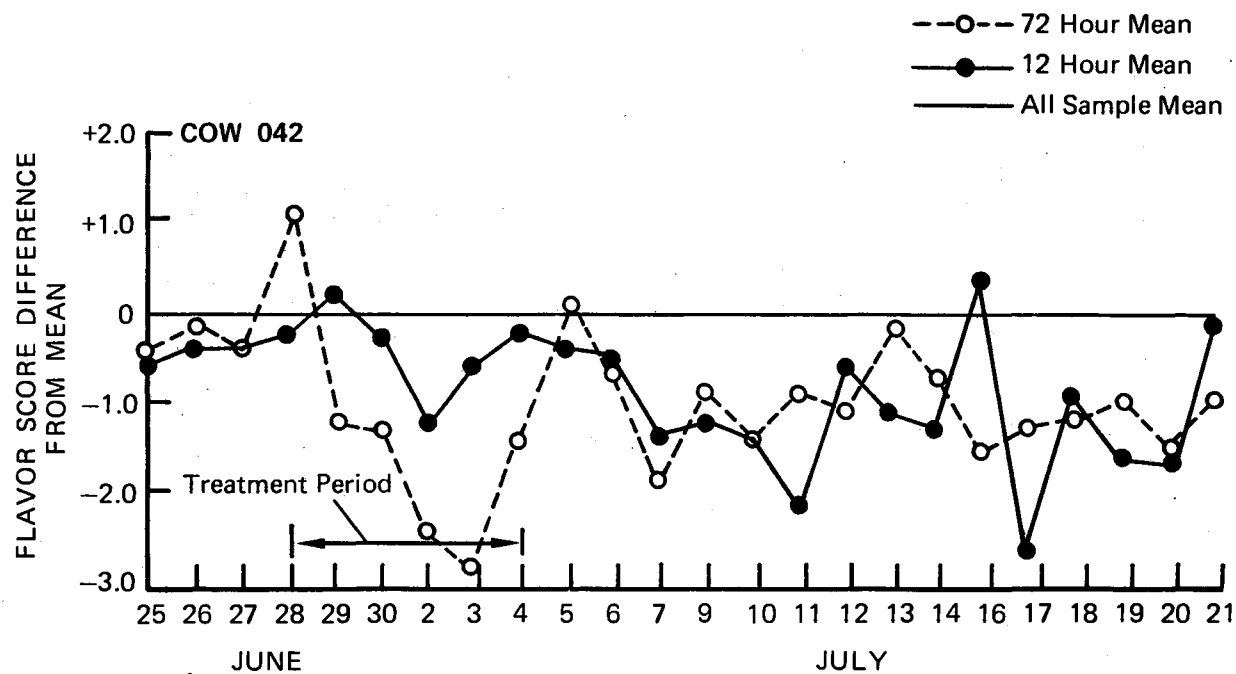


Figure 16. Difference from the Mean, Adjusted to Zero, of Daily Mean Flavor Scores for Milk from Cow No. 042, Tasted After 12 and 72 Hours and Plotted Over Days

