May 26, 1960

To the Graduate Council:

I am submitting herewith a thesis written by Douglas Howard Stamper entitled "The Free Fatty Acid Content of Herd Milk." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

W W Overcast
Major Professor

We have read this thesis
and recommend its
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THE FREE FATTY ACID CONTENT OF HERD MILK

A Thesis

Presented to

the Graduate Council of the

University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Douglas Howard Stamper

June 1960

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CHAPTER I

INTRODUCTION

During recent years the problem of rancidity in milk has been increasing rapidly. Rancidity, as it is applied to the dairy industry, has been confined to flavor defects resulting from hydrolysis of butterfat. The fat reacts with water to form free fatty acids, diglycerides, monoglycerides and possibly free glycerol. Short chain fatty acids give the characteristic odor to rancid milk. It is believed that the diglycerides and monoglycerides are responsible for the bitter taste that is often associated with rancid milk. Many judges identify rancid milk by the soapy, puckering sensation it leaves in the mouth. The frequent occurrence of these flavor defects has stimulated research on rancidity.

It has generally been accepted that all cows produce milk containing enzymes that are capable of splitting the fat molecule. Usually these enzymes are inactive; however, most workers have agreed that rancidity is brought about by the enzymatic activity of lipase or lipases. In view of this fact, the dairy industry has been faced with finding and isolating the conditions that activate the enzyme or enzymes.

The installation of pipeline milkers, farm bulk cooling tanks, and the practice of alternate day pick-up of the raw milk supply all have been associated with increased rancidity. There has been considerable disagreement among workers as to the effect of instantaneous cooling and temperature fluctuation of the raw milk, stage of lactation and gestation, feed and season of the year, atmospheric temperatures and other factors on the rate of lipolysis or lipase activity. Aware of the many factors influencing rancidity, this experiment was designed to obtain initial data on the free fatty acid content of raw milk from the University herd. The study was continued throughout the year to study the effect some of these factors have on rancidity.

CHAPTER II

REVIEW OF LITERATURE

The presence of lipase in raw milk is now an accepted fact in the dairy industry. In 1913 Eckles and Shaw (9) found an abnormal odor and flavor developing in milk stored as low as 10° C. In 1922 studies by Palmer (39) showed that the secretion of an active lipase in the milk caused a bitter taste and rancid odor to be present in milk secreted by cows in advanced lactation. Rice and Markley (41) also proved the presence of lipase in normal milk by incubating raw milk in a cream substate, saturated with sucrose as a preservative.

The amount of lipase present or the extent to which lipolysis has occurred in raw milk has been measured or estimated in various ways. As pointed out by Herrington (16), when milk fat is hydrolyzed, the fat reacts with water forming free fatty acids, diglycerides, monoglycerides and possibly free glycerol.

Among the methods used for estimating lipolysis, certain investigators (9, 10, 39, 43) have used the organoleptic examination, which detects free, short-chain fatty acids, mainly, butyric acid. Other workers

(3, 4, 49) have used surface tension measurements as an estimate of lipolysis. Tarassuk and Smith (49) reported that liberated fatty acids had a tendency to lower the surface tension.

Titration methods have probably been used most widely in estimating lipase activity. These methods usually involve two major steps: first, the separation of the fat portion of the milk; second, the measurement of the free fatty acids in the fat portion. Herrington and Krukovsky (21) obtained the fat by churning and then dissolved the fat in alcohol to be titrated for free fatty acid content. Thomas, et al. (50) used Bureau of Dairy Industry detergent (thirty grams of Triton X-100 and seventy grams of sodium tetraphosphate made up to one liter with distilled water) to recover the fat. Herrington (16) states that fat obtained in this manner yields less free fatty acids than fat prepared by churning.

Johnson and Gould (27) developed a method which yielded fat averaging thirty per cent higher in acidity than fat obtained by churning. They recovered the fat with ethanol, ethyl ether, and Skellysolve F. (petroleum ether). Johnson and Gould (28) later modified their solvent extraction procedure by acidifying the milk to

pH 2 with H_2SO_4 before extraction. They found an increase in the recovery of various fatty acids by adjusting the pH.

Several methods have been used to titrate the free fatty acids in butterfat. Breazeale and Bird (2) made a comparison of four different methods and found the alcoholic KOH methods to give the nearest values between duplicate samples and the titration end-points were the easiest observed.

In addition to the direct titration of the free fatty acids in the isolated butterfat, certain other methods have been employed to give a numerical estimate of lipolysis. Mattick and Kay (37) and Dunkley and Smith (8) used tributyrinase determinations as a measure of lipase activity. Harper, et al. (15) used the silica gel extraction procedure, which is a measure of the total fatty acids prior to the extraction of the fat.

There has been some uncertainty in the literature (1) as to whether milk lipase contains one or two enzymes. Herrington and Krukovsky (21) postulated that milk contains at least two enzymes on the basis of formaldehyde sensitivity. This work was later substantiated by Peterson, et al. (40) and Roahen and Sommer (42). Albrecht and Jaynes (1) observed four lipase enzymes based on formaldehyde sensitivity and substrate

specificity.

Schwartz et al. (44) studied the characteristics of lipolytic enzyme systems of milk by using freeze-dried skim milk as a source of lipase and milk fat as a substrate. The presence of three different lipases were observed with pH optima at 7.0, 7.5 to 7.9, and 8.5.

Tarassuk and Frankel (46) concluded that there are at least two different lipase systems. To distinguish between the two systems, they proposed to designate the naturally active lipase (that absorbed on the fat globule membrane material on cooling of milk) as the "membrane lipase" of milk; and they proposed to designate the lipase of normal milk (that which remains in the plasma and is associated with the caseinate fraction) as the "plasma lipase" of milk.

Tarassuk and Frankel (47) have reported that prolonged agitation of warm milk and temperature activation are among the chief contributors to rancidity. These two factors have initated research dealing with various milk handling procedures.