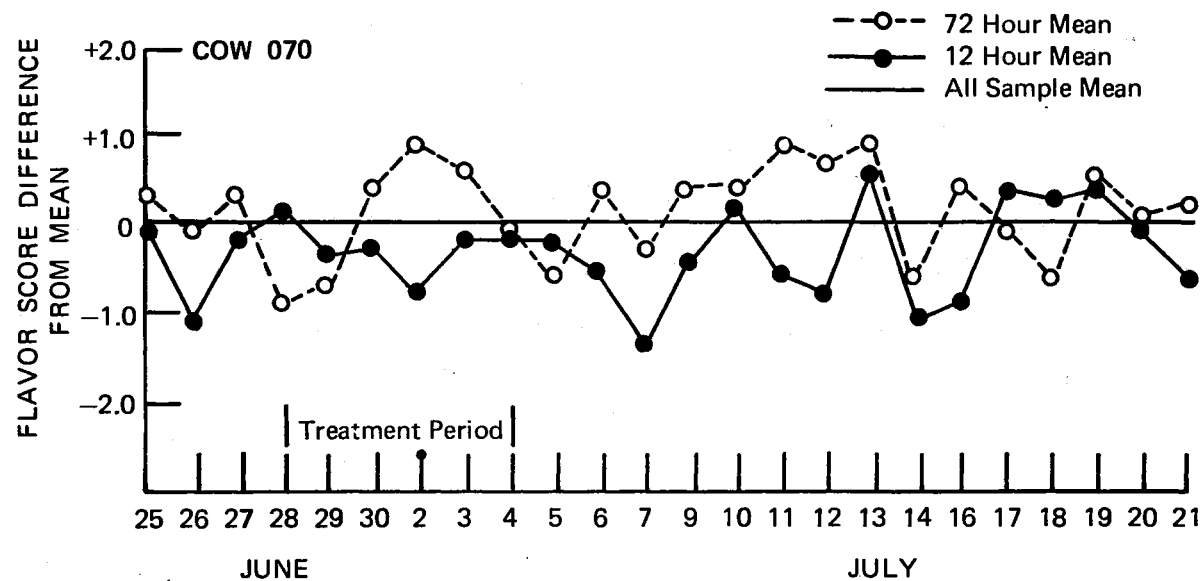


Figure 17. Difference from the Mean, Adjusted to Zero, of Daily Mean Flavor Scores for Milk from Cow No. 070, Tasted After 12 and 72 Hours and Plotted Over Days

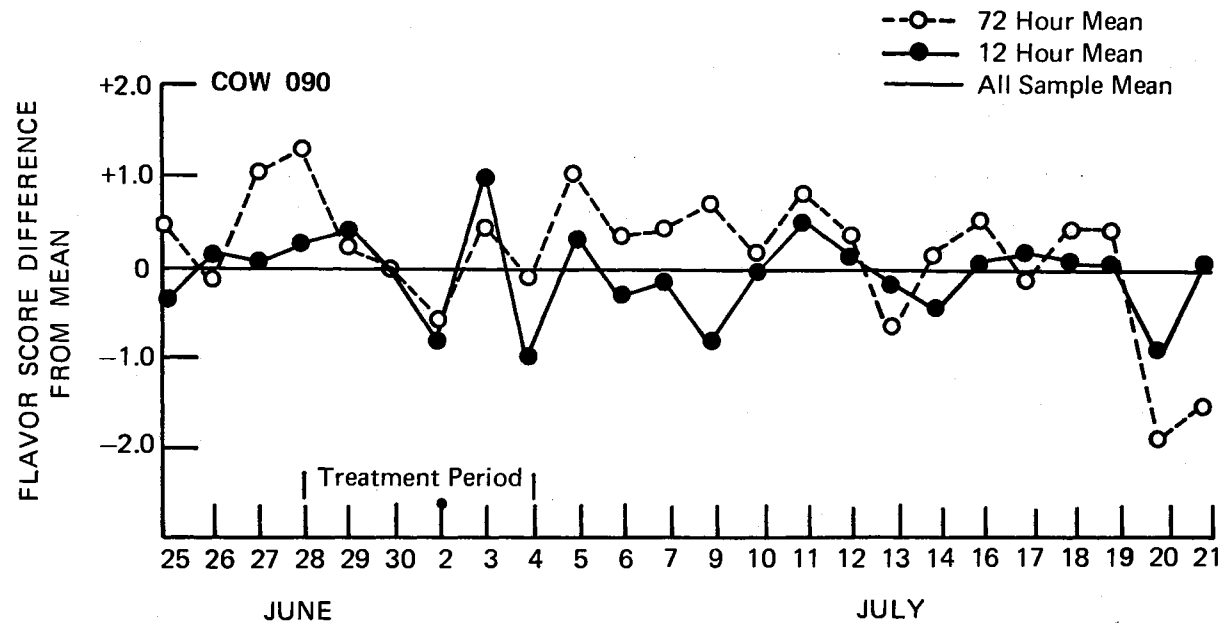


Figure 18. Difference from the Mean, Adjusted to Zero, of Daily Mean Flavor Scores for Milk from Cow No. 090, Tasted After 12 and 72 Hours and Plotted Over Days