Krukovsky and Sharp (35) have attributed the effect of agitation on induced rancidity to an alteration in the surface characteristics of the fat globules that create a condition more favorable for lipolysis. Dunkley and Kelley (7) states that fat in milk is

normally surrounded by a thin film of absorbed protein that appears to protect the fat from the lipase. If this protective film is disrupted or modified, the lipase is able to act on the fat, freeing fatty acids. Studies (24, 53) have revealed that the initial free fatty acid content and lipase activity of milk increases as the contact area between the fat and serum increases through smaller initial fat globules or sub-division of the globules present.

Because of a high incidence of lipolysis on dairy farms where pipeline milkers have been adopted, numerous studies have been made to better understand the mechanism of activation. Thomas, et al. (51) conducted a survey to compare the different types of equipment used for milk handling. Of all the combinations studied, they found excessive lipolysis to occur most frequently in pipeline milkers with bulk tanks.

Kelley and Dunkley (31) conducted an experiment to determine the activating effect of the individual parts of a pipeline milker. They found the more troublesome conditions causing induced rancidity to be the admission of air into the milk line, low milk flow-rate, elevation of warm milk under vacuum with air bubbling through it, inclusion of a filter and numerous fittings in the vacuum-section of the milk line and continuous

operation of a starved centrifugal pump. These results have been confirmed by other investigators (12, 26, 47, 51). Speer, et al. (45) reported that vertical risers in the pipe line are the primary cause of induced rancidity. Jokay and Jensen (30) found the number one cause of high fatty acid degree values to be excessive air intake, especially from leaks in the pipelines. They also found the free fatty acids to increase as the distance of flow and riser heights increased. Various workers have reported that the formation of foam is necessary or an important feature in the activation of lipolysis by agitation with air. Tarassuk and Frankel (47) reported that the condition necessary to produce rancidity appeared to be foaming with continuous mixing of the foam and milk at temperatures that keep the milk fat in the liquid state. They concluded that these conditions seemed to be ideally provided in pipeline milkers with risers. Herrington (17) states that a gentle treatment which produces foaming is more hazardous than violent agitations without foam production. He added that milk treatment which produces foaming will greatly increase the number of very small fat globules.

Herrington and Krukovsky (21) found that lipolysis in freshly drawn milk was decreased by rapid cooling of the milk. The rate of lipase activity in the

milk after storage is dependent on the rate at which the milk was cooled before storage. These same workers (22) found a critical temperature range in which lipase was accelerated by slow cooling. The upper limit of this range was found to be 20-25° C. and the lower limit was approximately zero in case of natural milk and 10° C. in case of temperature activated. Krukovsky and Sharp (36) and Krukovsky and Herrington (34) noted that lipolytic activity was increased when raw milk was cooled, warmed to 30° C. and then recooled. This optimum temperature for lipolysis in milk activated in this manner was found to be 5° to 10° C. Krukovsky and Sharp (35) have concluded that apparently all milk is capable of true lipolysis if subjected to suitable activating treatments.

Certain references in literature have led to the conclusion that rancidity will not develop at a temperature as low as 40° F. Davies (5) for instance, stated that lipase activity is suppressed at 0° to 3° C. (32-37° F.). Weaver (54) found that milk held at 40° F. for fifteen hours was far more rancid than a duplicate sample held for fifteen hours at 70° F. Tarassuk and Frankel (46) reported that milk containing naturally active lipase in appreciably amounts will exhibit spontaneous lipolysis with no involved treatment necessary for the development of rancidity. They concluded

the only condition necessary to initiate the hydrolysis of the fat is to cool the milk. Johnson and Von Gunten (29) reported higher acid degree values associated with delayed cooling than with rapid cooling of the milk. Krukovsky and Sharp (36) found the rate of lipase activity of resurfaced fat globules (re-emulsification produced by homogenization) increased as the temperature increased; whereas, the rate of lipolysis of original normal surfaced fat globules increased as the temperature decreased.

Studies (11, 33) have shown that individual cows maintained under the same conditions varied markedly in the susceptibility of their milk to rancidity and the trends they follow throughout their lactation periods. Milk from individual cows may vary considerably in its susceptibility to rancidity from day to day (11, 52) and even samples taken from individual quarters of the cows udder may show differences (6). These inconsistencies have made it difficult to establish definitely the influence of other variables on rancidity and have caused many conflicting reports in the literature.

There is considerable disagreement in the literature as to whether advanced lactation is associated with increased lipolytic activity. Mattick and Kay (37) noted that the concentration of lipase varied from cow

to cow. They found the highest concentration of the enzyme present in the colostrum, then the concentration falls to a minimum in about ten days, and then has a tendency to level off at a figure between the minimum and that of colostrum as the stage of lactation progresses. Their studies showed an unexpected slight decrease after three hundred days of lactation or just before drying off.

Thomas, et al. (52, 53) reported that the free fatty acid content may be related to the fat globule size, fat content, or fat aqueous ratio, but it is not necessarily related to the period of lactation. Herrington and Krukovsky (20) found no apparent relationship between the rate of lipolysis and the stage of lactation. Herrington and Guthrie (19) observed very low median values during the first week of lactation. The median values then passed through a maximum during the fourth and fifth months. They noted that after the first week of lactation, the individuality of the cow seemed more important than the stage of lactation. After the first week of lactation some samples showed lipase activity far above the median value.

Several earlier investigators (9, 10, 11, 39, 43) associated rancid milk with advanced lactation.

Colmey, et al. (3) found, using the free fatty acid analysis, a definite trend for the development of rancidity in milk from cows in later stages of lactation. Jensen (25) also found that acid degree values progressed as the stage of lactation progressed in three out of four cows. Studies by Fredeen, et al. (11) indicated that it was advanced lactation, not advanced gestation, which contributes to an increase in rancidity during late lactation; however, results reported by Weaver (54) indicate that advanced gestation may be more important than advanced lactation.