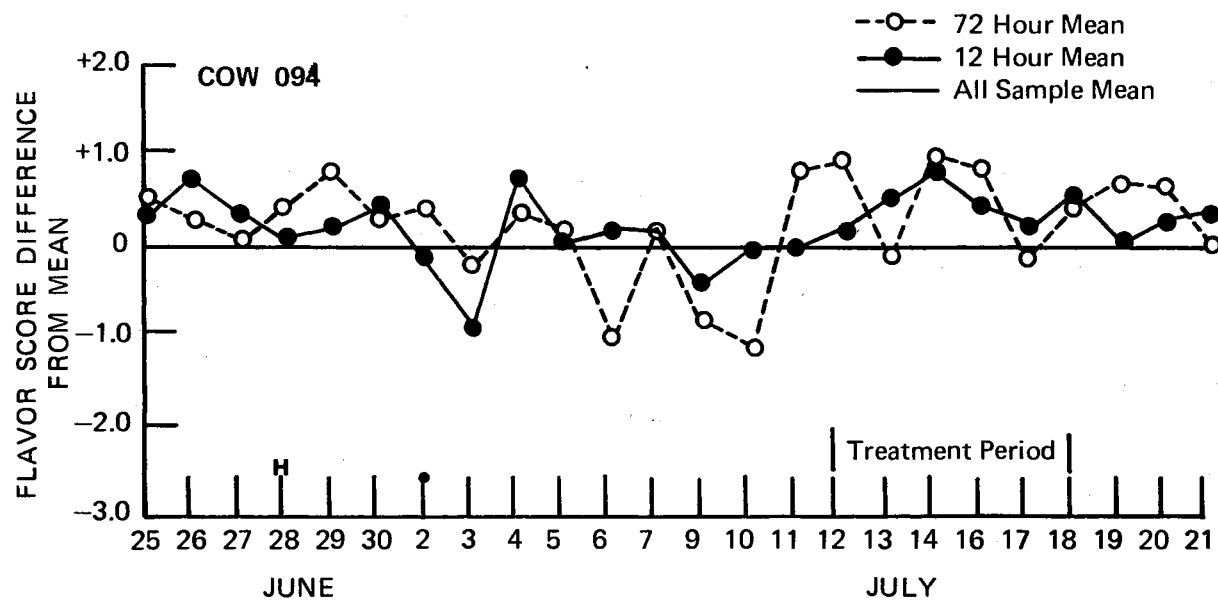


Figure 19. Difference from the Mean, Adjusted to Zero, of Daily Mean Flavor Scores for Milk from Cow No. 094, Tasted After 12 and 72 Hours and Plotted Over Days