Little consideration has been given to seasonal variables. Seasonal variations in lipolytic activity has in part been influenced by the stage of lactation (14, 54). Most workers (11, 13, 23, 43) have associated the season of the year with feeding practices. They have reported minimum lipolytic activity in early summer when cows are on pasture and a maximum values during the early winter when cows are on dry feed. However, Olson, et al. (38) found no significant change in acid degree values when the herd was shifted from dry feed to fifty per cent pasture and fifty per cent dry feed, and then to full pasture feeding. Other feeding experiments (48) and practical observations (10) have demonstrated that green pasture decreases and

dry feed increases the incidence of rancidity. Kelley (32) found lipase activity to be associated with the estrus cycle. He observed the greatest amount of lipolysis a few days before estrus, with a decrease during the heat period, and then a slow increase until the next estrus cycle.

CHAPTER III

METHODS

In this study samples of fresh, raw milk were taken three times per week for one year from the University herd. The cows were milked using DeLaval pipeline milkers. The milk was removed from the vacuum system by the DeLaval combine (diaphragm) vacuum pump which was operated continuously. The milk was pumped into a five hundred gallon, model R, Creamery Package, refrigerated farm tank. The farm tank was equipped with one rotating mechanical agitator. The milking operation started about two o'clock in the afternoon and ended about four P. M. During this two hour period the temperature of the milk was reduced and maintained between 40-45° F. It took from ten to fifteen minutes after the milking operation stopped to lower the milk in the bulk tank to below 40° F. The temperature was lowered to 33-36° F. by two o'clock the following morning when the milking operation began again. The temperature in the tank was never raised above 40° F. by the continuous addition of the morning milking. A Creamery Package centrifugal pump was used to pump the milk from the farm tank into a five hundred

gallon Walker trailer tank to be taken to the University Creamery.

When the milk reached the Creamery, it was agitated by air from an Ingersoll-Rand, air compressor. The air compressor was equipped with a carbon piston ring which runs against a dry cylinder wall, producing air free from oil. The milk was agitated using a stainless steel pipe extending almost to the bottom of the tank. The pipe was connected to a plastic hose coming from the air compressor. The air was bubbled through the milk for five minutes before the sample of milk was taken. The temperature at this time would range between 40-45° F. A pint sample was taken with a sanitized stainless steel dipper and stored at 40° F. in a quart milk bottle until the milk was taken to the laboratory to be analyzed for the free fatty acid content. This storage period ranged from eight to eleven hours.

The extraction-titration method of Frankel and Tarassuk (24), based on the procedure of Johnson and Gould (27), was used for the estimation of lipase activity in the raw whole milk. The butterfat content of the milk was first determined by the Babcock method. The sample of milk was then warmed to 20° C. by placing in a warm (20-25° C.) water bath. The milk was thoroughly mixed by pouring from one container to another four times.

Ten milliliters of the milk was then pipetted into a fifty milliliter centrifuge tube and ten milliliters of ninety-five per cent ethyl alcohol added. The tube was shaken for one minute and fifteen milliliters of a mixture of forty per cent ethyl ether and sixty per cent petroleum ether was added. The tube was then shaken for another minute and centrifuged at approximately 1,500 r. p. m. for three minutes. While the sample was centrifuging, five drops of one per cent alcoholic phenolphthalein was added to a fifty milliliter Erlenmeyer flask containing fifteen milliliters of ninety-five per cent ethyl alcohol. After centrifugation five milliliters of the supernatant clear ether layer was pipetted into the alcoholic solution containing the phenolphthalein indicator. The original pink color faded and this mixture was titrated back to the original pink color with 0.0250 N alcoholic KOH, using a two milliliter burette graduated to 0.01 milliliter. All samples were run in duplicate. The average titration reading was multiplied by three to obtain the volume of alkali required to neutralize the total supernatant ether layer. By using the butterfat percentage obtained in the Babcock test and the amount of 0.0250 N alkali necessary to neutralize the total ether layer, the number of milliliters of one N alkali required to neutralize an

ether extract of milk containing one hundred grams of fat was calculated. This value is expressed as "free fatty acidity" (F. F. A.). The results of this study are reported as F. F. A. numbers.

CHAPTER IV

RESULTS AND DISCUSSIONS

The main object of this research was to study the effect of feed, stage of lactation and the season of the year on the free fatty acid of fresh, raw milk in the University herd. It was realized that this experiment could not be designed to isolate the effect of any single factor influencing lipase activity; however, free fatty acid values over a complete year would give valuable information that may be useful in planning other research.

The findings of many earlier workers have been confirmed. Table I shows considerable fluctuation in the free fatty acid values obtained while the cows were on dry feed and silage. Fredeen, et al. (11) and Thomas, et al. (52) found that cows vary from day to day in their susceptibility to rancidity. The findings in Table I show that the free fatty acid values increased during the cold fall and winter months. Numerous investigators (11, 13, 23, 43) have reported that increased rancidity is associated with winter dry feeding.

Table II also shows variation from day to day

TABLE I

FREE FATTY ACID VALUES OF HERD WHILE ON DRY FEED AND SILAGE

Date	Ml of KOH used sample no. 1	2	Percent butterfat	Free fatty acid values
4-2-59	.240	.242	3.9	4.63
4-4-59	.237	.243	3.9	4.62
4-7-59	.200	.201	3.9	3.85
4-9-59	.188	.190	3.9	3.63
4-11-59	.240	.230	3.9	4.52
4-14-59	.276	.284	3.95	5.32
9-12-59	.316	.322	3.8	6.29
9-15-59	.308	.312	3.8	6.11
9-17-59	.270	.278	3.7	5.55
9-19-59	.340	.346	3.9	6.59
9-22-59	.307	.315	3.8	6.13
9-24-59	.310	.320	3.8	6.21
9-26-59	.286	.270	3.8	5.48
9-29-59	.195	.210	3.7	4.10
10- 1-59	.274	.282	3.8	5.48
10- 3-59	.290	.270	3.9	5.42
10- 6-59	.321	.327	4.0	6.07
10-8-59	.311	.313	3.9	6.00