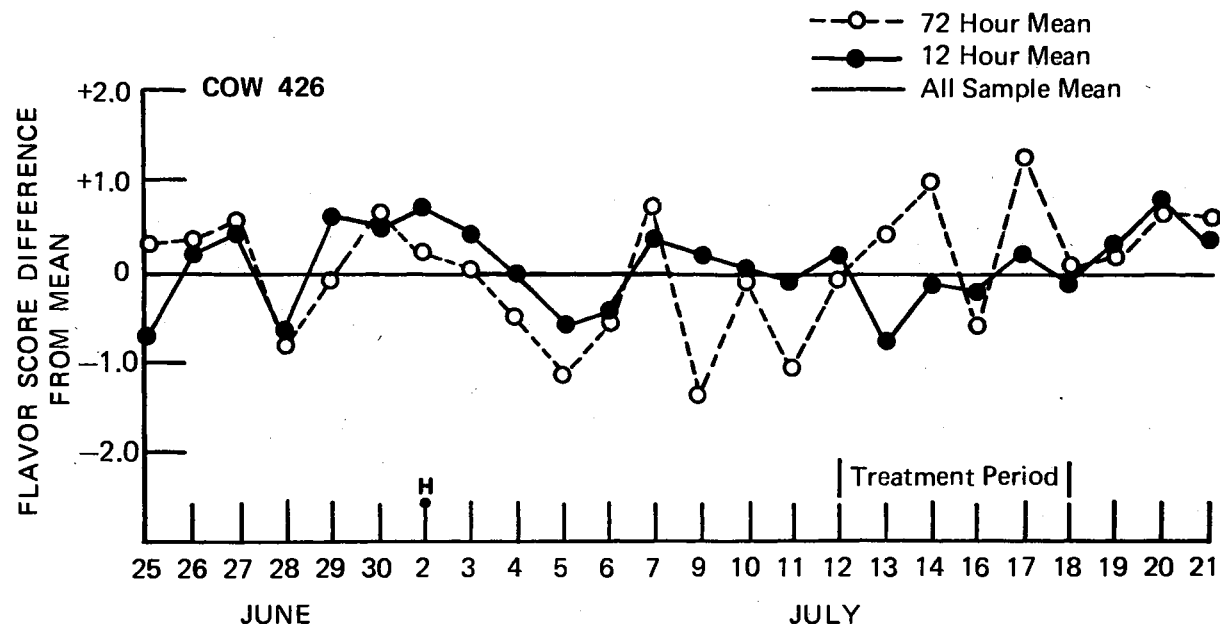


Figure 20. Difference from the Mean, Adjusted to Zero, of Daily Mean Flavor Scores for Milk from Cow No. 426, Tasted After 12 and 72 Hours and Plotted Over Days

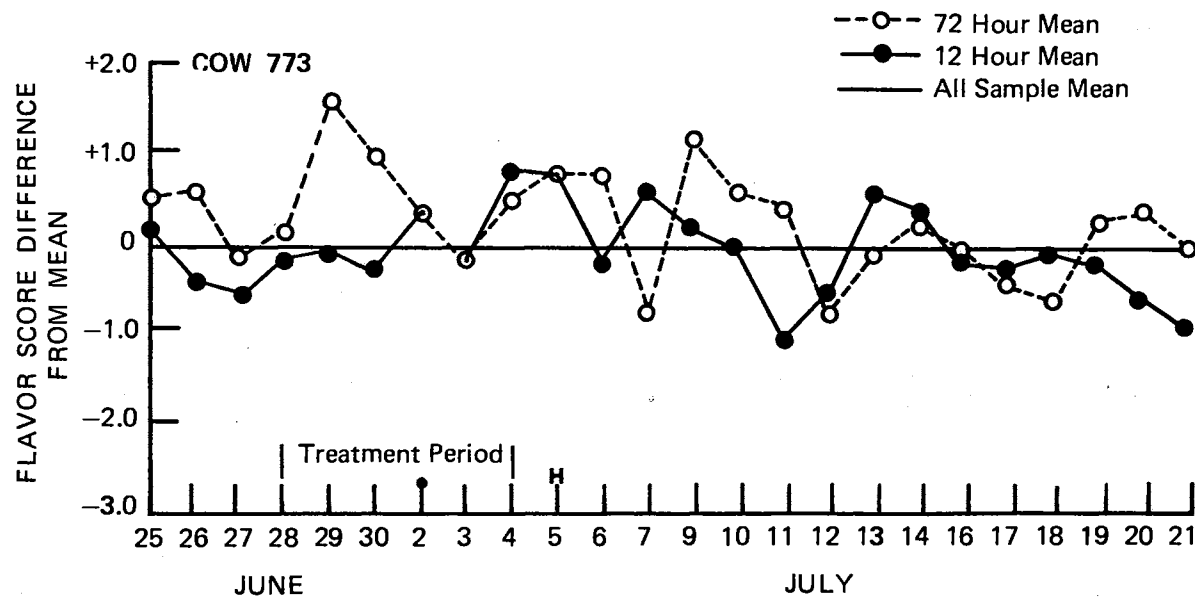


Figure 21. Difference from the Mean, Adjusted to Zero, of Daily Mean Flavor Scores for Milk from Cow No. 773, Tasted After 12 and 72 Hours and Plotted Over Days

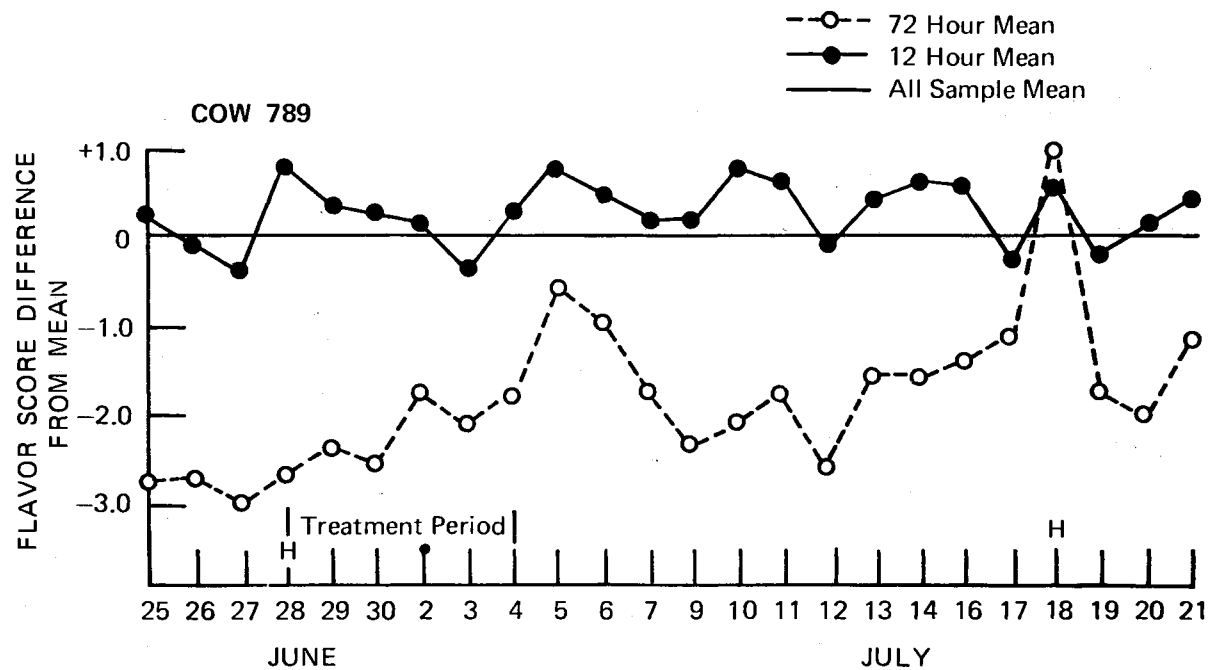


Figure 22. Difference from the Mean, Adjusted to Zero, of Daily Mean Flavor Scores for Milk from Cow No. 789, Tasted After 12 and 72 Hours and Plotted Over Days