TABLE I (continued)

Date	Ml of KOH used sample no. 1	2	Percent butterfat	Free fatty acid values
10-10-59	.365	.371	4.0	6.90
10-13-59	.376	.370	4.0	4.99
10-15-59	.356	.360	4.2	4.39
10-17-59	.320	.326	4.1	5.90
10-20-59	.316	.312	4.1	5.74
10-22-59	.284	.280	3.9	5.42
10-24-59	.298	.300	4.0	5.62
10-27-59	.410	.418	4.5	6.90
10-29-59	.366	.360	4.1	6.64
10-31-59	.346	.340	4.2	6.12
11- 3-59	.321	.325	3.9	6.21
11- 5-59	.363	.357	4.0	6.75
11- 7-59	.292	.294	4.2	5.23
11-10-59	.282	.284	4.2	5.05
11-12-59	.305	.301	4.2	5.41
11-14-59	.355	.361	4.3	6.24
11-17-59	.327	.339	4.1	6.09
11-19-59	.349	.361	4.1	6.49
11-21-59	.321	.319	4.1	5.85

TABLE I (continued)

Date	Ml of KOH used sample no. 1	2	Percent butterfat	Free fatty acid values
11-24-59	.264	.270	4.0	5.00
11-28-59	.368	.372	4.2	6.60
12- 1-59	.387	.383	4.2	6.87
12- 3-59	.331	.337	4.0	6.26
12- 5-59	.431	.427	4.2	7.66
12- 8-59	.427	.435	4.3	7.51
12-10-59	.424	.410	4.2	7.44
12-12-59	.390	.374	4.3	6.66
12-15-59	.402	.388	4.4	6.73
12-17-59	.389	.387	4.4	6.61
12-19-59	.351	.363	4.3	6.22
12-22-59	.360	.352	4.2	6.35
12-24-59	.320	.326	4.2	5.76
12-26-59	.340	.336	4.2	6.03
12-29-59	.327	.329	4.2	5.84
12-31-59	.261	.275	4.1	4.90
1- 2-60	.367	.382	4.4	6.37
1- 5-60	.357	.375	4.2	6.53
1- 7-60	.436	.450	4.1	8.10

TABLE I (continued)

Date	Ml of KOH used sample no. 1	2	Percent butterfat	Free fatty acid values
1- 9-60	.475	.477	4.2	8.50
1-12-60	.403	.405	4.1	7.39
1-14-60	.410	.424	4.0	7.81
1-16-60	.418	.398	4.1	7.46
1-19-60	.440	.434	4.2	7.80
1-21-60	.480	.476	4.2	8.53
1-23-60	.498	.510	4.3	9.13
1-26-60	.456	.460	4.3	7.98
1-28-60	.394	.396	4.2	7.05
1-30-60	.476	.480	4.2	8.53
2- 2-60	.498	.492	4.2	8.83
2- 4-60	.551	.533	4.2	9.67
2- 6-60	.390	.402	4.1	7.24
2- 9-60	.420	.432	4.3	7.43
2-11-60	.440	.444	4.1	8.08
2-13-60	.488	.496	4.2	8.78
2-16-60	.535	.545	4.3	9.41
2-18-60	.566	.558	4.5	9.36
2-20-60	.448	.450	4.3	7.83

TABLE I (continued)

Date	Ml of KOH used sample no. 1	2	Percent butterfat	Free fatty acid values
2-23-60	.499	.487	4.2	8.80
2-25-60	.525	.511	4.2	9.25
2-27-60	.490	.482	4.1	8.89
3- 1-60	.428	.434	4.0	8.08
3- 8-60	.550	.546	4.2	9.78
3-10-60	.486	.492	4.2	9.78
3-12-60	.415	.417	4.2	8.73
3-15-60	.378	.390	4.1	7.02
3-17-60	.386	.398	4.1	7.17
3-19-60	.412	.422	4.1	7.62
3-22-60	.540	.546	4.2	9.69
3-24-60	.504	.522	4.2	9.16
3-26-60	.498	.480	4.2	8.72
3-29-60	.560	.572	4.3	9.37
3-31-60	.530	.536	4.2	9.51

TABLE II

FREE FATTY ACID VALUES OF HERD WHILE ON PASTURE AND DRY FEED

Date	Ml of KOH used sample no. 1	2	Percent butterfat	Free fatty acid values
4-16-59	.209	.213	3.9	4.06
4-18-59	.258	.265	3.8	5.03
4-21-59	.229	.219	3.8	4.42
4-23-59	.315	.307	3.8	6.13
4-25-59	.179	.185	3.8	3.59
4-28-59	.202	.196	3.8	3.92
4-30-59	.193	.197	3.7	3.95
5- 2-59	.197	.173	3.8	3.44
5- 5-59	.310	.293	3.8	5.94
5- 7-59	.207	.205	3.7	4.17
5- 9-59	.198	.196	3.8	3.88
5-12-59	.237	.229	3.7	4.72
5-14-59	.220	.220	3.7	4.46
5-16-59	.261	.265	3.8	5.19
5-19-59	.295	.285	3.7	5.87
5-21-59	.230	.248	3.7	4.84
5-23-59	.292	.275	3.7	5.74

TABLE II (continued)

Date	Ml of KOH used sample no. 1	2	Percent butterfat	Free fatty acid values
5-26-59	.242	.236	3.7	4.84
5-28-59	.209	.213	3.8	4.16
5-30-59	.203	.213	3.7	4.21
6- 2-59	.218	.222	3.7	4.46
6- 4-59	.239	.227	3.7	4.72
6- 6-59	.244	.242	3.7	4.92
6- 9-59	.243	.226	3.7	4.74
6-11-59	.234	.240	3.8	4.67
6-13-59	.250	.262	3.7	5.18
6-16-59	.298	.282	3.7	5.87
6-18-59	.310	.316	3.75	6.26
6-20-59	.352	.368	3.7	7.29
6-23-59	.308	.312	3.7	6.28
6-25-59	.271	.265	3.7	5.42
6-27-59	.262	.264	3.7	5.33
6-30-59	.274	.268	3.7	5.44
7- 2-59	.249	.255	3.7	5.10
7- 4-59	.245	.261	3.7	5.12
7- 7-59	.189	.201	3.5	4.17

TABLE II (continued)

Date	Ml of KOH used sample no. 1	2	Percent butterfat	Free fatty acid values
7- 9-59	.279	.285	3.4	6.22
7-11-59	.236	.244	3.5	5.14
7-14-59	.245	.261	3.5	5.42
7-16-59	.278	.260	3.6	5.62
7-18-59	.255	.269	3.7	5.31
7-21-59	.332	.340	3.75	6.72
7-23-59	.274	.288	3.8	5.54
7-25-59	.306	.310	3.9	5.92
7-28-59	.310	.324	4.0	5.94
7-30-59	.272	.278	3.95	5.22
8- 1-59	.250	.248	3.95	4.72
8- 4-59	.272	.288	3.8	5.52
8- 6-59	.222	.230	3.8	4.46
8- 8-59	.220	.236	3.7	4.62
8-11-59	.328	.342	4.0	6.28
8-13-59	.315	.300	3.8	6.06
8-15-59	.199	.217	3.7	4.21
8-18-59	.245	.263	3.8	5.01
8-20-59	.308	.318	3.9	6.01

TABLE II (continued)

Date	Ml of KOH used sample no. 1	2	Percent butterfat	Free fatty acid values
8-22-59	.290	.276	3.85	5.51
8-25-59	.313	.305	3.8	6.09
8-27-59	.298	.290	3.8	5.80
8-29-59	.220	.234	4.1	4.15
9- 1-59	.262	.268	3.65	5.44
9- 3-59	.270	.274	3.85	5.29
9- 5-59	.300	.288	3.75	5.88
9- 8-59	.325	.321	3.75	6.46
9-10-59	.330	.336	3.9	6.40

in the free fatty acid values while the herd was on pasture. In general, however, the values during this period are lower than those obtained while the herd had no pasture. The overall average free fatty acid value was 5.2 while on pasture as compared to 7.0 when off pasture. Table II shows minimum values during April, May and June with increasing values in July, August and September or in the latter part of the pasturing season. Other investigators (10, 53) have reported minimum lipolytic activity during the early summer when cows were on pasture.

After reviewing the literature dealing with rancidity, many conditions or situations have been observed and recognized that may have contributed to the fluctuations in the free fatty acid values obtained from the University herd. Considerable daily variations may be due to the individuality of the cows. The milk could have received different treatment in the pipeline milking system, varying with the techniques employed in operating the system by different operators and the installation of the equipment. Jokay and Jensen (30) reported that the major cause of high fatty acid degree values was excessive air intake, especially from leaks in pipelines. The continuous operated diaphragm pump may have caused fluctuations, depending on the

extent to which it was starved during the milking operation.

Weather conditions, rate of cooling and variation in storage time and temperature could have affected the degree of lipolysis. Samples of milk were taken every Tuesday, Thursday and Saturday. Samples taken on Thursday and Saturday represented two milkings, the morning and the evening before, with a total storage period ranging from twelve to fifteen hours. The sample taken on Tuesday represented Monday morning, evening and Tuesday morning milkings with a storage period of twenty-four to twenty-seven hours. The storage temperature ranged from 33° F. to 45° F. Certain workers (46, 54) have reported that lipolysis may be accelerated by low storage temperatures.

Daily fluctuations in free fatty acid values may be associated with the daily variations in average lactation days. The individual cows varied from 3 to 584 days of lactation. Mattick and Kay (37) found that lipase is concentrated in colostrum milk. Numerous investigators (9, 10, 11, 25, 39, 43) have reported increased rancidity associated with advanced lactation. The estrus cycle and stage of gestation may may have shown some effect. Kelley (32) found lipase activity associated with the estrus cycle. Work by

Weaver (54) also indicated that gestation may influence the rate of lipolysis.

Figure 1, 2, 3 and 4 show that the free fatty acid values (F. F. A.) during April through June and July increased as the average days of lactation for the herd progressed. The average days of lactation were computed by totaling the number of days each cow had been milking and then dividing the total days by the number of cows milking at any given time. Figure 1 represents approximately the tenth of each month; Figure 2 the twentieth; Figure 3 the thirtieth; Figure 4 represents an average of the values for the tenth, twentieth, and the thirtieth of each month. All figures show that the acid degree values remained comparatively low during the pasture period (Table II) even though the herd was in advanced lactation.

Figures 1 and 3 show that the free fatty acid values continued to increase during August through October and November, while the average days of lactation dropped sharply. This may be associated with a shortage of pasture and an increase in the utilization of dry feed. Figure 1 shows a decrease in free fatty acid values during November along with a sudden decrease in average days of lactation. Figure 4 shows a slight increase in free fatty acid values during