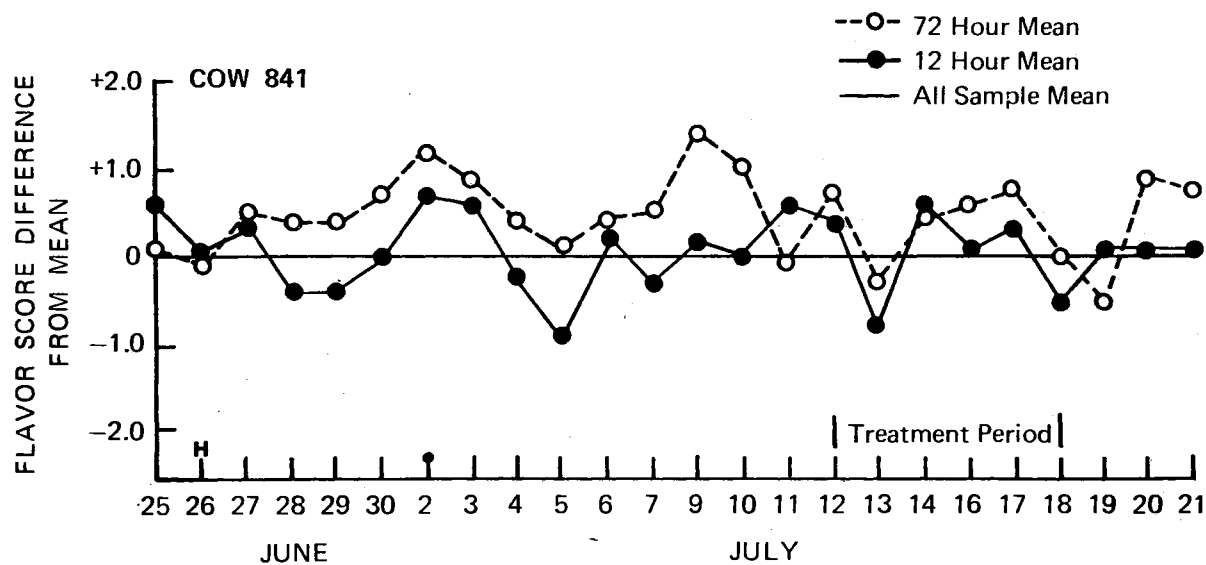


Figure 23. Difference from the Mean, Adjusted to Zero, of Daily Mean Flavor Scores for Milk from Cow No. 841, Tasted After 12 and 72 Hours and Plotted Over Days

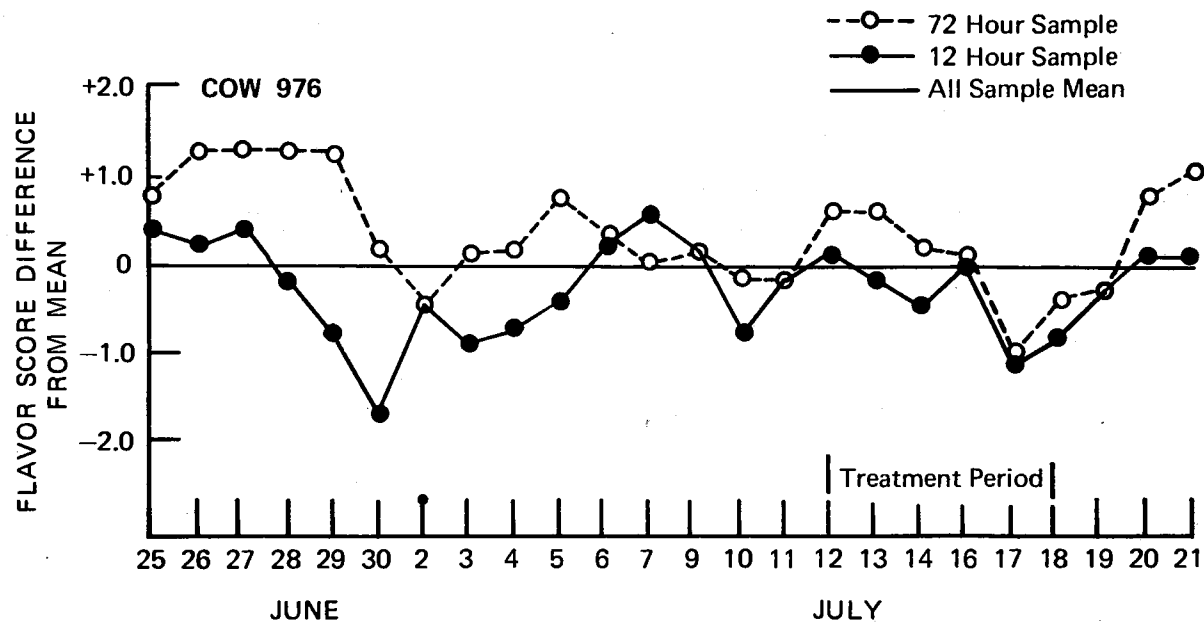


Figure 24. Difference from the Mean, Adjusted to Zero, of Daily Mean Flavor Scores for Milk from Cow No. 976, Tasted After 12 and 72 Hours and Plotted Over Days.

VITA ✓

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