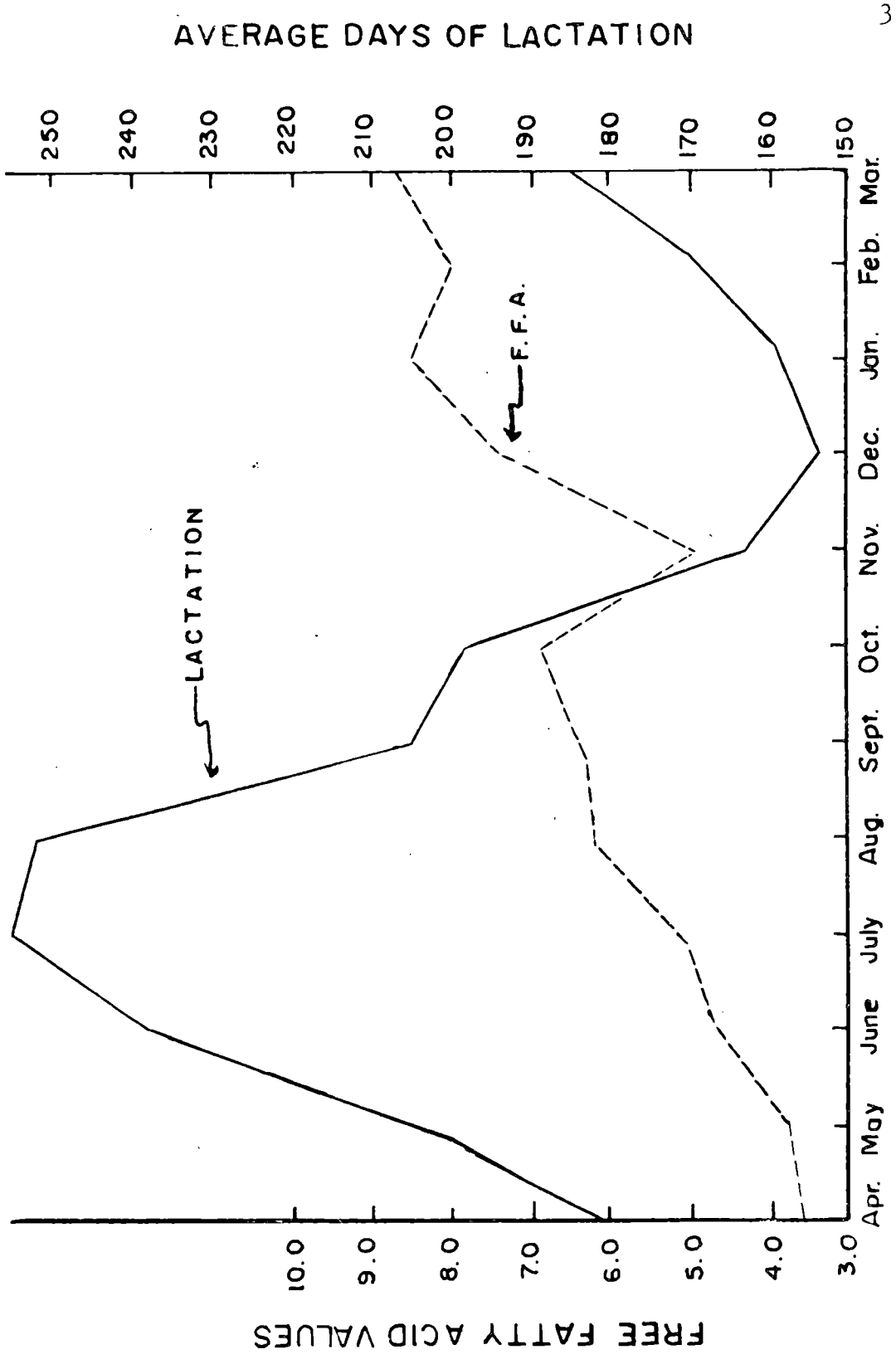

Figure 1. Comparison of free fatty acid values and average days of lactation throughout the year.

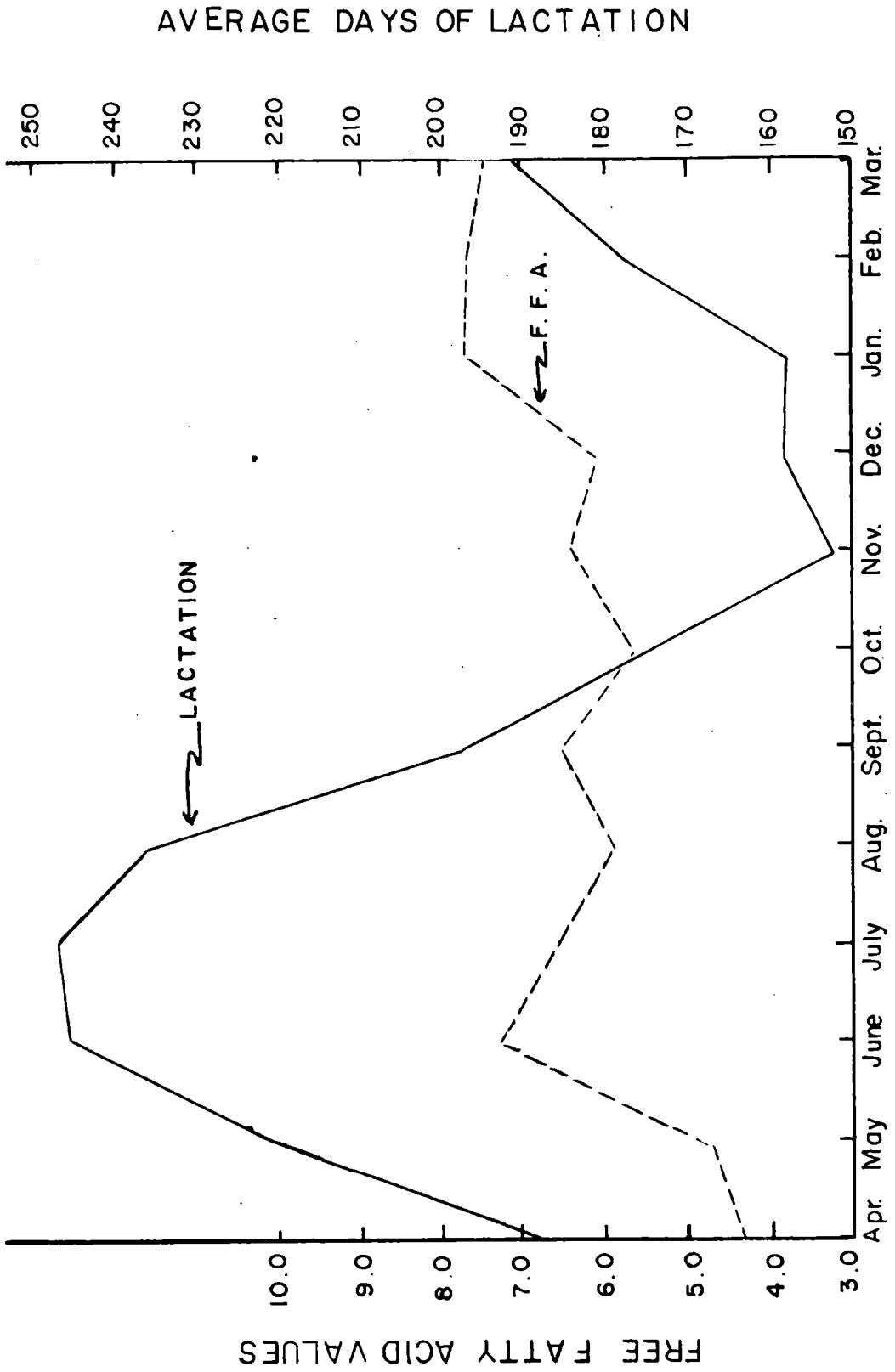

Figure 2. Comparison of free fatty acid values and average days of lactation throughout the year.

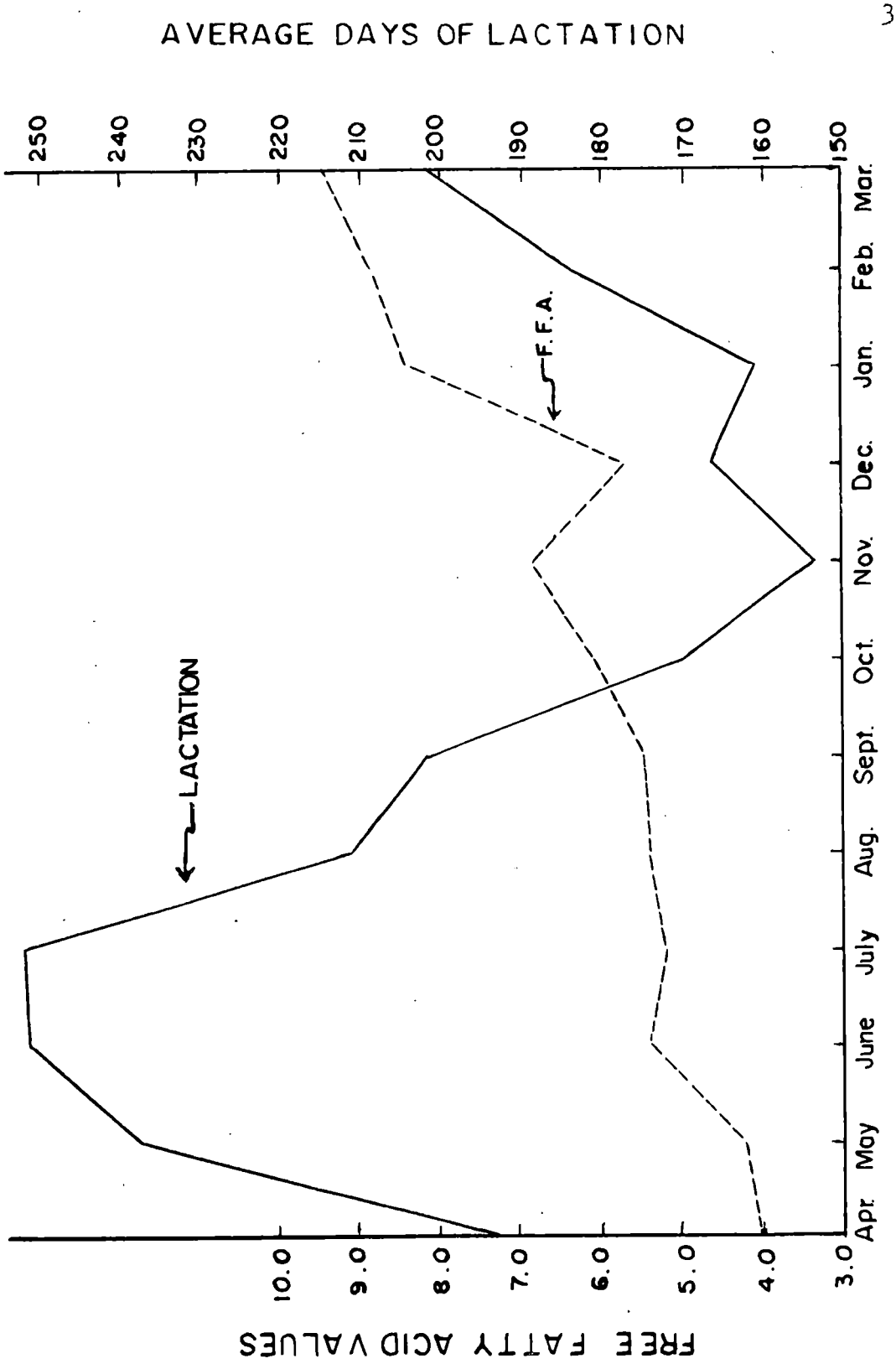

Figure 3. Comparison of free fatty acid values and average days of lactation throughout the year.

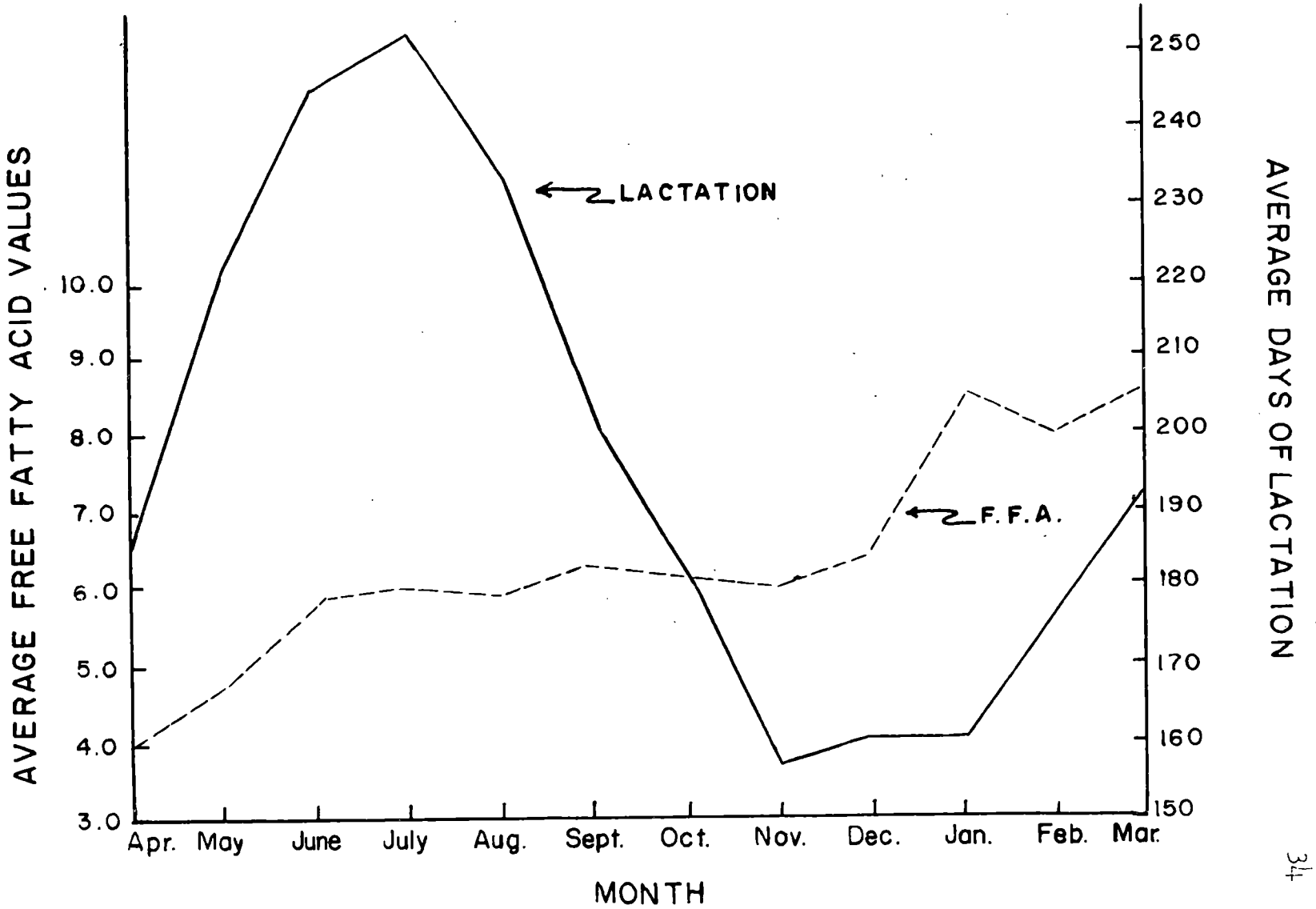

Figure 4. Comparison of the averages of the free fatty acid values and the average days of lactation for the 10th, 20th, and 30th of each month.

December which is accompanied by a slight increase in average days of lactation. Figure 4 also shows that the free fatty acid values were rather constant from September through November despite the reduction in average days of lactation. There might have been an increase in the free fatty acid values in September when the herd went off pasture if there had not also been the decrease in the stage of lactation.

Considerable fluctuations in free fatty acid values were noted during the winter months. It was observed that during extremely cold, snowy weather there was a marked increase in the free fatty acid values and during warm, mild periods there was a decided decrease. For example, Figure 3 shows a sudden drop in the free fatty acid values in December with a sharp increase in January, neither of which can be associated with feed or stage of lactation. However, during the last week of December the temperature was unseasonably high. Hansen and Theophilus (14) postulated that the activating effects of lower temperatures during the winter months may contribute to increased lipolysis. Herrington(18) also reported that spontaneous rancidity was much more common during the colder months.

Figures 1, 3 and 4 show that the free fatty acid values increased rapidly from December and January

through March. This rapid increase was probably brought about by a combination of reasons. The herd was on dry feed during this period. There was a rapid increase in the average days of lactation and the temperatures in Knoxville were unusually low through these months.

CHAPTER V

SUMMARY AND CONCLUSIONS

Samples of mixed, raw milk were taken from the University herd three times per week throughout the year. The free fatty acid content of the samples was estimated by extracting the fat with ether and titrating with alcoholic KOH.

The research findings show considerable fluctuations in the free fatty acid values from day to day with an average of 5.2 while the herd was on pasture and an average of 7.0 while the herd had no pasture. Minimum values were found during April, May and June while the cows were on fresh pasture and maximum values when the temperatures were unseasonably low and the herd was on winter feeding. Increased values were noted during extremely cold periods.

The free fatty acid values ran high throughout the year as compared with other reported values. Several factors were observed that may have contributed to high values. The use of pipeline milkers, the rate of cooling and temperature fluctuations, storing the raw milk from twenty-four to twenty-seven hours at low

temperatures (33°-45° F.), unusually cold winter and the fact that some of the cows were in extremely advanced lactation (584 days) all may have contributed to the high free fatty acid values.

The data in this trial suggests that the type of feed may have had a greater influence than the stage of lactation on the free fatty acid content of the milk. Lower free fatty acid values were found during the spring and summer months while the herd was on pasture; however, the average days of lactation were at a maximum during this period. In general, an increase in the average days of lactation was accompanied by an increase in the free fatty acid values.

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